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The Measure of Confidence

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Increasing Sample Throughput with High-Speed Megabore Columns

Application

Greater than 20% More Plates Per Meter

Improved Resolution and/or Faster Run Times Compared to 0.53-mm ID Columns

No Special Hardware Required

Decreasing the diameter of a capillary column is an effective way of increasing column efficiency. This increase in the number of theoretical plates per meter (N/m) can be utilized to improve compound resolution. A significant decrease in analysis time can also be achieved by adjusting the analysis conditions or shortening the column length.

For the chromatographer using Megabore (that is, 0.53-mm ID) columns, going to smaller internal diameter columns has not always been an option, due in part to capacity issues and injector and/or detector hardware incompatibilities. The 0.45-mm ID, High-Speed Megabore column introduces the traditional Megabore chromatographer to a column that can increase the resolution of analytes and/or reduce some analysis times by as much as 45%. Because Agilent's High-Speed Megabore columns retain the same outer diameter as 0.53-mm ID columns, no special ferrules or adaptors are required.

High-Speed Megabore columns also have the same phase ratio (ß) as 0.53-mm ID columns, making it very easy to translate the method conditions. Methods can easily be optimized for speed or resolution using free method translation software available from the Agilent Web site or by speaking with our Technical Support Department (call 800-227-9770 in the U.S. or Canada or visit www.agilent.com/chem).

On average, the High-Speed Megabore provides 24% more theoretical plates per meter than the comparable 0.53-mm ID column (Table 1). At some point, increasing a column's length can begin to work against chromatographic efficiency gain due to high carrier gas pressure drop in long capillaries. This is exemplified with the 105 m, DB-502.2. Figure 1 compares the two DB-502.2 columns for the analysis of volatile organics by purge and trap (for example, EPA Method 502.2). Most notable in these chromatograms are the essentially identical resolution of analytes and the 23-minute decrease in run time with the High-Speed Megabore column.

High-Speed Megabore columns are ideally suited to applications where dual 0.53-mm columns are currently being used. Figure 2a and 2b show one such application.

Table 1. Column Efficiencies

Column phase	Column length	Internal diameter	Film thickness [1]	Plates/meter (% increase) [2]
DB-VRX	75 meters	0.449 mm	2.55 µm	1997 (28)
	75 meters	0.540 mm	3.00 µm	1447
DB-624	75 meters	0.446 mm	2.55 µm	1402 (22)
	75 meters	0.546 mm	3.00 µm	1090
DB-502.2	75 meters	0.453 mm	2.55 µm	1526 (20)
	105 meters	0.544 mm	3.00 µm	873
DB-WAX	30 meters	0.447 mm	0.85 µm	1656 (25)
	30 meters	0.544 mm	1.00 µm	1357
DB-1	30 meters	0.455 mm	1.30 µm	1477 (27)
	30 meters	0.551 mm	1.50 µm	1357
DB-5	30 meters	0.446 mm	1.30 µm	1895 (23)
	30 meters	0.540 mm	1.50 µm	1454
DB-608	30 meters	0.450 mm	0.71 µm	1477 (23)
	30 meters	0.535 mm	0.83 µm	1134

[1] Phase ratio (ß) held constant for all columns

[2] Average 24%

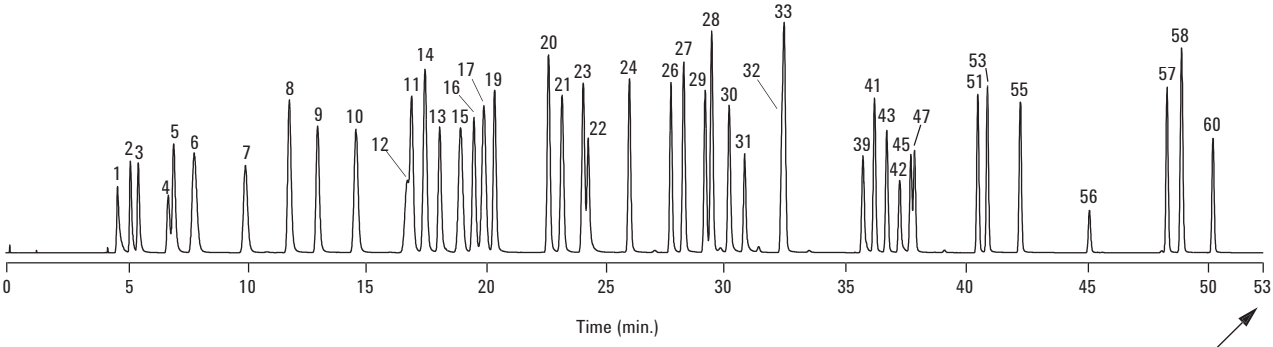
Compound List for all Chromatograms

1. Dichlorodifluoromethane
2. Chloromethane
3. Vinyl chloride
4. Bromomethane
5. Chloroethane
6. Trichlorofluoromethane
7. 1,1-Dichloroethene
8. Methylene chloride
9. trans-1,2-Dichloroethene
10. 1,1-Dichloroethane
11. cis-1,2-Dichlorethene
12. 2,2-Dichloropropane
13. Bromochloromethane
14. Chloroform
15. 1,1,1-Trichloroethane
16. 1,1-Dichloropropene
17. Carbon Tetrachloride
18. Benzene
19. 1,2-Dichloroethane
20. Silica trichloroethene
21. 1,2-Dichloropropane
22. Dibromomethane
23. Bromodichloromethane
24. cis-1,3-Dichloropropene
25. Toluene
26. trans-1,3-Dichloropropene
27. 1,1,2-Trichloroethane
28. Tetrachloroethene
29. 1,3-Dichloropropane
30. Dibromochloromethane
31. 1,2-Dibromomethane
32. Chlorobenzene
33. 1,1,1,2-Tetrachloroethane
34. Ethylbenzene
35. meta-Xylene
36. para-Xylene
37. ortho-Xylene
38. Styrene
39. Bromoform
40. Isopropylbenzene
41. 1,1,2,2-Tetrachloroethane
42. Bromobenzene
43. 1,2,3-Trichloropropane
44. n-Propylbenzene
45. 2-Chlorotoluene
46. 1,2,3-Trimethylbenzene
47. 4-Chlorotoluene
48. tert-Butylbenzene
49. 1,2,4-Trimethylbenzene
50. sec-Butylbenzene
51. 1,3-Dichlorobenzene
52. para-Isopropyltoluene
53. 1,4-Dichlorobenzene
54. n-Butylbenzene
55. 1,2-Dichlorobenzene
56. 1,2-Dibromo-3-chloropropane
57. 1,2,4-Trichlorobenzene
58. Hexachlorobutadiene
59. Naphthalene
60. 1,2,3-Trichlorobenzene

Conditions

Column: **DB-502.2, 105 m x 0.53-mm ID, 3.0 µm**
Part no.: 125-14A4
Carrier: Helium at 10 mL/min, measured at 35 °C
Oven: 35 °C for 10 min
35 °C - 200 °C at 4 °C/min
200 °C for 5 min

Injector: Purge and trap (OIA 4560)
40 ppb per component in 5 mL water
Trap: Tenax™/Silica gel/Charcoal
Detector: Electrolytic conductivity detector (ELCD) (OIA 4420) with NiCat™ reaction tube in the halogen mode



Conditions

Column: **DB-502.2, 75 m x 0.45-mm ID, 2.55 µm**
Part no.: 124-1474
Carrier: Helium at 9 mL/min, measured at 35 °C
Oven: 35 °C for 6 min
35 °C - 200 °C at 8 °C/min
200 °C for 3.5 min

Injector: Purge and trap (OIA 4560)
40 ppb per component in 5 mL water
Trap: Tenax/Silica gel/Charcoal
Detector: ELCD (OIA 4420) with NiCat reaction tube in the halogen mode

High-Speed Megabore saves 23 minutes!

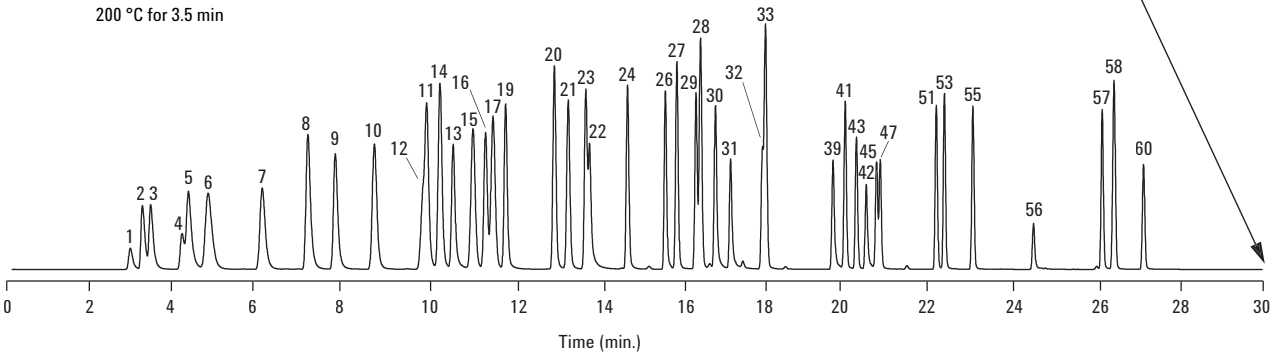


Figure 1. Analysis time comparison

Conditions
Figure 2a and 2b

Columns:	**DB-624** 75m x 0.45-mm ID, 2.55 µm Part no.: 124-1374 **DB-VRX** 75m x 0.45-mm ID, 2.55 µm Part no.: 124-1574
Guard Column:	5m x 0.53-mm ID deactivated fused silica tubing 3-way universal glass union
Carrier:	Helium at 9 mL/min (18 mL/min total), measured at 35 °C
Oven:	35 °C for 12 min 35 °C - 60 °C at 5 °C/min 60 °C for 1 min 60 °C - 200 °C at 17 °C/min 200 °C for 4 min
Injector:	Purge and trap (OIA 4560) 40 ppb per component in 5 mL water
Trap:	Tenax/Silica gel/Charcoal
Detector A:	Photoionization detector (PID) (OIA 4430) at 220 °C
Detector B:	Electrolytic conductivity detector (ELCD) (OIA 4420) with NiCat reaction tube in the halogen mode

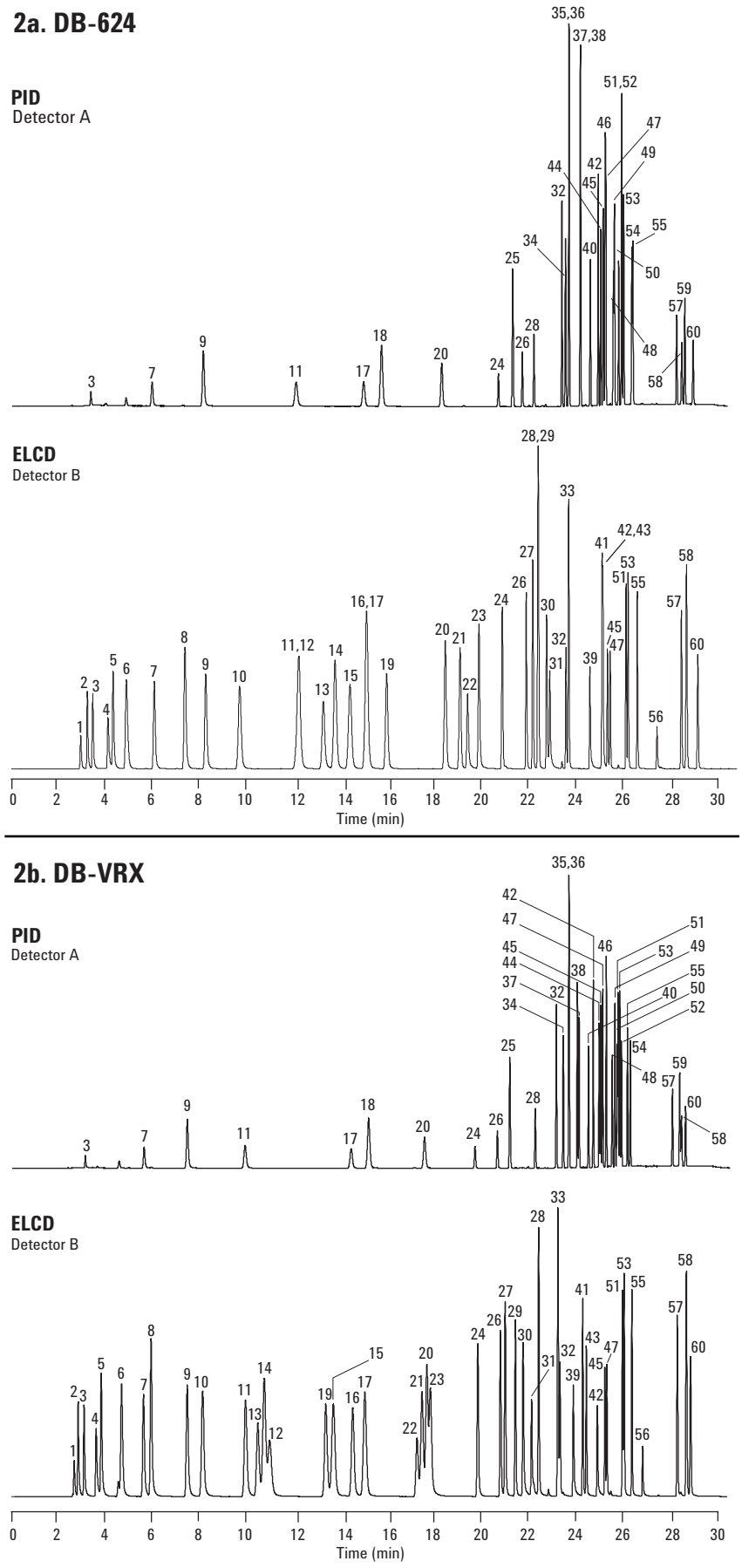


Figure 2a and 2b. High-Speed Megabore dual column applications.

0.45-mm ID High-Speed Megabore Column Order Guide

Phase[1]	Inner diameter (mm)	Length (meter)	Film thickness (µm)	Temperature limits (°C)	Part number
DB-1	0.45	15	1.27	-60 to 300/320	124-1012
DB-1	0.45	15	2.55	-60 to 260/280	124-1014
DB-1	0.45	30	0.42	-60 to 300/320	124-1037
DB-1	0.45	30	1.27	-60 to 300/320	124-1032
DB-1	0.45	30	2.55	-60 to 260/280	124-1034
DB-1	0.45	30	4.25	-60 to 260/280	124-1005
DB-1	0.45	60	1.27	-60 to 300/320	124-1062
DB-5	0.45	15	1.27	-60 to 300/320	124-5012
DB-5	0.45	30	0.42	-60 to 300/320	124-5037
DB-5	0.45	30	1.27	-60 to 300/320	124-5032
DB-5	0.45	30	4.25	-60 to 260/280	124-5035
DB-17	0.45	15	0.85	40 to 260/280	124-1712
DB-17	0.45	30	0.85	40 to 260/280	124-1732
DB-1701	0.45	30	0.42	-20 to 260/280	124-0737
DB-1701	0.45	30	0.85	-20 to 260/280	124-0732
DB-200	0.45	30	0.85	30 to 280/300	124-2032
DB-210	0.45	30	0.85	45 to 220/240	124-0232
DB-2887	0.45	10	2.55	-60 to 350	124-2814
DB-502.2	0.45	75	2.55	0 to 260/280	124-1474
DB-502.2	0.45	105	2.55	0 to 260/280	124-14A4
DB-608	0.45	30	0.42	40 to 260/280	124-6837
DB-608	0.45	30	0.70	40 to 260/280	124-1730
DB-624	0.45	30	2.55	-20 to 260	124-1334
DB-624	0.45	75	2.55	-20 to 260	124-1374
DB-FFAP	0.45	15	0.85	40 to 250/250	124-3212
DB-FFAP	0.45	30	0.85	40 to 250	124-3232
DB-MTBE	0.45	30	2.55	35 to 260/280	124-0034
DB-TPH	0.45	30	1.00	-10 to 290/290	124-1632
DB-VRX	0.45	30	2.55	-10 to 260	124-1534
DB-VRX	0.45	75	2.55	-10 to 260	124-1574
DB-WAX	0.45	60	0.85	20 to 230/240	124-7062
DB-WAX	0.45	15	0.85	20 to 230/240	124-7012
DB-WAX	0.45	30	0.85	20 to 230/240	124-7032
DB-WAXetr	0.45	5	1.70	50 to 230/250	124-7304
DB-XLB	0.45	30	1.27	30 to 320/340	124-1232

[1]Additional phases, lengths, and film thickness can be made with a 0.45-mm ID High-Speed Megabore column. If you do not find the column you are looking for, ask for a custom column quote (order part number 100-2000 and specify the phase, ID, length, and film thickness).

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Printed in the USA
February 13, 2002
5988-5271EN

Agilent Technologies

DuraGuard Columns: GC Columns with Built-In Protection

Application

Guard columns/retention gaps without the use of unions

- **Minimize front-end contamination of the column and increase column lifetime**
- **Aid in focusing sample onto the front end of the column for excellent peak shape**
- **Minimize the amount of mass selective detector (MSD) source contamination originating from the column**

All this with no leaks, no added activity, and no hassle

Deactivated fused silica tubing is commonly added to the front of an analytical column to act as a guard column or retention gap. It can also be added to the back of the analytical column as a transfer line into the MSD to minimize the amount of source contamination originating from the column.

Historically, deactivated tubing has been connected to the analytical column by using a union. These are difficult to install requiring a great deal of care and skill to ensure they will work properly. With incorrect installation unions can cause leaks resulting in column degradation, dead volume resulting in peak shape problems, or activity problems resulting in peak shape problems and/or response loss. Leaks are especially a problem when the union is located close to the MSD when using deactivated fused silica for the transfer line.

DuraGuard columns, with a built in length of deactivated fused silica tubing, avoid these potential problems. The deactivated fused silica and the analytical column are made with a single, continuous piece of fused silica tubing, thus eliminating the need for the union. Installation hassles, peak shape problems and leaks associated with unions are history. Samples containing difficult analytes such as pesticides or drugs can be chromatographed without any undesirable contributions from the union.

Guard Columns

DuraGuard columns are especially beneficial as guard columns when analyzing samples containing low levels of chemically active compounds. Unions can be active towards these analytes and can cause peak-shape problems, which in turn result in poor detection limits. DuraGuard columns eliminate the potentially active union by using a single piece of fused silica tubing. Agilent Technologies' special deactivation process results in extremely inert columns and tubing for a broad range of analyte types.

Guard columns are used when samples contain nonvolatile residues that contaminate the column. The nonvolatile residues deposit in the guard column and not in the analytical column. This greatly reduces the interaction between the residues and the sample since the guard column does not retain the solutes (because it contains no stationary phase). Also, the residues do not coat the stationary phase which often results in poor peak shapes. Periodic cutting or trimming of the guard column is usually required upon a build-up of residues. Guard columns 5–10 meters in length allow for substantial trimming before the entire guard column requires replacement. The onset of peak shape problems is the usual indicator that the guard column needs trimming or changing.

Retention Gaps

DuraGuard columns offer the user the benefits of a retention gap without the hassle of making critical clean column cuts and installing the fused silica tubing to the front of their analytical column with a union. By avoiding the union there are no additional sources of leaks or activity. The only difference is the improved peak shape of the analytes.

Retention gaps are used to improve peak shape for some types of samples, columns and GC conditions. Use of 3–5 meters of tubing is required to obtain the benefits of a retention gap. The situations that benefit the most from retention gaps are large volume injections (>2 µL) and solvent-stationary phase polarity mismatches for splitless, Megabore direct and on-column injections. Peak shapes are sometimes distorted when using combinations of these conditions. Polarity mismatches occur when the sample solvent and column stationary phase are very different in polarity. The greatest improvement is seen for the peaks eluting closest to the solvent front or solutes very similar to the solvent in polarity. The benefits of a retention gap are often unintentionally obtained when using a guard column.

MSD Transfer Line

DuraGuard columns help minimize source contamination without the potential for leaks. The vacuum system of the MSD makes it especially difficult to maintain a leak free system - particularly the closer the connection is to the MSD. The use of unions with Mass Spec Detectors has always been tricky and prone to leakage. By using a single piece of fused silica, there are no additional connections to cause leaks.

Using a piece of deactivated fused silica as the transfer line to an MSD can reduce the frequency of source cleaning. Often the MSD transfer line temperature is at or above the columns upper temperature limit and thermal degradation of the stationary phase occurs. Volatile polymer breakdown products are carried into the MSD and can deposit in the MSD ion source. Using deactivated fused silica tubing as the MSD transfer line eliminates the presence of polymer in the heated zone and decreases the amount of material that can contaminate the MSD source thus decreasing the frequency of required source cleanings.

Results

Figure 1 is an FID chromatogram of a complex test mixture separated using a combination DuraGuard column. Note the peak shape quality for notoriously difficult to analyze compounds.

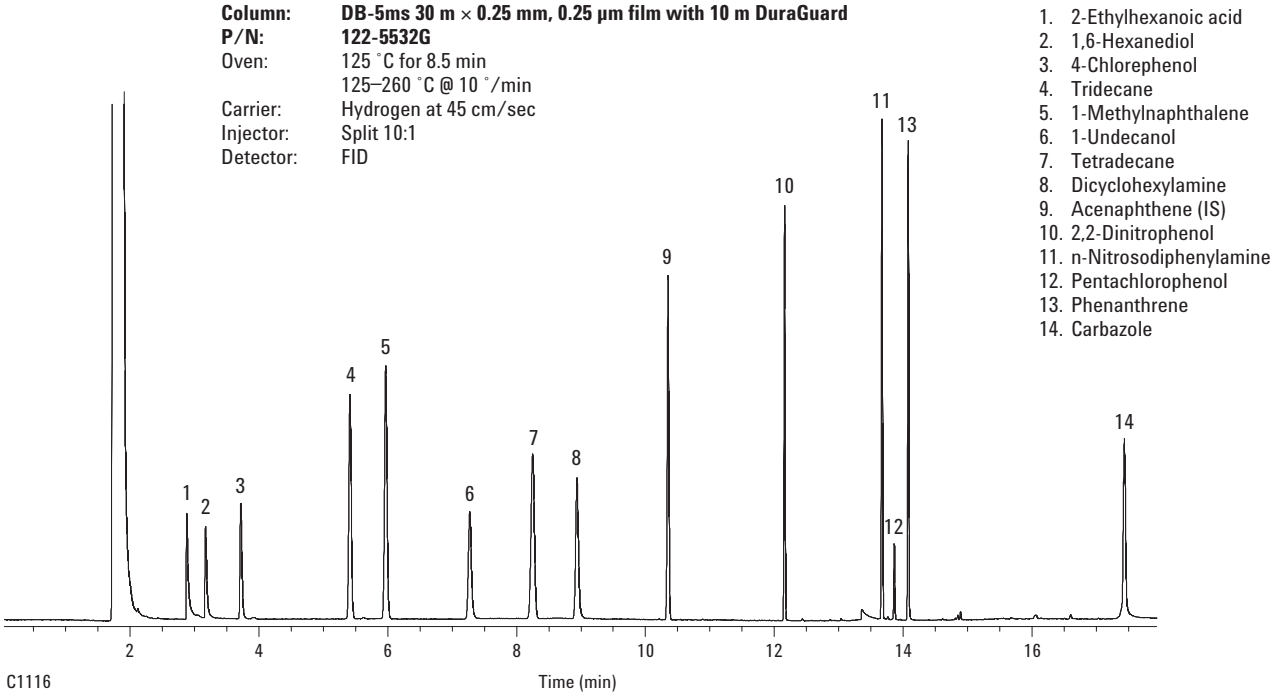

Figure 1. Chromatogram of test mixture using combination guard and analytical columns.

Want a Guard Column or Retention Gap of a Different Internal Diameter?

If you would prefer a guard column with a different diameter than your analytical column, save yourself the hassle of assembling union connections and let us do it for you! Agilent Technologies offers the dependable Leak-free connection service to meet your analytical needs: short guard columns, long guard columns, different diameters, or dual columns. Whatever you need, Agilent Technologies can provide through our Custom Column shop.

Our Leak-free connection service results in a dependable, long lasting leak-free connection. We use high quality glass press fit unions with polyimide sealing resin to ensure the connection will last. See Figure 2. At Agilent Technologies our technicians have years of experience in creating leak-free connections and in using special techniques to keep the polyimide sealing resin out of the flow path. Once the connection is carefully made, the resin is cured and the product is tested for leaks.

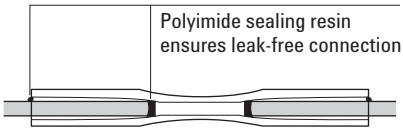

Figure 2. Detail of glass press fit union with polyimide sealing resin.

DuraGuard Column Order Guide

Part number	Phase	Inner diameter (mm)	Length (m)	Film thickness (µm)	DRGD Length (m)
122-1032G	DB-1	0.25	30	0.25	10
122-5532G	DB-5ms	0.25	30	0.25	10
122-5536G	DB-5ms	0.25	30	0.5	10
122-5533G	DB-5ms	0.25	30	1	10
122-5562G	DB-5ms	0.25	60	0.25	10
125-5537G	DB-5ms	0.53	30	0.5	10
122-1232G	DB-XLB	0.25	30	0.25	10
125-0732G	DB-1701	0.53	30	1	10
125-1334G5	DB-624	0.53	30	3	5

DuraGuard columns of different phases and dimensions are available through Agilent Technologies custom column shop. Any DB polysiloxane or low bleed phase can be made as a DuraGuard column with 0.18 mm id or larger fused silica tubing. Ask for a custom column quote (part number 100-2000 and specify the phase, id, length, and film thickness of analytical column, and desired length of DuraGuard).

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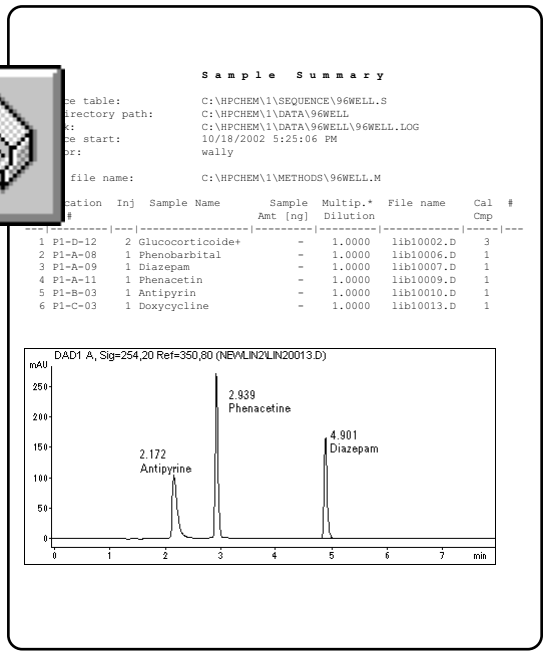
Printed in the USA
August 5, 2002
5988-7215EN

Agilent Technologies

Using Agilent ChemStation to generate summary reports for a single analysis or a sequence of analyses

Application

Angelika Gratzfeld-Huesgen



Sample Summary

ce table:	C:\HPCHEM\1\SEQUENCE\96WELL.S
irectory path:	C:\HPCHEM\1\DATA\96WELL
t:	C:\HPCHEM\1\DATA\96WELL\96WELL.LOG
ce start:	10/18/2002 5:25:06 PM
or:	wally
file name:	C:\HPCHEM\1\METHODS\96WELL.M

cation #	Inj	Sample Name	Sample Amt [ng]	Multip.* Dilution	File name	Cal Cmp #
1 P1-D-12	2	Glucocorticoide+	-	1.0000	lib10002.D	3
2 P1-A-08	1	Phenobarbital	-	1.0000	lib10006.D	1
3 P1-A-09	1	Diazepam	-	1.0000	lib10007.D	1
4 P1-A-11	1	Phenacetin	-	1.0000	lib10009.D	1
5 P1-B-03	1	Antipyrin	-	1.0000	lib10010.D	1
6 P1-C-03	1	Doxycycline	-	1.0000	lib10013.D	1

Introduction

The Agilent ChemStation base software includes a wide range of built-in report styles and types. For example, it provides standard reports such as area percent (AREA%), external standard (ESTD), internal standard (ISTD), and normalized (NORM) reports as well as system suitability reports and sequence summary reports with statistical evaluation of retention times, areas, heights and more.

For each type of report the user can determine the amount of information that is included in the report. The ChemStation base software also provides a report editor for customizing reports — a topic that is beyond the scope of this note.

This Application Note describes how to set up the different report types, explaining the software screens and giving example reports. The main objective is to give guidelines and to provide strategies on how to use the different built-in reports in the ChemStation base software.

Agilent Technologies

Equipment

The data for the report examples was generated using an Agilent 1100 Series HPLC system comprising the following modules.

- high pressure gradient pump
- micro-vacuum degasser
- well plate sampler
- thermostatted column compartment
- diode array detector

The Agilent ChemStation base software including the 3D data evaluation module, revision A.08.04, was used for instrument control, data acquisition, data handling, sample tracking, and reporting.

Report setup on ChemStation

The standard reporting function in the ChemStation base software provides for single run reports or sample-set reports for a full sequence of runs, whereby these so-called sequence summary reports can only be generated after completion of the sequence. The content of the sequence summary reports is defined by the acquisition sequence.

Further, the ChemStation base software includes a wide range of built-in standard reports that allow users to define the content and amount of printed information. Whereas this functionality meets the requirements of most standard applications to a large extent, it does not have the flexibility to create additional table elements for non-chromatographic information, charts or custom calculations.

If such extended reporting capabilities are required, it is recommended to use the ChemStation Plus data system including the ChemStore data organization module.

The ChemStation base software offers four types of report.

- Individual run reports, which can be generated automatically after each run or sequence, provide quick and easy printouts of results.

- Sequence summary reports provide comprehensive infomation for a full set of samples, including full GLP/GMP details. They are generated automatically at the end of a sequence and may include individual reports as well as statistical summary reports.

- Batch reports provide direct printouts of first-pass review modifications and results. They are generated during reprocessing of data from a complete sequence or of a subset of one sequence using ChemStation batch review.

- Advanced custom reports for requirements that go beyond the scope of the previous types. These include customized reports for individual runs or complete sequences and can also be obtained automatically after each run or sequence.

The following sections focus on the individual-run and sequence-summary report types, which are built-in as standard in the ChemStation base software, and explain in detail how to use and set up these report types.

Qualitative reports for individual runs

Qualitative reports are used mainly during the development of a separation or when a quick decision is needed as to whether a compound is present or not. Here the separation of peaks is of primary interest and a short AREA% report is sufficient. Particularly during method development it does not make sense to obtain reports with quantitative results.

Setup

To obtain an automated printout of an individual report such as a short AREA% report, the item *Standard Data Analysis* must be selected in the *Run Time Checklist*, which is part of the overall method for acquisition, data analysis and reporting, see figure 1. This screen is part of the *Edit Entire Method* dialog or can be accessed directly from the *Method* menu of the *Method and Run Control* view.

The item shown in figure 1 must be selected when the calculation of results is required, such as for printing reports, including sequence summary reports, with or without individual run reports.

Configuration

To obtain qualitative reports the item *Calculate* in the group *Quantitative Results* must be set to *Percent* as shown in figure 2.

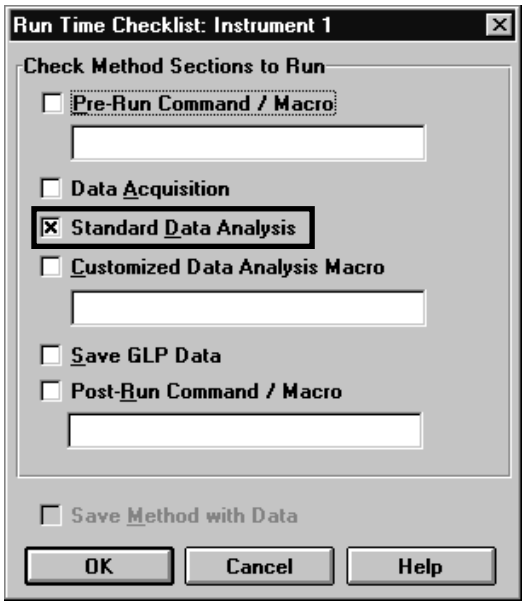


Figure 1
Activating *Standard Data Analysis*, including integration and quantification as part of the ChemStation method, is mandatory to obtain automated printouts of all report types available in the ChemStation base software

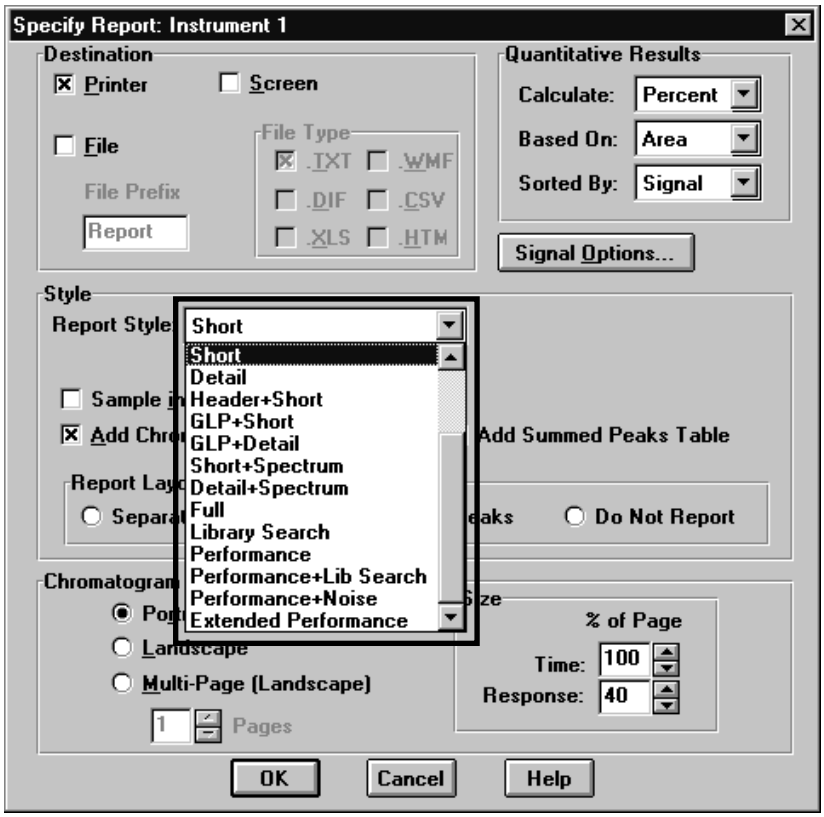


Figure 2
Specifying individual run reports

There are three ways to set up reports for individual runs.

1 Using the report smart icon in the *Method and Run Control* view.
2 Using part of the *Edit Entire Method* wizard
3 Using the *Data Analysis* view by selecting *Report* and then *Specify Report*.

Figure 2 shows the setup screen for run reports. Several report styles are available, covering a broad spectrum of report types. The report output can be sent to a printer, displayed on the screen or saved to a file. Multiple report destinations can be selected at a time. Other report parameters allow to include chromatograms, in landscape or portrait format or even distributed over several pages, and to define the way unknown compounds are reported.

An example of an AREA% report is given on page 12, containing information about the used method, data filename, time of injection, chromatogram and report.

The report styles that are available depend on the installed software modules. For example, the report styles Short+Spectrum, Detail+Spectrum and Library Serach are only available when the 3D data evaluation module is installed.

During method development the combination of *Percent* and *Performance* in reporting can be a valid tool to find out about k', resolution, selectivity, peak width and, for isocratic runs, the number of plates. An example is given on page 19.

Calculation procedures such as **Percent** (for others such as ESTD and ISTD, see section "Quantitative reports for individual runs") can be combined with any of the available standard reports shown in figure 2.

Qualitative reports can not use calculations based on standards such as ESTD and ISTD.

Quantitative reports for individual runs

Quantitative reports offer compound identification and compound quantification. They are mainly used with known samples or reference results in method optimization and quality control areas.

Setup

Before a quantitative report can be generated, standard samples with known compound concentrations have to be run and a calibration table has to be set up.

Peak integration should always be optimized before a peak is used as a reference in the calibration table and before the calibration tasks are done. To optimize integration, load a sample file with known sample concentration and then use the *Integration* tool set in the *Data Analysis* screen. When integration is optimized and saved, the calibration table can be created.

The calibration table is set up in *Data Analysis* from the *Calibration* menu, see figure 3.

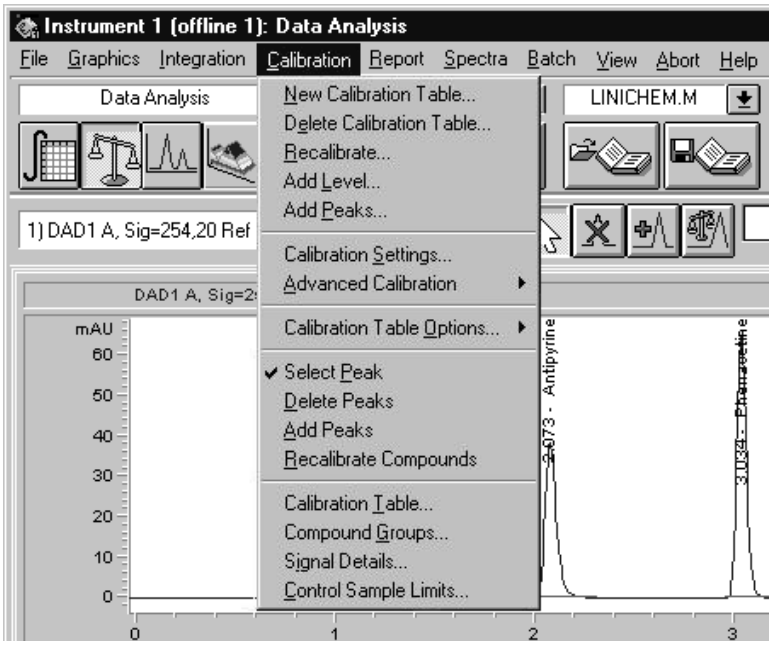


Figure 3
Calibration setup menu

In the following example we set up a multilevel calibration with four calibration levels. Multilevel calibrations use multiple files to complete the calibration. One file defines one level—completion of a four-level calibration thus requires four files. The steps involved are as follows.

1 Load the first file and click on *New Calibration Table*.

2 Calibrate each peak by selecting the peak (left mouse click), and filling in compound name and compound amount.

3 Repeat step 2 for all peaks.

4 When all peaks in the file are calibrated, load the next file with the next concentration. Use the *Add Level* tool to fill in the amounts for the next concentration level (level two).

5 Repeat step 4 for level three and four.

The calibration is stored as part of the ChemStation method. It is saved by simply saving the method. Every calibration update is easily accessible by loading the method, modifying (for example, updating) the calibration files and saving the new method revision.

Setup

When the calibration is complete all prerequisites for generating a quantitative report are met. The first step in generating a report is to specify the report style as described in the section "Qualitative reports for individual runs." The calibration of the method now offers access to all predefined report styles such as standards reports or normalized reports or, when running a sequence, to sequence summary reports (see separate section later.)

The calculation of results can be a normalized (NORM) area determination or based on an external standard (ESTD) or internal standard (ISTD). Result calculations can be based on area or height. Figure 4 shows selection of *External Standard Method* as calculation procedure and *Short* as *Report Style*. An example is given on page 13.

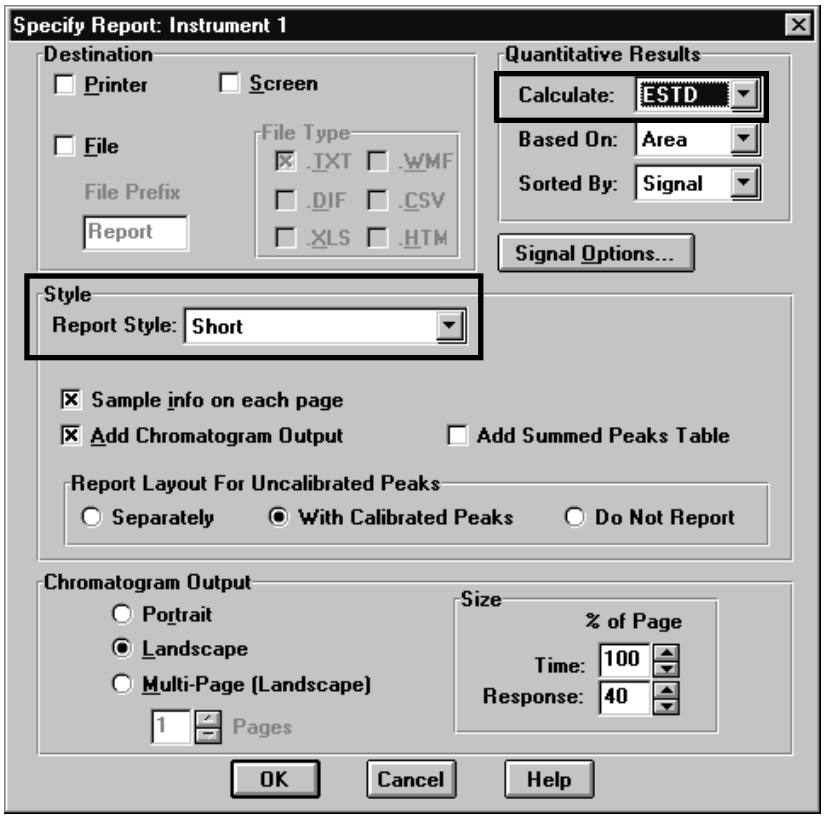


Figure 4
Selection of external standard report and short report style

Configuration

Additional report features can be specified such as output format for the chromatogram (including multipage outputs), picture size and the documentation of uncalibrated (which means unknown) peaks in the *Specify Report* screen as shown in figure 4. Any report style (see figure 2) can also be combined with any calculation procedure. Examples are given on pages 13 through 21.

- ESTD combined with report style *Short* (p 13)
- ESTD combined with report style *Library Search* (p 14)
- ESTD combined with report style *GLP+Short* (p 16)
- ESTD combined with report style *Performance* (p 19)
- ESTD combined with report style *Detail* (p 20)

Similar to the calibration, the report configuration is saved with the ChemStation method. Thus all data analysis steps for integration, calibration, result calculation and reporting are saved under one "umbrella" tool. Once setup, reuse of all steps is automated by simply reapplying the method to any sample under investigation.

The method that has been set up for data acquisition, integration, calibration and reporting has to be saved under a unique name to ensure that samples are analyzed and evaluated using the correct conditions.

Final report output

Final report outputs are quick and easy to obtain with ChemStation. Both qualitative and quantitative reports offer the same options and use identical tools to generate the final report.

Reports can be
- sent to a printer
- displayed on the screen for a quick review or preview when setting up report options
- saved to a file in HTML, CSV, XML, TXT, WMF, or DIF format

It is possible to combine all output types, for example, to get a printed copy on paper, an online report display on the screen and a file copy on the local hard disk.

The user can choose either
- automated report output at the end of each sample analysis (or reanalysis), or
- interactive report output at user request

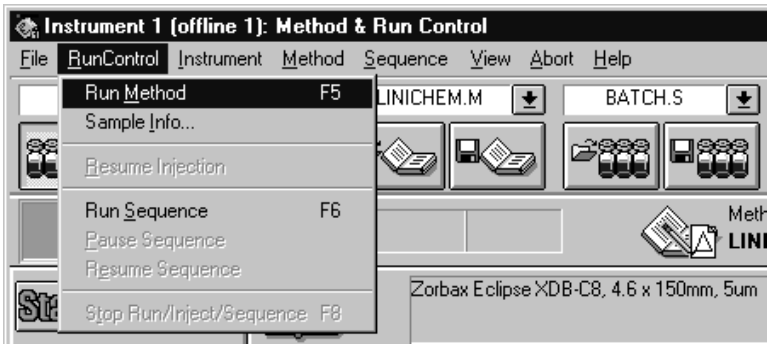


Figure 5
Run method for automated method execution and result output

Automated report output

An automated report is output whenever the ChemStation method is executed and at least one report destination is selected in the *Specify Report* screen, see figure 4. If no report output is desired, simply leave all report destination check boxes blank.

Method execution typically is used to analyze a sample or to reapply changes in calculations or calibration during data analysis. To execute a method, simply press *F5* or select *Run method* from the ChemStation *Run control* menu as shown in figure 5.

If the user wants to re-analyze data without data acquisition, *Data Acquisition* must be disabled in the *Run Time Checklist*, see figure 1.

Interactive report printout

Manual report output is available from the ChemStation *Data Analysis* view. It is designed to preview report outputs on the screen during report configuration or to get an individual sample report during interactive result analysis or result review.

The *Data Analysis* view is designed to set up advanced reports such as library searches, detailed spectrum reports and others. It has a separate report menu and additional smart icons for report setup, preview and output to a printer as shown in figure 6.

When the user wants a report during their data review session, they simply press the preview or print button and immediately get the report on the screen or on paper.

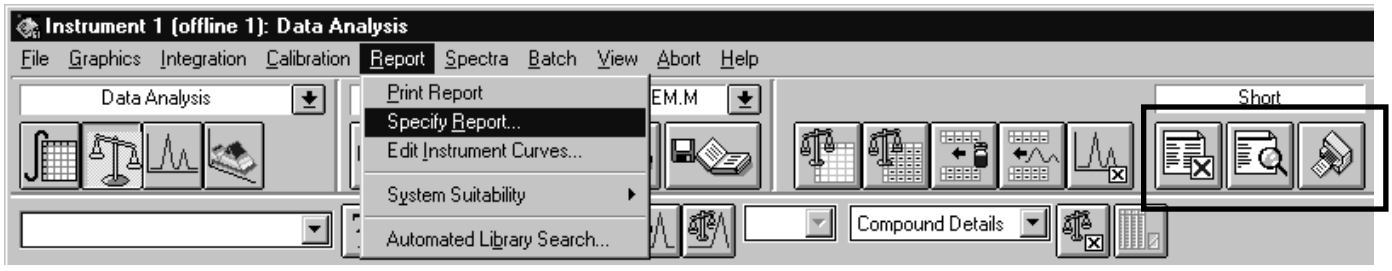


Figure 6
Report menu and smart icons (far right) in ChemStation Data Analysis view

Sequence summary reports

In contrast to individual run reports, sequence summary reports can only be generated for a complete set of samples that have been analyzed in one continuos sequence. The sequence summary report (also referred to as a system suitability report) is designed to meet the specific needs of GLP and GMP regulations in the pharmaceutical industry as well as comparable ISO and DIN regulations in other industries.

In addition to result calculation and result documentation, all regulations require additional documentation on how the results have been obtained and how "well" the analytical system behaved during analysis. The sequence summary report is a single all-inclusive report style, combining the analytical result with full documentation of how the result was obtained and the system suitability information, thereby providing a comprehensive report that addresses all regulatory requirements.

Sequence summary reports are frequently used in quality control work. These reports include the analytical results along with documented evidence of the system's suitability for the analytical purpose. System suitability is defined in the various Pharmacopoeia guidelines and it typically includes system performance information based on parameters such as peak width, theoretical plate number, resolution and others.

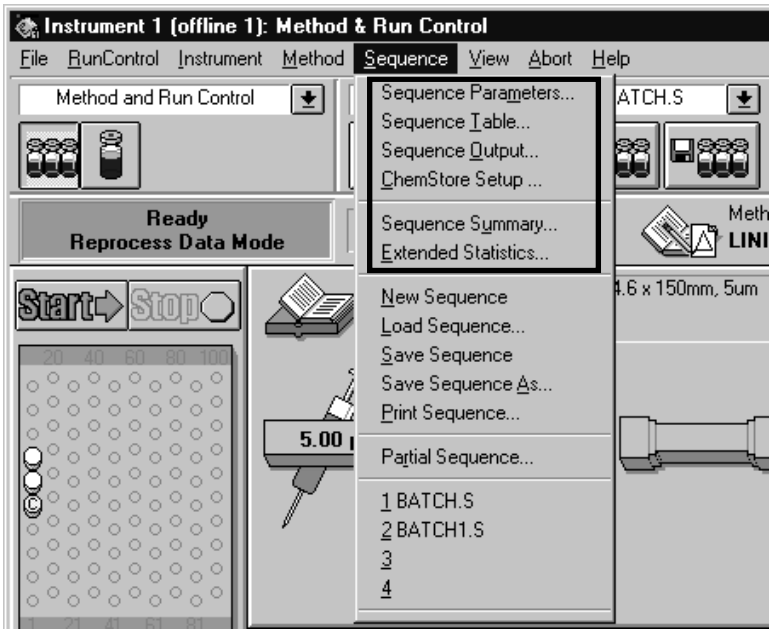


Figure 7
Entries need to be made in these sections to obtain automatically a sequence summary report at the end of a sequence

All these parameters are available in the report style, but the user must configure the report to suit their own specific needs. The following section describes setup and configuration of a sequence summary report in ChemStation.

Setup and configuration

After each sequence of runs a sequence summary report can be printed. Typically this is done to obtain statistical results and determine system suitability. In addition to the entries in the sequence table and before the report can be calculated and printed, several data inputs for sequence parameter and sequence output are required, see figure 7.

In the *Sequence Parameters* screen (figure 8) the item *Parts of Method to Run* must be set to *According to Runtime Checklist*. This entry determines which part of a method is executed during a sequence and *According to Runtime Checklist* refers to the run-time checklist configuration that was previously edited as part of the method in order to obtain integration and quantitative results.

If data acquisition is completed and the user wants to reanalyze a sequence of samples without data acquisition, the option *Reprocessing Only* allows to recalculate the sequence summary report easily.

In the *Sequence Output* screen the report destination and the content of a sequence summary report are defined by selecting the appropriate check boxes, see figure 9.

The content of the sequence summary report is defined by the items on the right side of the scrreen shown in figure 9. Selecting *Setup* in the *Sequence Output* dialog box accesses this configuration screen. The sequence summary report allows a variety of informations to be printed in one continuously enumerated report.

In addition to a wide selection of statistical results from sample and/or calibration runs, other items can be selected such as sample summary reports that list all acquired samples, com-

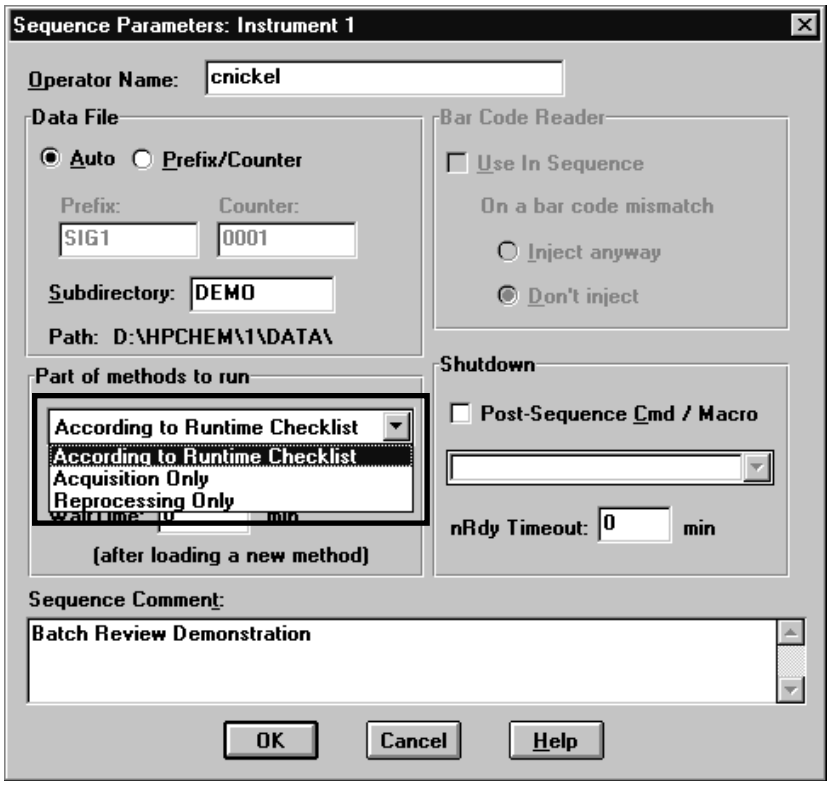


Figure 8
Sequence parameters screen

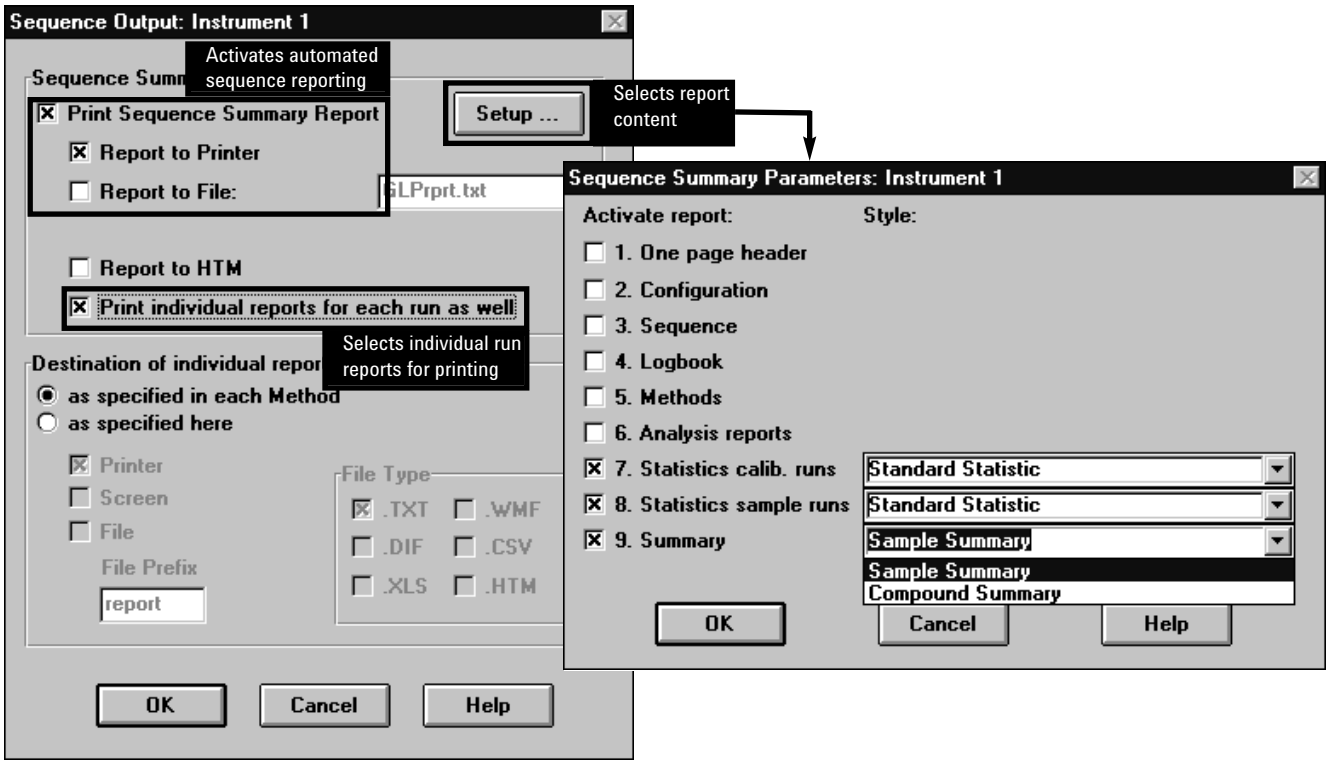


Figure 9
Selection of report destination and content of a sequence summary report

plete printouts of all parameters in the methods that were used, printouts of sequence logbooks and so on.

It is also possible to include the individual result reports for each run as part of the summary report instead of individual printouts after the end of each run.

The statistical evaluation of sequence runs is defined in the *Extended Statistic Parameter* screen, see figure 10. Statistical results can be obtained for all parameter shown in this dialog box. Either standard deviation or relative standard deviation or 95% confidence interval can be applied and upper/lower limits for each parameter can be specified.

A calibrated method is necessary to be able obtain statistical results.

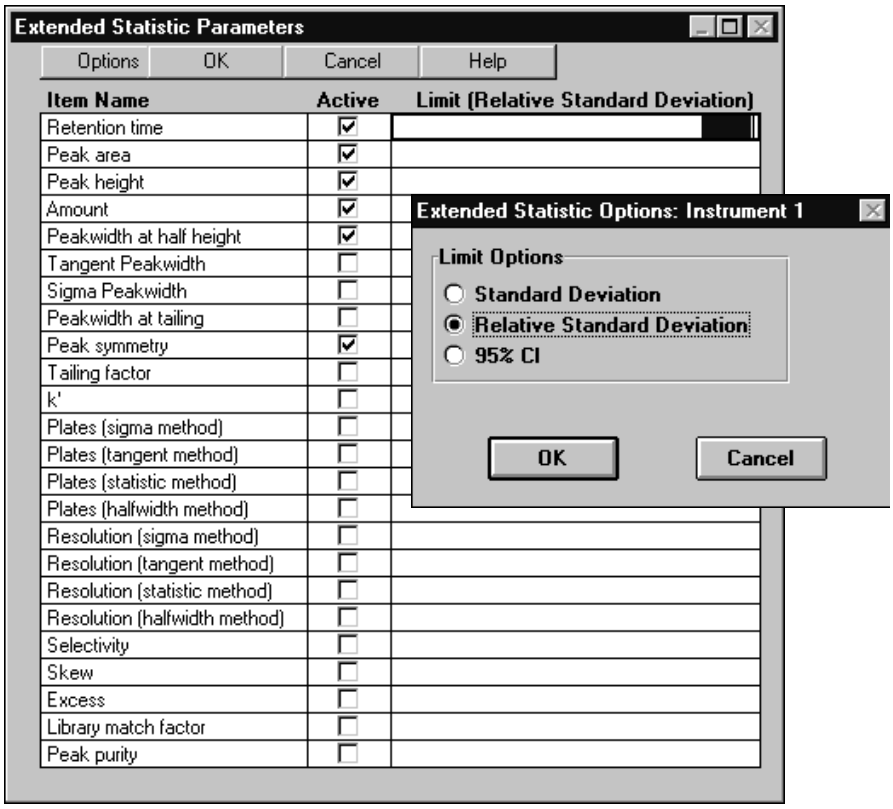


Figure 10
Setup of statistical calculations for sequence runs

Figure 11 shows the *Sequence Table* screen, in which it is important to ensure that the sample type is correctly set to *Sample*, *Calibration* or *Control Sample*, because statistical calculations can be selected based on sample type.

Figure 12 shows an example of a sequence summary report. It contains information about the analyzed samples such as location, sample name, filename, and so on. The header includes information such as operator name, the used chromatographic method, and date of acquisition.

Further report examples can be found on pages 11 through 35.

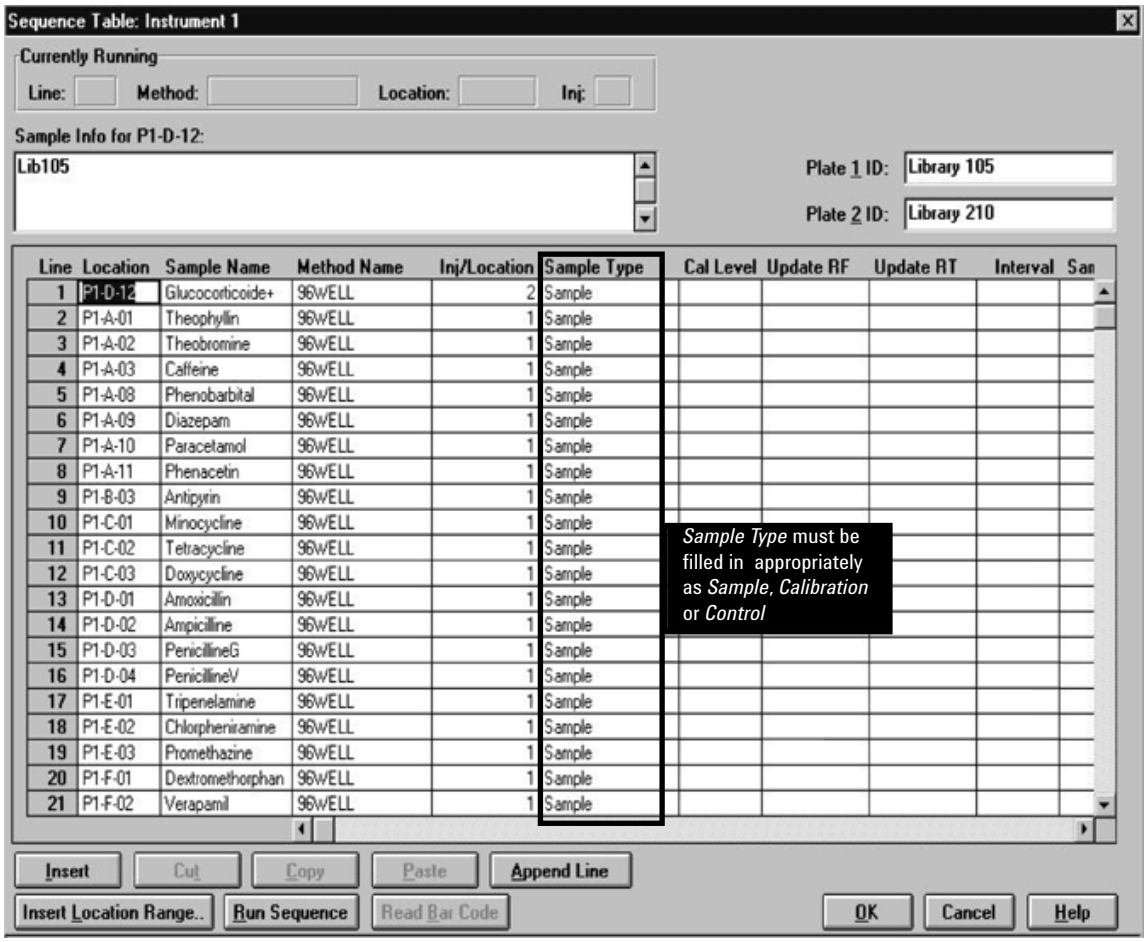


Figure 11
The Sequence Table screen

S a m p l e S u m m a r y

Sequence table: C:\HPCHEM\1\SEQUENCE\96WELL.S
Data directory path: C:\HPCHEM\1\DATA\96WELL
Logbook: C:\HPCHEM\1\DATA\96WELL\96WELL.LOG
Sequence start: 10/18/2002 5:25:06 PM
Operator: agratz

Method file name: C:\HPCHEM\1\METHODS\96WELL.M

Run #	Location #	Inj	Sample Name	Sample Amt [ng]	Multip.* Dilution	File name	Cal #	Cmp
1	P1-D-12	2	Glucocorticoide+	-	1.0000	lib10002.D	3	
2	P1-A-08	1	Phenobarbital	-	1.0000	lib10006.D	1	
3	P1-A-09	1	Diazepam	-	1.0000	lib10007.D	1	
4	P1-A-11	1	Phenacetin	-	1.0000	lib10009.D	1	
5	P1-B-03	1	Antipyrin	-	1.0000	lib10010.D	1	
6	P1-C-03	1	Doxycycline	-	1.0000	lib10013.D	1	

Figure 12
Example of a sequence sample summary report

Conclusion

The built-in single-run and sequences summary reports that are available in the ChemStation base software offer a wide range of reporting capabilities. The various reports give access to all important sample-related information quickly and easily. For all report types the user can select the amount of information to be included, from a simple qualitative report on one page through detailed quantitative reports to comprehensive and powerful sequence summary reports. Knowledge of a report editor is not required to be able to set up the ChemStation reports.

Reports can be obtained after each run or at the end of a sequence. With the ChemStation Method concept users starting from scratch can have a printed result copy of any type in less than 10 minutes – once set up the report is available within seconds after run completion. ChemStation reports are easy to configure, fast to obtain and quickly stored and managed.

Appendix

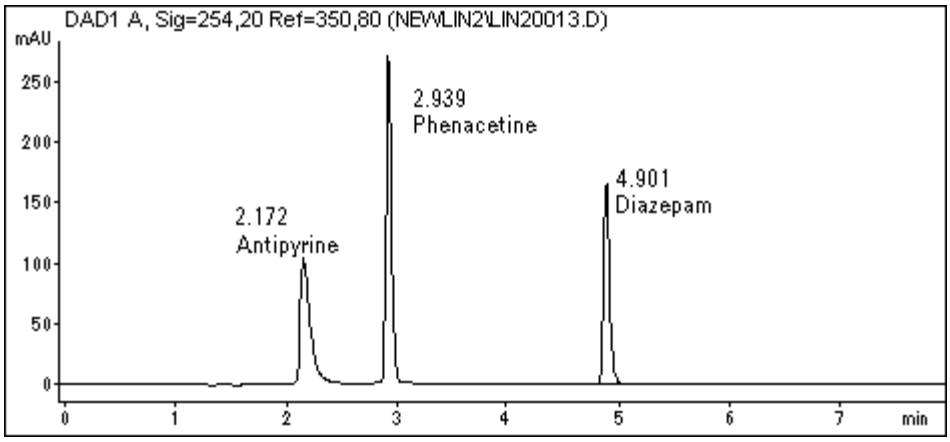
The following pages show examples of summary reports that can be generated with the ChemStation base software. The examples were generated using the print-to-file function and may have different pagination than a report printed directly from the ChemStation. Reports shown include:

- Short Area Percent Report
- Short ESTD Report
- Spectral Library Search Report
- Short GLP Report
- Performance Report
- Detail Report
- Extended Performance Report
- Sequence Summary Report – Compound Summary
- Sequence Summary Report – Standard Statistics for Sample Runs

Short Area Percent Report

```
Data File D:\HPCHEM\1\DATA\NEWLIN2\LIN20013.D
Instrument 1 1/24/02 8:54:14 AM agratz

==================================================================
Injection Date  : 10/25/00 8:47:20 AM            Seq. Line :   7
Sample Name     : sample1                         Location : Vial 2
Acq. Operator   : agratz                             Inj :   1
                                                 Inj Volume :  1 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\LINI2.M
Last changed    : 10/25/00 6:57:17 AM by agratz
Analysis Method : D:\HPCHEM\1\METHODS\LINICHEM.M
Last changed    : 1/24/02 8:53:08 AM by agratz
Zorbax Eclipse XDB-C8, 4.6 x 150 mm, 5 µm
==================================================================
```




```
                          Area Percent Report
==================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Thursday, January 24, 2002 8:52:20 AM
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: DAD1 A, Sig=254,20 Ref=350,80

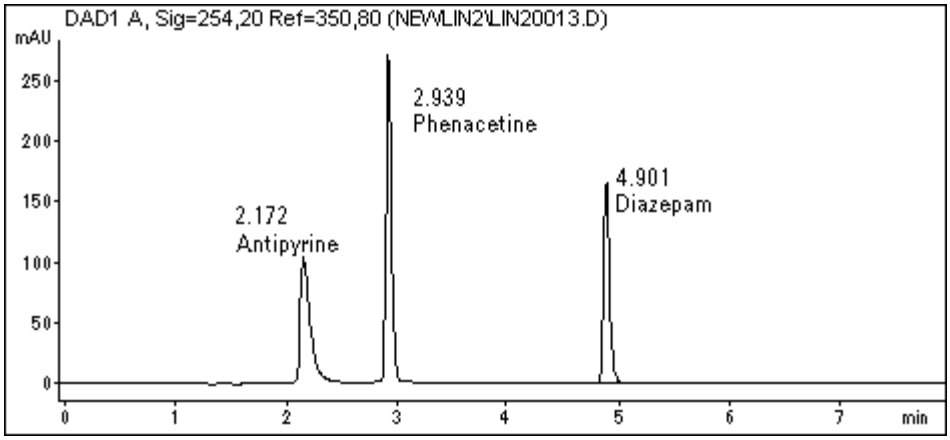
Peak RetTime  Type    Width      Area       Area     Name
  #    [min]           [min]    [mAU*s]       %
----|-------|------|-------|----------|--------|--------------------
  1  1.424    BV     0.0829    10.51506   0.4743 ?
  2  2.172    BB     0.0933   661.70422  29.8443 Antipyrine
  3  2.939    BB     0.0535   934.32690  42.1402 Phenacetine
  4  4.901    BB     0.0566   610.64050  27.5412 Diazepam
Totals :                    2217.18669

==================================================================
                          *** End of Report ***
```

Short ESTD Report

```
Data File D:\HPCHEM\1\DATA\NEWLIN2\LIN20013.D
Instrument 1 1/24/02 9:09:23 AM agratz

=====================================================================
Injection Date  : 10/25/00 8:47:20 AM             Seq. Line :   7
Sample Name     : sample1                          Location : Vial 2
Acq. Operator   : agratz                                Inj :   1
                                                  Inj Volume : 1 µl
Different Inj Volume from Sequence !       Actual Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\LINI2.M
Last changed    : 10/25/00 6:57:17 AM by agratz
Analysis Method : D:\HPCHEM\1\METHODS\LINICHEM.M
Last changed    : 1/24/02 9:09:14 AM by agratz
                  (modified after loading)
Zorbax Eclipse XDB-C8, 4.6 x 150 mm, 5 µm
=====================================================================
```




```
                        External Standard Report
=====================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Thursday, January 24, 2002 9:09:12 AM
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: DAD1 A, Sig=254,20 Ref=350,80

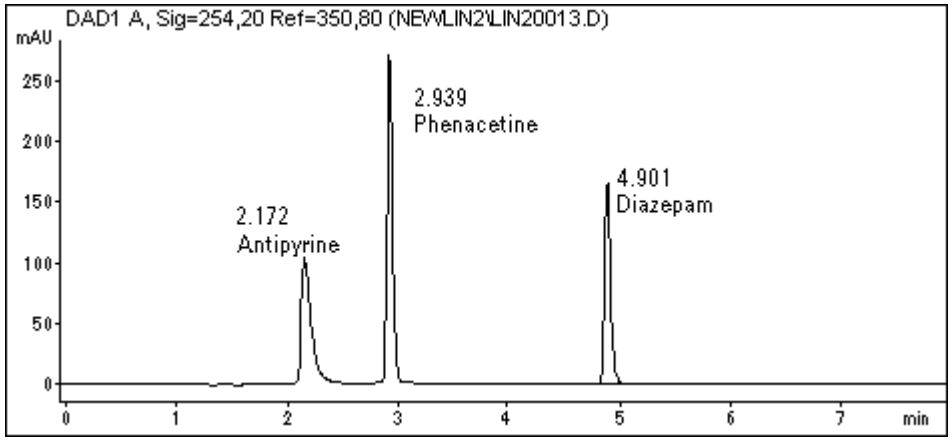
RetTime  Type      Area      Amt/Area     Amount    Grp    Name
 [min]           [mAU*s]                  [ng]
-------|------|----------|----------|----------|--|------------------
  2.172 BB      661.70422 6.62986e-1   438.70069     Antipyrine
  2.939 BB      934.32690    1.00317   937.28787     Phenacetine
  4.901 BB      610.64050 9.81915e-1   599.59734     Diazepam
Totals :                             1975.58590

=====================================================================
                            *** End of Report ***
```

Spectral Library Search Report

```
Data File D:\HPCHEM\1\DATA\NEWLIN2\LIN20013.D
Instrument 1 1/24/02 9:28:46 AM agratz

=====================================================================
Injection Date  : 10/25/00 8:47:20 AM             Seq. Line :   7
Sample Name     : sample1                           Location : Vial 2
Acq. Operator   : agratz                                 Inj :   1
                                                    Inj Volume : 1 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\LINI2.M
Last changed    : 10/25/00 6:57:17 AM by agratz
Analysis Method : D:\HPCHEM\1\METHODS\LINICHEM.M
Last changed    : 1/24/02 9:28:26 AM by agratz
                  (modified after loading)
Zorbax Eclipse XDB-C8, 4.6 x 150 mm, 5 µm
=====================================================================
```




```
                    External Standard Report
=====================================================================

Calib. Data Modified  :       Thursday, January 24, 2002 9:09:12 AM
Multiplier            :       1.0000
Dilution              :       1.0000

Library search mode:     Automatic library search

Library file No.       : 1
Library file name      : D:\HPCHEM\1\METHODS\LINICHEM.M\PHARMA.UVL
Match threshold        :     950        Purity threshold: Calculated
Time window  left [%] :    5.00         Case sensitive : No
Time window right [%] :    5.00             Whole word : No
Wavelength shift       :     0.0       Compare spectrum : Yes
Absorbance threshold   :     0.0           Search logic : OR
Search range           :     All
```

Spectral Library Search Report (continued)

Signal 1: DAD1 A, Sig=254,20 Ref=350,80
 Results obtained with standard integrator!
Calibrated compounds:

Meas. RetTime [min]	Library RetTime [min]	CalTbl RetTime [min]	Sig	Amount [ng]	Purity Factor	#	Library Match	Name
2.172	2.177	2.071	1	438.70069	1000	1	1000	Antipyrine
2.939	2.944	3.038	1	937.28787	1000	1	1000	Phenacetine
4.901	4.904	5.090	1	599.59734	1000	1	1000	Diazepam

Note(s):
u: compound identified at upslope. Purity factor exceeds threshold.
d: compound identified at downslope. Purity factor exceeds threshold.

==========
*** End of Report ***

Short GLP Report

```
Data File D:\HPCHEM\1\DATA\NEWLIN2\LIN20013.D
Instrument 1 1/24/02 9:31:21 AM agratz

This is a special file, named RPTHEAD.TXT, in the directory of a method
which allows you to customize the report header page.
It can be used to identify the laboratory which uses the method.

This file is printed on the first page with the report styles:

   Header+Short, GLP+Short, GLP+Detail, Short+Spec, Detail+Spec, Full


                  XXXX    XXX
                 XX  XX    XX
                XX         XX        XXXXX    XXX XX
                XX         XX XXX   XX     X   XX X XX
                XX     X   XXX XX   XXXXXXX   XX X XX
                 XX  XX    XX  XX   XX         XX    XX
                  XXXX    XXX  XXX   XXXXX    XXX   XXX


    XXXXXX      X                   X          XX
   XX     X     XX                  XX
   XX          XXXXX     XXXXX     XXXXX      XXX       XXXX     XX XXX
    XXXXX      XX            X      XX          XX      XX  XX    XXX XX
        XX     XX       XXXXXX      XX          XX      XX  XX    XX  XX
   X    XX     XX XX   X    XX      XX XX       XX      XX  XX    XX  XX
   XXXXXX       XXX    XXXXX X      XXX       XXXX      XXXX     XX  XX


                                                                  X
         XX XXX     XXXXX    XX XXX      XXXX    XX XXX     XXXXX
          XXX XX   XX     X    XX  XX    XX  XX    XXX XX     XX
          XX       XXXXXXX    XX  XX    XX  XX    XX         XX
          XX       XX          XXXXX     XX  XX    XX         XX XX
         XXXX       XXXXX     XX          XXXX    XXXX         XXX
                              XXXX


         XXX                                XXX
          XX                                 XX
          XX         XXXXX     XXXXX         XX     XXXXX    XX XXX
          XX XXX   XX     X         X      XXXXX    XX     X    XXX XX
          XXX XX   XXXXXXX   XXXXXX     XX  XX    XXXXXXX    XX
          XX  XX   XX         X    XX    XX  XX    XX          XX
         XXX  XXX   XXXXX    XXXXX X    XXXX X    XXXXX    XXXX
```

Short GLP Report (continued)

```
==================================================================
Injection Date  : 10/25/00 8:47:20 AM             Seq. Line :   7
Sample Name     : sample1                         Location : Vial 2
Acq. Operator   : agratz                               Inj :   1
                                                 Inj Volume : 1 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\LINI2.M
Last changed    : 10/25/00 6:57:17 AM by agratz
Analysis Method : D:\HPCHEM\1\METHODS\LINICHEM.M
Last changed    : 1/24/02 9:31:10 AM by agratz
                  (modified after loading)
Zorbax Eclipse XDB-C8, 4.6 x 150 mm, 5 µm
==================================================================
Module                                Firmware revision  Serial number
-----------------------------------|------------------|---------------
1100 Wellplate Autosampler            A.04.08            DE02700294
1100 Column Thermostat                A.04.06            DE53400174
1100 Diode Array Detector             S.03.91            DE00900051
1100 Binary Pump                      A.04.06            DE53500104
1100 Sample Thermostat                n/a                DE82203241

Software Revisions for:
- Acquisition:   Rev. A.08.03 [847] Copyright © Agilent Technologies
- Data Analysis: Rev. A.08.04 [1008] Copyright © Agilent Technologies
==================================================================

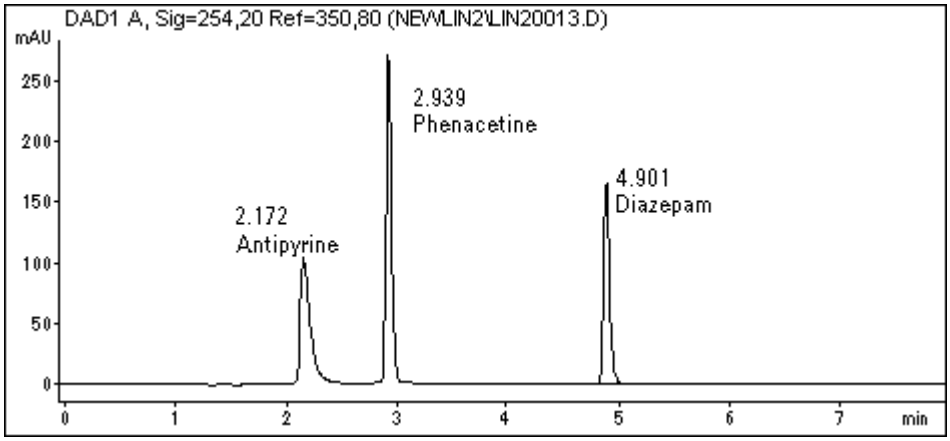
Instrument Conditions   :    At Start            At Stop
Air Temperature (Tray)  :      20.1   °C
Column Temp. (left)     :      40.0                40.0   °C
Column Temp. (right)    :      40.0                40.0   °C
Pressure                :      69.8                75.7   bar
Flow                    :       1.200               1.200 ml/min

Detector Lamp Burn Times: Current On-Time  Accumulated On-Time
DAD 1, UV Lamp          :       2.44              454.9   h
DAD 1, Visible Lamp     :       2.44              424.1   h

Solvent Description     :
PMP1, Solvent A         : Water
PMP1, Solvent B         : acn
==================================================================
```

Short GLP Report (continued)

```
                                Run Logbook
=====================================================================
Method        Method started:  line# 7 vial# 2 inj# 1    10:46:18 10/25/00
Method        Instrument running sample Vial 2           10:46:18 10/25/00
1100 ALS    1 Air temperature (tray) = 20.1 °C           10:47:21 10/25/00
1100 PMP    1 Pressure = 69.8 bar                        10:47:21 10/25/00
1100 THM    1 Column temperature = 40.0 °C               10:47:21 10/25/00
1100 THM    1 Column temperature = 40.0 °C               10:55:21 10/25/00
1100 PMP    1 Pressure = 75.7 bar                        10:55:21 10/25/00
Method        Instrument run completed                  10:55:23 10/25/00
Method        Method completed                           10:55:23 10/25/00
=====================================================================
=====================================================================
```




```
                    External Standard Report
=====================================================================

Sorted By             :       Signal
Calib. Data Modified  :       Thursday, January 24, 2002 9:09:12 AM
Multiplier            :       1.0000
Dilution              :       1.0000

Signal 1: DAD1 A, Sig=254,20 Ref=350,80

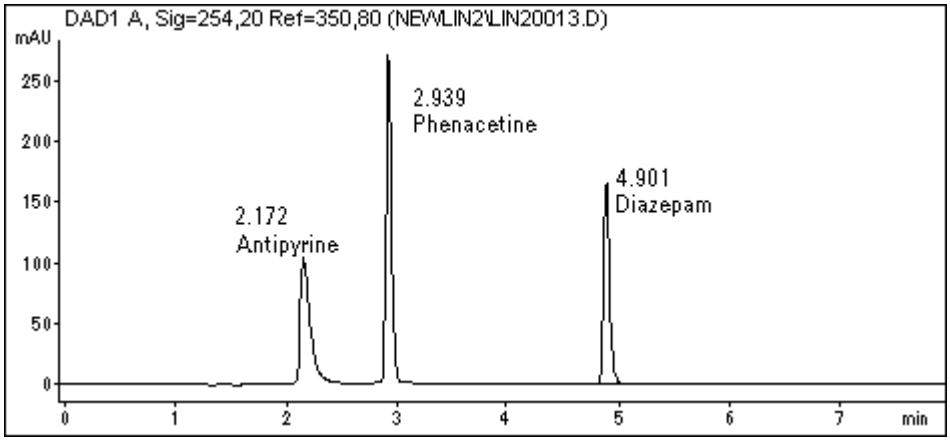
RetTime  Type      Area      Amt/Area     Amount    Grp    Name
 [min]           [mAU*s]                  [ng]
-------|------|----------|----------|----------|--|------------------
  2.172 BB       661.70422 6.62986e-1   438.70069     Antipyrine
  2.939 BB       934.32690     1.00317  937.28787     Phenacetine
  4.901 BB       610.64050 9.81915e-1   599.59734     Diazepam
Totals :                                1975.58590

=====================================================================
                            *** End of Report ***
```

Performance report

```
Data File D:\HPCHEM\1\DATA\NEWLIN2\LIN20013.D
Instrument 1 1/24/02 9:36:38 AM agratz

================================================================
Injection Date  : 10/25/00 8:47:20 AM             Seq. Line :   7
Sample Name     : sample1                           Location : Vial 2
Acq. Operator   : agratz                                 Inj :   1
                                                    Inj Volume : 1 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\LINI2.M
Last changed    : 10/25/00 6:57:17 AM by agratz
Analysis Method : D:\HPCHEM\1\METHODS\LINICHEM.M
Last changed    : 1/24/02 9:36:32 AM by agratz (modified after loading)
Zorbax Eclipse XDB-C8, 4.6 x 150mm, 5µm
================================================================
```




```
     External Standard Report with Performance
================================================================

Calib. Data Modified  :       Thursday, January 24, 2002 9:09:12 AM
Multiplier            :       1.0000
Dilution              :       1.0000

Signal 1: DAD1 A, Sig=254,20 Ref=350,80
 Results obtained with standard integrator!

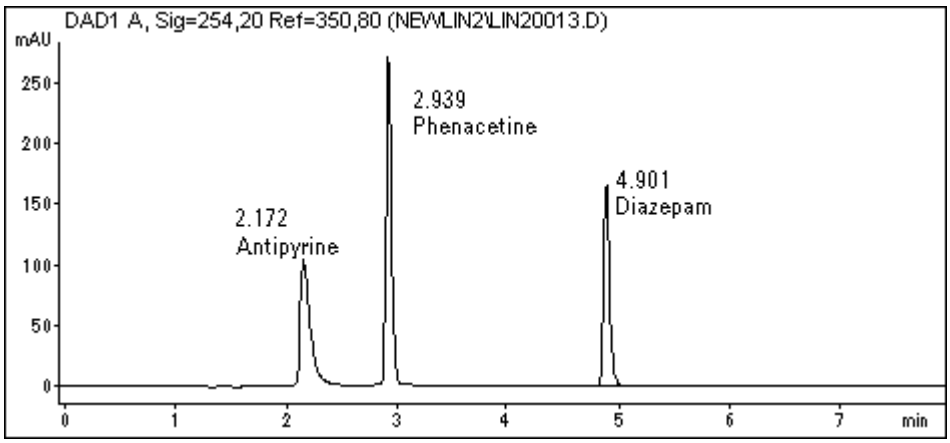
RetTime     k'  Sig    Amount     Symm.  Width    Plates Resol  Name
 [min]                  [ng]             [min]           ution
-------|------|---|----------||-----|-------|-------|-----|----------
 2.172    0.81  1    438.70069    0.44  0.0883     3351  4.47 Antipyrine
 2.939    1.45  1    937.28787    0.83  0.0524    17435  6.40 Phenacetine
 4.901    3.08  1    599.59734    0.80  0.0550    43990 21.47 Diazepam

================================================================
                          *** End of Report ***
```

Detail report

```
Data File D:\HPCHEM\1\DATA\NEWLIN2\LIN20013.D
Instrument 1 1/24/02 9:51:47 AM agratz

================================================================
Injection Date  : 10/25/00 8:47:20 AM             Seq. Line :   7
Sample Name     : sample1                         Location : Vial 2
Acq. Operator   : agratz                                 Inj :   1
                                                  Inj Volume : 1 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\LINI2.M
Last changed    : 10/25/00 6:57:17 AM by agratz
Analysis Method : D:\HPCHEM\1\METHODS\LINICHEM.M
Last changed    : 1/24/02 9:51:35 AM by agratz
                  (modified after loading)
Zorbax Eclipse XDB-C8, 4.6 x 150 mm, 5 µm
================================================================
```




```
                  External Standard Report
================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Thursday, January 24, 2002 9:09:12 AM
Multiplier            :      1.0000
Dilution              :      1.0000

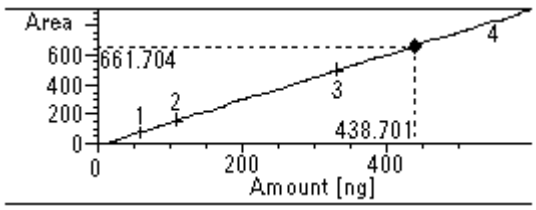
Signal 1: DAD1 A, Sig=254,20 Ref=350,80

RetTime  Type      Area      Amt/Area    Amount    Grp    Name
 [min]           [mAU*s]                 [ng]
-------|------|----------|----------|----------|--|------------------
  2.172 BB       661.70422 6.62986e-1  438.70069     Antipyrine
  2.939 BB       934.32690    1.00317  937.28787     Phenacetine
  4.901 BB       610.64050 9.81915e-1  599.59734     Diazepam
Totals :                               1975.58590
================================================================
```

Detail report (continued)

```
======================================================================
Injection Date  : 10/25/00 8:47:20 AM             Seq. Line :   7
Sample Name     : sample1                         Location : Vial 2
Acq. Operator   : agratz                                 Inj :   1
                                                  Inj Volume : 1 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 10 µl
Acq. Method     : C:\HPCHEM\1\METHODS\LINI2.M
Last changed    : 10/25/00 6:57:17 AM by agratz
Analysis Method : D:\HPCHEM\1\METHODS\LINICHEM.M
Last changed    : 1/24/02 9:51:35 AM by agratz
                  (modified after loading)
Zorbax Eclipse XDB-C8, 4.6 x 150 mm, 5 µm
======================================================================

======================================================================
----------------------------------------------------------------------
                        Calibration Curves
======================================================================
```




```
Antipyrine at exp. RT: 2.071
DAD1 A, Sig=254,20 Ref=350,80
Correlation:          1.00000
Residual Std. Dev.:   0.00000
Formula: y = ax^3 + bx^2 + cx + d
      a:     1.00818e-7
      b:     9.51014e-5
      c:     1.57593
      d:   -19.85331
      x: Amount (ng)
      y: Area
```

:
:
:

The header information and calibration curve is repeated for each peak

```
======================================================================
                      *** End of Report ***
```

Extended Performance Report

```
Data File D:\HPCHEM\1\DATA\SYSSUI\CONOOO05.D

                  Extended Performance Report

Instrument: Instrument 1

Module                          Firmware revision         Serial number
-------------------------------------------------------------------------
1100 Quaternary Pump            A.04.11                   DEl 1116042
1100 Wellplate Autosampler      A.04.13                   DE02700294
1100 Column Thermostat          A.04.11                   DE53400174
1100 Diode Array Detector       A.04.11                   DEO0900051
1100 Sample Thermostat          n/a                       DE82203241

Specials:
micro column switching valve installed in oven

Software Revisions for:
-Acquisition: Rev. A.08.04 [982] Copyright @ Agilent Technologies
-Data Analysis: Rev. A.08.04 [1008] Copyright @ Agilent Technologies

Column Description: XDB-C8
Product# Zorbax Batch#: b99024
Serial# USLLO00162
Diameter 2.1 mm Length: 30.0 mm
Particle size 3.5 mm Void volume 0.08 ml
Maximum Pressure 350 bar Maximum pH : 9
Maximum Temperature: 60 °C
Comment: system suitability

Analysis method: D:\HPCHEM\l\METHODS\SYSSUIP.M

Sample information for vial#: 21

  Sample Name:           calanti+     Multiplier:  1.00
  Injection#:            5            Dilution:    1.00
  Injection volume:      3 µl

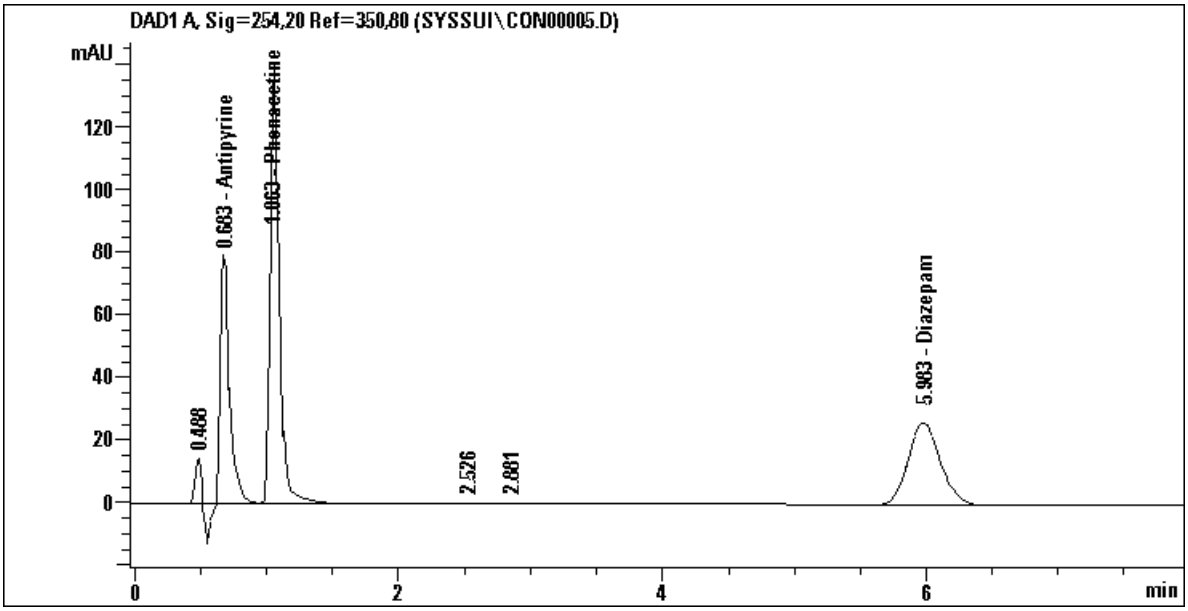
Acquisition information:
  Operator:              agratz
  Date/Time:             2/11/029:06:34 AM
  Data file name:        D:\HPCHEM\1\DATA\SYSSUI\CONOOO05.D
  Method file name:      D:\HPCHEM\l\METHODS\SYSSUIP.M

  Flow:                  0.200 ml/min
  Pressure at start:     85 bar              Pressure at end:    88 bar
  Temperature at start:  25.1°C Temperature  at end:            25.0°C
```

Extended Performance Report (continued)

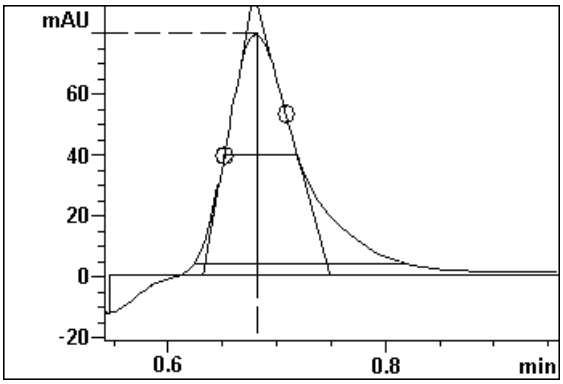
```
Solvents:              PMP1, Solvent A     water
                       PMP1, Solvent B     ACN
                       PMP1, Solvent C
                       PMP1, Solvent D

Signal description: DAD1 A, Sig=254,20 Ref=350,80
```




```
Compound# 2    :  Antipyrine
Amount [ng]:         51.1385

Peak description [min]:
      Signal: DAD1 A, Sig=254,20 Ref=350,80
      RetTime: 0.583      K':    0.706
      Height:  79.78  Area:      371.2
      Start:      0.546   End:   0.956
      Skew:    0.898   Excess:   1.643
      Width at half height:      0.067
            5 sigma:             0.196
            tangent:             0.117
            tailing:             0.190
      Symmetry:                  0.483
      USP Tailing:               1.657
      Integration type:            HV
      Time increment [macc]:     400.0
      Data points:                  66
```



Extended Performance Report (continued)

```
Statistical moments (BB peak detection):    Efficiency:  Plates per ...
      M0:  514.1                                        column       meter
      M1:  0.699               Tangent method             541        18020
      M2:  0.00341             Halfwidth method           581        19360
      M3:  0.000179            5 sigma method             385        10153
      M4: 0.000054             Statistical                143        4782

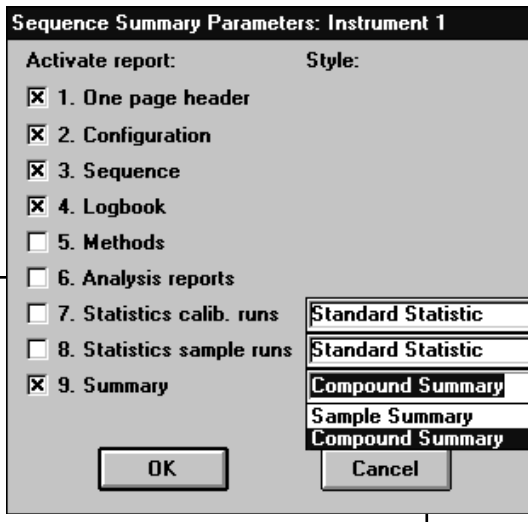
Relationship to preceeding peak:            Selectivity: 3.217
Resolution Tangent method: 2.015            5 sigma method 1.700
Halfwidth method 2.034                      Statistical method 1.067

                                        :
                                        :
                                        :

================================================================
                          *** End of Report ***
```

The peak description and statistical moments are repeated for each compound

Sequence Summary Report – Compound Summary




```
  XXXXXX      XX        XXXXXX
 XX    XX     XX        XX    XX
 XX           XX        XX    XX
 XX           XX        XXXXXX
 XX    XX     XX        XX
 XX    XX     XX        XX
 XX    XX     XX        XX
  XXXXXX      XXXXXX    XX
```

S E Q U E N C E

S U M M A R Y

R E P O R T

A.G Huesgen

.....
Date/Signature

I n s t r u m e n t C o n f i g u r a t i o n

Instrument: Instrument 1

Module	Firmware revision	Serial number
1100 Wellplate Autosampler	A.04.08	DE02700294
1100 Column Thermostat	A.04.06	DE53400174
1100 Diode Array Detector	S.03.91	DE00900051
1100 Binary Pump	A.04.06	DE53500104
1100 Sample Thermostat	n/a	DE82203241

Software Revisions for:
- Acquisition: Rev. A.08.03 [847] Copyright © Agilent Technologies
- Data Analysis: Rev. A.08.04 [1008] Copyright © Agilent Technologies

Sequence Summary Report – Compound Summary (continued)

S e q u e n c e

Sequence Parameters:

Operator:	agratz
Data File Naming:	Prefix/Counter
Signal 1 Prefix:	Lin2
Counter:	0001
Data Directory:	D:\HPCHEM\1\DATA\
Data Subdirectory:	NEWLIN2
Part of Methods to run:	Reprocessing only
	Use SAMPLE.MAC
Wait Time after loading Method:	0 min
Barcode Reader:	not used
Sequence Timeout:	0 min
Shutdown Cmd/Macro:	none
Sequence Comment:	Linearity Test

Sequence Table:

Sample Information Part:

Line	Location	Sample Information
1	Vial 1	1:10 diluted stock solution
2	Vial 1	1:10 diluted stock solution
3	Vial 1	1:10 diluted stock solution
4	Vial 1	1:10 diluted stock solution
5	Vial 1	1:10 diluted stock solution
6	Vial 1	1:10 diluted stock solution
7	Vial 2	1:100 diluted stock solution
8	Vial 2	1:100 diluted stock solution
9	Vial 2	1:100 diluted stock solution
10	Vial 2	1:100 diluted stock solution
11	Vial 2	1:100 diluted stock solution

Sequence Summary Report – Compound Summary (continued)

```
     Method and Injection Info Part:

     Line Location SampleName        Method   Inj SampleType InjVolume DataFile
     ==== ======== ================ ======== === ========== ========= ==========

     1    Vial 1   1:10dil.         LINICHEM 2   Sample        0.1
     2    Vial 1   1:10dil.         LINICHEM 2   Sample        0.5
     3    Vial 1   1:10dil.         LINICHEM 2   Sample        1
     4    Vial 1   1:10dil.         LINICHEM 2   Sample        3
     5    Vial 1   1:10dil.         LINICHEM 2   Sample        5
     6    Vial 1   1:10dil.         LINICHEM 2   Sample        10
     7    Vial 2   1:100dil.        LINICHEM 2   Sample        25
     8    Vial 2   1:100dil.        LINICHEM 2   Sample        50
     9    Vial 2   1:100dil.        LINICHEM 2   Sample        75
     10   Vial 2   1:100dil.        LINICHEM 2   Sample        100
     11   Vial 2   1:100dil.        LINICHEM 2   Sample        0.1


     Calibration Part:

     Line Location SampleName        Method   CalLev Update RF Update RT Interval
     ==== ======== ================ ======== ====== ========= ========= =========



     Quantification Part:

     Line Location SampleName        SampleAmount ISTDAmt   Multiplier Dilution
     ==== ======== ================ ============ =======   ========== =========

     1    Vial 1   1:10dil.
     2    Vial 1   1:10dil.
     3    Vial 1   1:10dil.
     4    Vial 1   1:10dil.
     5    Vial 1   1:10dil.
     6    Vial 1   1:10dil.
     7    Vial 2   1:100dil.
     8    Vial 2   1:100dil.
     9    Vial 2   1:100dil.
     10   Vial 2   1:100dil.
     11   Vial 2   1:100dil.


Sequence Output Parameters:

      Print Sequence Summary Report (SSR):              Yes
           SSR to Printer:                              Yes
           SSR to File:                                 Yes
           SSR File Name:                               GLPrprt.txt
           SSR to HTML:                                 No
           Print individual reports for each run:       No
```

Sequence Summary Report – Compound Summary (continued)

```
Sequence Summary Parameters:

One page header:                        Yes
Print Configuration:                    Yes
Print Sequence:                         Yes
Print Logbook:                          Yes
Print Method(s):                        No
Print Analysis reports:                 No
Print Statistics for Calib. runs:       No
Statistic Sample runs style:            No
Summary style:                          Compound Summary


                              L o g b o o k
24 Jan 02  10:48 AM
Logbook File: D:\HPCHEM\1\DATA\NEWLIN2\LIN2.LOG

Module      # Event Message                                          Time      Date
-------------------------------------------------------------------------------------
Sequence      LIN2.S started                                         10:47:06 01/24/02
Method        Loading Method LINICHEM.M                              10:47:07 01/24/02
Method        Method started:  line# 1 vial# 1 inj# 1                10:47:08 01/24/02
CP Macro      Analyzing rawdata Lin20001.D                           10:47:08 01/24/02
Method        Method completed                                       10:47:10 01/24/02
Method        Method started:  line# 1 vial# 1 inj# 2                10:47:11 01/24/02
CP Macro      Analyzing rawdata Lin20002.D                           10:47:11 01/24/02
Method        Method completed                                       10:47:13 01/24/02
Method        Method started:  line# 2 vial# 1 inj# 1                10:47:14 01/24/02
CP Macro      Analyzing rawdata Lin20003.D                           10:47:14 01/24/02
Method        Method completed                                       10:47:16 01/24/02
Method        Method started:  line# 2 vial# 1 inj# 2                10:47:17 01/24/02
CP Macro      Analyzing rawdata Lin20004.D                           10:47:18 01/24/02
Method        Method completed                                       10:47:19 01/24/02
Method        Method started:  line# 3 vial# 1 inj# 1                10:47:21 01/24/02
CP Macro      Analyzing rawdata Lin20005.D                           10:47:21 01/24/02
Method        Method completed                                       10:47:22 01/24/02
Method        Method started:  line# 3 vial# 1 inj# 2                10:47:24 01/24/02
CP Macro      Analyzing rawdata Lin20006.D                           10:47:24 01/24/02
Method        Method completed                                       10:47:26 01/24/02
Method        Method started:  line# 4 vial# 1 inj# 1                10:47:27 01/24/02
CP Macro      Analyzing rawdata Lin20007.D                           10:47:27 01/24/02
Method        Method completed                                       10:47:29 01/24/02
Method        Method started:  line# 4 vial# 1 inj# 2                10:47:30 01/24/02
CP Macro      Analyzing rawdata Lin20008.D                           10:47:30 01/24/02
Method        Method completed                                       10:47:32 01/24/02
Method        Method started:  line# 5 vial# 1 inj# 1                10:47:33 01/24/02
CP Macro      Analyzing rawdata Lin20009.D                           10:47:34 01/24/02
Method        Method completed                                       10:47:35 01/24/02
Method        Method started:  line# 5 vial# 1 inj# 2                10:47:37 01/24/02
CP Macro      Analyzing rawdata Lin20010.D                           10:47:37 01/24/02
Method        Method completed                                       10:47:39 01/24/02
Method        Method started:  line# 6 vial# 1 inj# 1                10:47:40 01/24/02
```

Sequence Summary Report – Compound Summary (continued)

```
CP Macro      Analyzing rawdata Lin20011.D                          10:47:40 01/24/02
Method        Method completed                                      10:47:42 01/24/02
Method        Method started:  line# 6 vial# 1 inj# 2               10:47:43 01/24/02
CP Macro      Analyzing rawdata Lin20012.D                          10:47:43 01/24/02
Method        Method completed                                      10:47:45 01/24/02
Method        Method started:  line# 7 vial# 2 inj# 1               10:47:46 01/24/02
CP Macro      Analyzing rawdata Lin20013.D                          10:47:47 01/24/02
Method        Method completed                                      10:47:48 01/24/02
Method        Method started:  line# 7 vial# 2 inj# 2               10:47:50 01/24/02
CP Macro      Analyzing rawdata Lin20014.D                          10:47:50 01/24/02

24 Jan 02  10:48 AM
Logbook File: D:\HPCHEM\1\DATA\NEWLIN2\LIN2.LOG

Module      # Event Message                                         Time      Date
-------------------------------------------------------------------------------------
Method        Method completed                                      10:47:51 01/24/02
Method        Method started:  line# 8 vial# 2 inj# 1               10:47:53 01/24/02
CP Macro      Analyzing rawdata Lin20015.D                          10:47:53 01/24/02
Method        Method completed                                      10:47:55 01/24/02
Method        Method started:  line# 8 vial# 2 inj# 2               10:47:56 01/24/02
CP Macro      Analyzing rawdata Lin20016.D                          10:47:56 01/24/02
Method        Method completed                                      10:47:58 01/24/02
Method        Method started:  line# 9 vial# 2 inj# 1               10:47:59 01/24/02
CP Macro      Analyzing rawdata Lin20017.D                          10:47:59 01/24/02
Method        Method completed                                      10:48:01 01/24/02
Method        Method started:  line# 9 vial# 2 inj# 2               10:48:02 01/24/02
CP Macro      Analyzing rawdata Lin20018.D                          10:48:03 01/24/02
Method        Method completed                                      10:48:04 01/24/02
Method        Method started:  line# 10 vial# 2 inj# 1              10:48:06 01/24/02
CP Macro      Analyzing rawdata Lin20019.D                          10:48:06 01/24/02
Method        Method completed                                      10:48:08 01/24/02
Method        Method started:  line# 10 vial# 2 inj# 2              10:48:09 01/24/02
CP Macro      Analyzing rawdata Lin20020.D                          10:48:09 01/24/02
Method        Method completed                                      10:48:11 01/24/02
Method        Method started:  line# 11 vial# 2 inj# 1              10:48:12 01/24/02
CP Macro      Analyzing rawdata Lin20021.D                          10:48:13 01/24/02
Method        Method completed                                      10:48:14 01/24/02
Method        Method started:  line# 11 vial# 2 inj# 2              10:48:16 01/24/02
CP Macro      Analyzing rawdata Lin20022.D                          10:48:16 01/24/02
Method        Method completed                                      10:48:18 01/24/02
Sequence      LIN2.S completed                                      10:48:19 01/24/02
```

Sequence Summary Report – Compound Summary (continued)

C o m p o u n d S u m m a r y

Sequence table: D:\HPCHEM\CORE\LIN2.S
Data directory path: D:\HPCHEM\1\DATA\NEWLIN2
Logbook: D:\HPCHEM\1\DATA\NEWLIN2\LIN2.LOG
Sequence start: 10/25/00 6:58:26 AM
Operator: agratz

Method file name: D:\HPCHEM\1\METHODS\LINICHEM.M

Sample Name	Sample Amt [ng]	Multip.* Dilution	FileName .D	RetTime [min]	Amount [ng]	Compound
sample1	0.00000	1.0000	Lin20001	2.071	-	-
				3.005	41.80740	Phenacetine
				5.061	27.57288	Diazepam
sample2	0.00000	1.0000	Lin20002	2.071	-	-
				2.927	37.71584	Phenacetine
				4.931	24.68503	Diazepam
sample3	0.00000	1.0000	Lin20003	2.159	113.94044	Antipyrine
				2.921	249.65462	Phenacetine
				4.927	162.09926	Diazepam
sample4	0.00000	1.0000	Lin20004	2.138	115.89423	Antipyrine
				2.888	254.19389	Phenacetine
				4.893	167.32050	Diazepam
sample5	0.00000	1.0000	Lin20005	2.071	-	-
				2.967	533.16102	Phenacetine
				4.977	350.64724	Diazepam
sample6	0.00000	1.0000	Lin20006	2.071	-	-
				2.935	555.34634	Phenacetine
				4.885	359.02135	Diazepam
sample7	0.00000	1.0000	Lin20007	2.120	770.88338	Antipyrine
				2.932	1659.61614	Phenacetine
				4.939	1090.77773	Diazepam
sample8	0.00000	1.0000	Lin20008	2.156	766.86882	Antipyrine
				2.978	1658.25754	Phenacetine
				4.990	1088.46781	Diazepam
sample9	0.00000	1.0000	Lin20009	2.112	1298.20959	Antipyrine
				2.956	2780.26621	Phenacetine
				4.874	1801.76061	Diazepam
sample10	0.00000	1.0000	Lin20010	2.125	1265.65752	Antipyrine
				2.931	2753.00356	Phenacetine
				4.917	1784.44912	Diazepam
sample11	0.00000	1.0000	Lin20011	2.070	2206.34622	Antipyrine
				2.928	4737.72659	Phenacetine
				4.931	3055.52966	Diazepam
sample12	0.00000	1.0000	Lin20012	2.157	2219.77978	Antipyrine
				2.959	4771.25573	Phenacetine
				4.905	3043.14819	Diazepam
sample13	0.00000	1.0000	Lin20013	2.172	438.70069	Antipyrine
				2.939	937.28787	Phenacetine
				4.901	599.59734	Diazepam

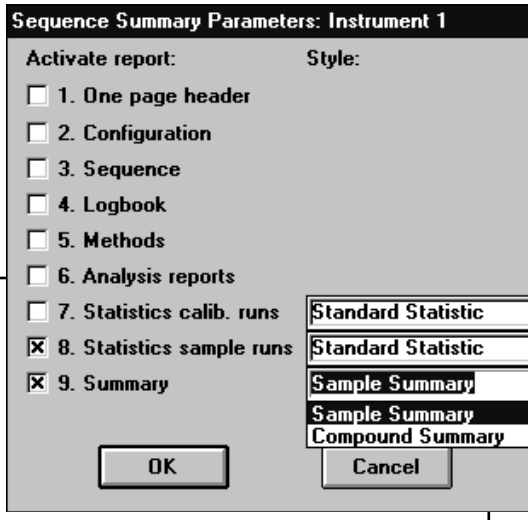
Sequence Summary Report – Compound Summary (continued)

sample14	0.00000	1.0000	Lin20014	2.137	431.19756	Antipyrine
				2.920	922.41613	Phenacetine
				4.914	598.82718	Diazepam
sample15	0.00000	1.0000	Lin20015	2.130	1050.21043	Antipyrine
				2.956	2257.23577	Phenacetine
				4.946	1454.09021	Diazepam
sample16	0.00000	1.0000	Lin20016	2.071	-	-
				3.062	2266.63554	Phenacetine
				4.914	1450.54300	Diazepam
sample17	0.00000	1.0000	Lin20017	2.112	1860.82017	Antipyrine
				2.958	4083.57167	Phenacetine
				4.943	2601.71134	Diazepam
sample18	0.00000	1.0000	Lin20018	2.114	1846.79895	Antipyrine
				2.970	4045.19575	Phenacetine
				4.970	2576.86650	Diazepam
sample19	0.00000	1.0000	Lin20019	2.152	2485.47770	Antipyrine
				3.019	5268.86688	Phenacetine
				4.973	3410.01754	Diazepam
sample20	0.00000	1.0000	Lin20020	2.135	2489.66113	Antipyrine
				2.975	5298.02094	Phenacetine
				4.943	3415.39103	Diazepam
sample21	0.00000	1.0000	Lin20021	2.155	2961.16799	Antipyrine
				3.010	6013.24563	Phenacetine
				5.003	4037.60722	Diazepam
sample22	0.00000	1.0000	Lin20022	2.156	2983.41614	Antipyrine
				3.042	6012.35737	Phenacetine
				4.988	4010.73532	Diazepam

==========

*** End of Report ***

Sequence Summary Report – Standard Statistics for Sample Runs

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                    S t a t i s t i c    R e p o r t

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Operator:                agratz

Method file name:        D:\HPCHEM\1\METHODS\LINI2.M
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2	Vial 2	2	8/24/00 12:51:09 AM	new00062.D	sample2
3	Vial 2	3	8/24/00 1:00:14 AM	new00063.D	sample3
4	Vial 2	4	8/24/00 1:09:18 AM	new00064.D	sample4
5	Vial 2	5	8/24/00 1:18:21 AM	new00065.D	sample5
6	Vial 2	6	8/24/00 1:27:25 AM	new00066.D	sample6
7	Vial 2	7	8/24/00 1:36:30 AM	new00067.D	sample7
8	Vial 2	8	8/24/00 1:45:34 AM	new00068.D	sample8
9	Vial 2	9	8/24/00 1:54:38 AM	new00069.D	sample9
10	Vial 2	10	8/24/00 2:03:42 AM	new00070.D	sample10

Compound: Antipyrine (Signal: DAD1 A, Sig=254,20 Ref=350,80)

Run #	Type	RetTime [min]	Amount [ng]	Area [mAU*s]	Height [mAU]	Width [min]	Symm.
1	BV	2.071	26.23064	834.52417	215.75279	0.0594	0.74
2	BV	2.071	26.28149	836.14185	216.26503	0.0594	0.74
3	BV	2.070	26.22879	834.46539	215.85945	0.0594	0.74
4	BV	2.070	26.27553	835.95233	216.52124	0.0594	0.74
5	BV	2.070	26.21720	834.09644	215.51944	0.0594	0.74
6	BV	2.070	26.19317	833.33203	216.02470	0.0593	0.74
7	BV	2.070	26.27779	836.02423	216.93185	0.0592	0.74
8	BV	2.072	26.29524	836.57941	216.89178	0.0593	0.74
9	BV	2.072	26.22549	834.36017	216.09763	0.0593	0.74
10	BV	2.071	26.21184	833.92590	216.06882	0.0593	0.74
Mean:		2.071	26.24372	834.94019	216.19327	0.0594	0.74
S.D.:		6.81e-4	3.53636e-2	1.12509	4.66512e-1	6.63e-5	1e-3
RSD :		0.033	1.34751e-1	1.34751e-1	2.15784e-1	0.1117	0.20
95% CI:		4.87e-4	2.52976e-2	8.04838e-1	3.33722e-1	4.74e-5	1e-3

Sequence Summary Report – Standard Statistics for Sample Runs

Compound: Phenacetine (Signal: DAD1 A, Sig=254,20 Ref=350,80)

Run #	Type	RetTime [min]	Amount [ng]	Area [mAU*s]	Height [mAU]	Width [min]	Symm.
1	BB	3.035	12.05932	1203.01074	357.49438	0.0528	0.88
2	BB	3.035	12.07862	1204.93591	357.76285	0.0527	0.87
3	BB	3.035	12.05487	1202.56653	357.16501	0.0527	0.88
4	BB	3.035	12.07567	1204.64221	357.80615	0.0527	0.88
5	BB	3.036	12.05951	1203.02979	356.62448	0.0528	0.87
6	BB	3.036	12.02965	1200.05090	356.52957	0.0528	0.88
7	BB	3.037	12.08083	1205.15625	357.92139	0.0527	0.88
8	BB	3.037	12.06433	1203.51099	357.60211	0.0527	0.88
9	BB	3.039	12.05340	1202.42065	356.89868	0.0527	0.87
10	BB	3.038	12.04430	1201.51282	356.41678	0.0528	0.88
Mean:		3.036	12.06005	1203.08368	357.22214	0.0527	0.88
S.D.:		1.35e-3	1.59266e-2	1.58880	5.70986e-1	3.70e-5	6e-3
RSD :		0.045	1.32061e-1	1.32061e-1	1.59840e-1	0.0702	0.68
95% CI:		9.69e-4	1.13932e-2	1.13656	4.08458e-1	2.65e-5	4e-3

Compound: Diazepam (Signal: DAD1 A, Sig=254,20 Ref=350,80)

Run #	Type	RetTime [min]	Amount [ng]	Area [mAU*s]	Height [mAU]	Width [min]	Symm.
1	BB	5.085	17.51478	820.56067	228.97469	0.0556	0.84
2	BB	5.086	17.54309	821.88702	229.58243	0.0557	0.84
3	BB	5.085	17.51162	820.41229	229.04759	0.0557	0.84
4	BB	5.084	17.54478	821.96600	229.60602	0.0557	0.84
5	BB	5.086	17.51105	820.38562	229.37668	0.0556	0.84
6	BB	5.087	17.47411	818.65503	228.69946	0.0556	0.85
7	BB	5.088	17.54951	822.18774	229.63567	0.0556	0.84
8	BB	5.088	17.51423	820.53491	229.10289	0.0556	0.84
9	BB	5.090	17.51381	820.51508	229.17131	0.0557	0.84
10	BB	5.090	17.50570	820.13525	228.79688	0.0556	0.84
Mean:		5.087	17.51827	820.72396	229.19936	0.0556	0.84
S.D.:		2.12e-3	2.24801e-2	1.05318	3.38200e-1	3.77e-5	2e-3
RSD :		0.042	1.28324e-1	1.28324e-1	1.47557e-1	0.0678	0.29
95% CI:		1.52e-3	1.60813e-2	7.53401e-1	2.41934e-1	2.70e-5	2e-3

Sequence Summary Report – Standard Statistics for Sample Runs

```
                  S a m p l e    S u m m a r y


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Statistic report on calibration runs:  1
Operator:                                agratz

Method file name:                        D:\HPCHEM\1\METHODS\LINI2.M

Run Location Inj Sample Name Sample Amt Multip.* File name Cal # Page
 #             #                    [ng]    Dilution               Cmp  #
---|--------|---|----------------|----------|--------|----------|-|---|---
  1 Vial 2    1  sample1               -    1.0000  new00061.D *   3  -
  2 Vial 2    2  sample2               -    1.0000  new00062.D *   3  -
  3 Vial 2    3  sample3               -    1.0000  new00063.D *   3  -
  4 Vial 2    4  sample4               -    1.0000  new00064.D *   3  -
  5 Vial 2    5  sample5               -    1.0000  new00065.D *   3  -
  6 Vial 2    6  sample6               -    1.0000  new00066.D *   3  -
  7 Vial 2    7  sample7               -    1.0000  new00067.D *   3  -
  8 Vial 2    8  sample8               -    1.0000  new00068.D *   3  -
  9 Vial 2    9  sample9               -    1.0000  new00069.D *   3  -
 10 Vial 2   10  sample10              -    1.0000  new00070.D *   3  -


================================================================================
                           *** End of Report ***
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www.agilent.com/chem/nds

Published March 1, 2003
Publication Number 5988-9011EN

Agilent Technologies

Applying the 5975 inert MSD to the Higher Molecular Weight Polybrominated Diphenyl Ethers (PBDEs)

Application

Environmental, Component Testing

Author

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USA

Abstract

A previous application note presented results for analysis of the polybrominated diphenyl ethers (PBDEs) in polymers using the 5973N inert MSD [1]. Mass spectra were presented and interpreted for all of the important PBDEs. The new 5975 inert MSD provides many new features and improvements with expanded mass range to 1050 u being but one. This note presents the full spectra of the octa-, nona and decabrominated biphenyls ethers including ions that appear beyond the mass range of the previous 5973 MSD platform.

Introduction

PBDEs have become the "new PCBs" due to their widespread detection throughout the ecosystem. They have some structural and consequently mass spectral features in common with the polychlorinated biphenyls (PCBs) as well. The series of fragments formed by loss of chlorines (M-nCl_2) generates a number of intense ions useful in their determination. The PCBs also show relatively intense molecular ion clusters that assist in distinguishing the congeners. Similar attributes are expected and hoped for the PBDEs which show much more analytical difficulty than the PCBs.

This note presents the full scan spectra obtained for the PBDEs over the extended mass range of the 5975 inert MSD. The polymeric sample preparation and extraction protocols are cited elsewhere and supply two approaches to PBDE determinations [1].

Experimental

PBDE standards were acquired from Cambridge Isotope Laboratories (Andover, MA) and AccuStandard (New Haven, CT).

Instrumental Configuration and Conditions

The 6890 GC configuration and conditions are given in the previous application note [1]. The 5975 inert MSD system was operated in scan mode for acquisition of the PBDE spectra. The MSD scan operating parameters are cited in Table 1.

Table 1. 5975 inert MSD Configuration and Parameters

Mass spectrometer parameters	
Ionization mode	Electron impact
Ionization energy	70 eV
Tune parameters	Autotune
Electron multiplier voltage	Autotune + 400V
Scan mode	200–1000 u
Quadrupole temperature	150 °C
Inert source temperature	300 °C

Full conditions and parameters, as appropriate to the polymer analysis cited in reference 1, are available in the eMethod for this analysis (www.agilent.com/chem/emethods).

Results

EI Spectra of the Higher Molecular Weight PBDEs

Figures 1, 2, and 3 present the full-scan spectra of an octa-, nona- and the decabromodiphenyl ether. Note that most intense ions in all cases are the $[M–Br_2]^+$ and the corresponding to $[M–Br_2]^{+2}$ ions. The relative abundance of the molecular ion clusters $[M]^+$ are under 30%. Figure 4 compares the theoretical isotopic pattern to that experimentally obtained by the 5975 inert MSD. Agreement is good in both the abundance of the isotopes and the mass accuracy using the standard system Autotune. Mass accuracy agrees to within 0.2 *m/z* of the theoretical and experimental values. Table 2 presents the important ions for the PBDEs greater than the dibromoDE. These ions are those most important to characterizing the technical mixtures used as additives to polymers.

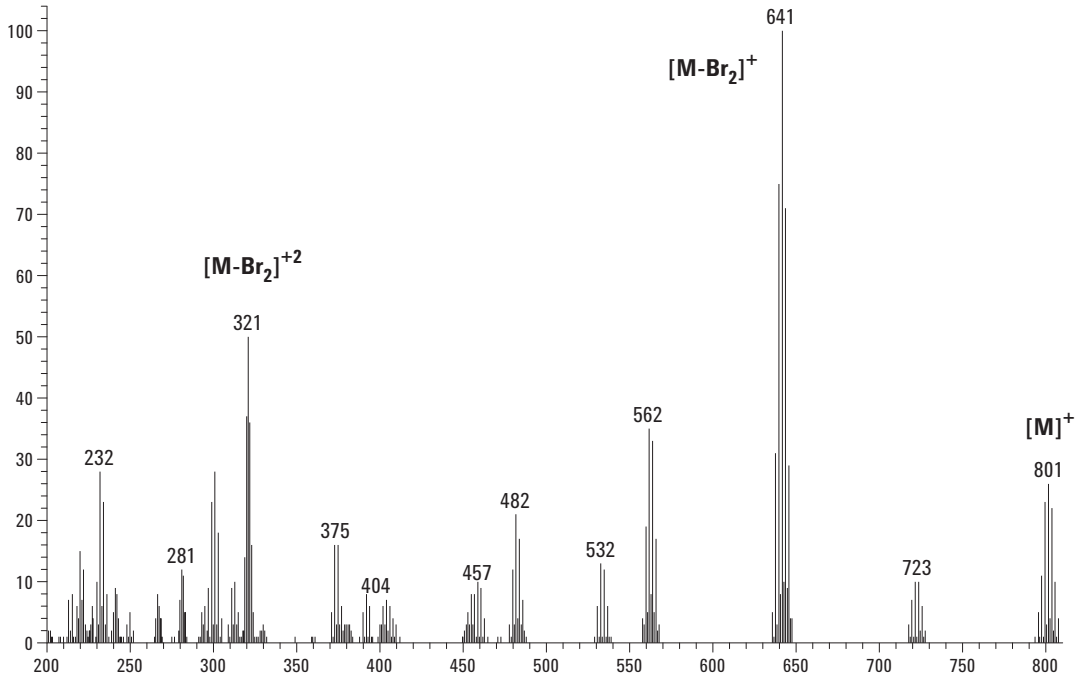

Figure 1. Electron impact ionization spectrum of an octabromodiphenyl ether (PBDE-203) from 200 to 810 *m/z*.

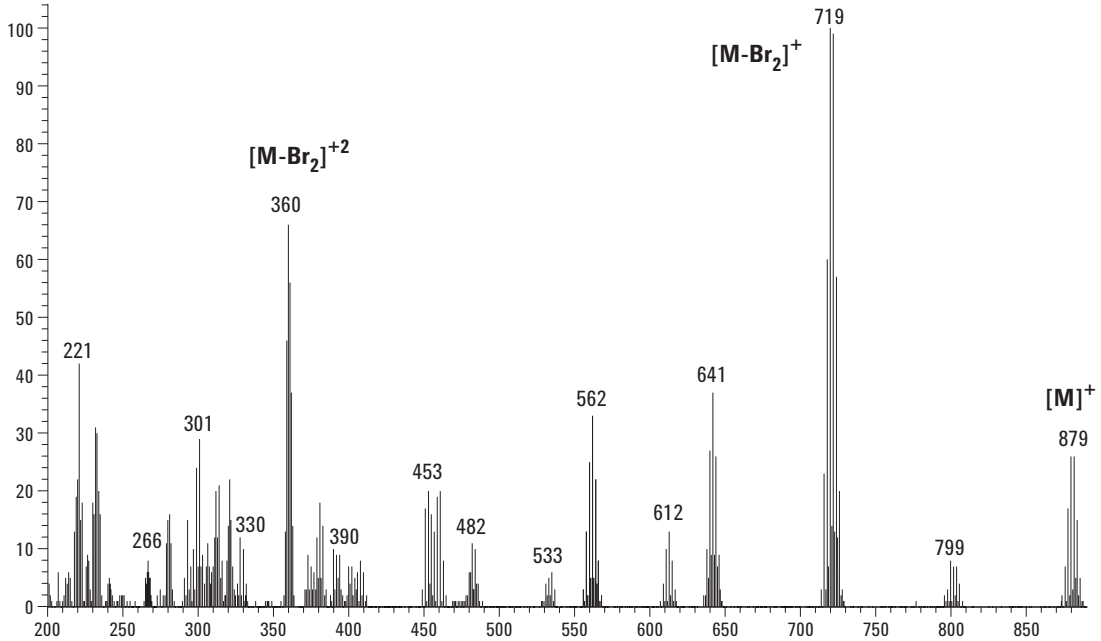

Figure 2. Electron impact ionization spectrum of a nonabromodiphenyl ether (PBDE-208) from 200 to 890 *m/z*.

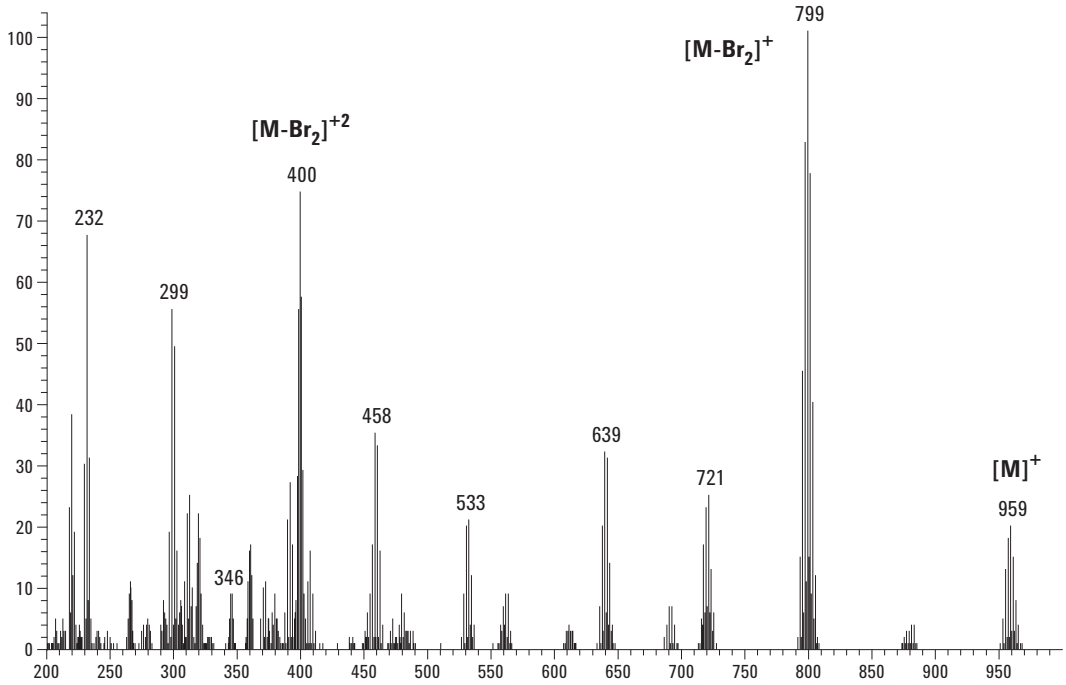

Figure 3. Electron impact ionization spectrum of the decabromodiphenyl ether (PBDE-209) from 200 to 1000 *m/z*.

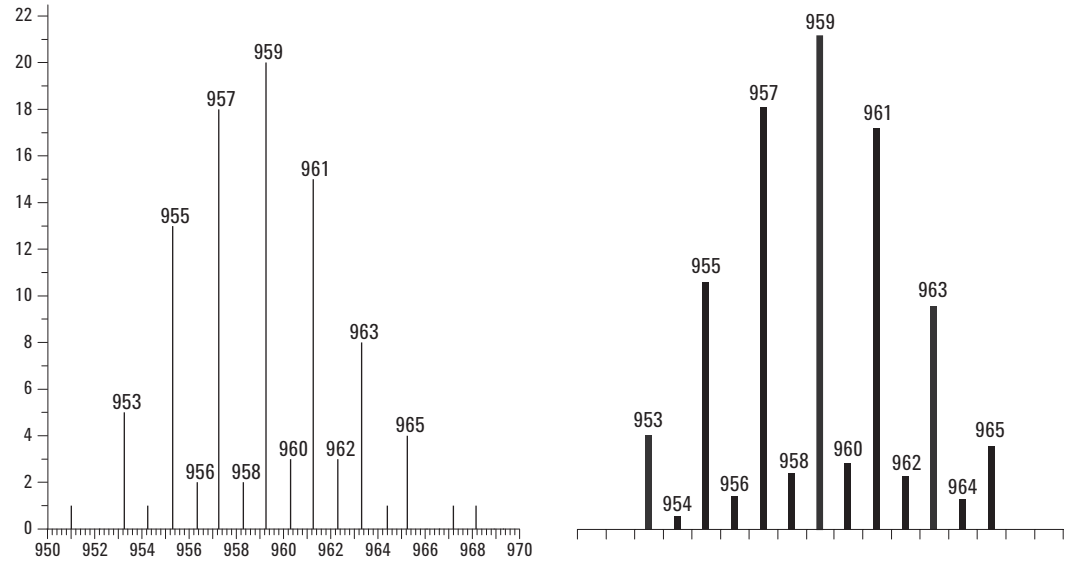

Figure 4. Experimental spectrum of the decabromodiphenyl ether (PBDE-209) molecular ion cluster $[M]^+$ versus theory.

Table 2. Important Ions for the PB_nDEs (n>2)

PBDE bromination	$[M]^+$	$[M\text{-}Br_2]^+$	$[M\text{-}Br_2]^{+2}$
3	405.8	246.0	123.0
4	485.7	325.9	162.9
5	563.6	403.8	201.9
6	643.5	483.7	241.9
7	721.5	561.6	(280.8 **)
8	801.4	641.5	320.8
9	879.3	719.4	359.7
10	959.2	799.3	399.7

**The 280.8 and 281.8 *m/z* ions can be compromised by column bleed interferences so these have not been used in acquisition although they provide a useful diagnostic for column degradation.

The user should note the ion source and quadrupole temperature settings in Table 1. Figure 5 presents SIM acquisitions of several higher molecular weight PBDEs at source temperatures of 300 °C and 230 °C. Notice the signal height roughly doubles on average for the PBDEs at the higher ion source temperature. The insert in the figure shows the improvement in the peak shape for the hexabrominated diphenyl ether. This peak sharpening accounts for the increase in signal height. Since these compounds elute at higher temperatures

among other high boiling components that belong to the matrix, heating the quadrupole is important for robust and low maintenance operation in samples.

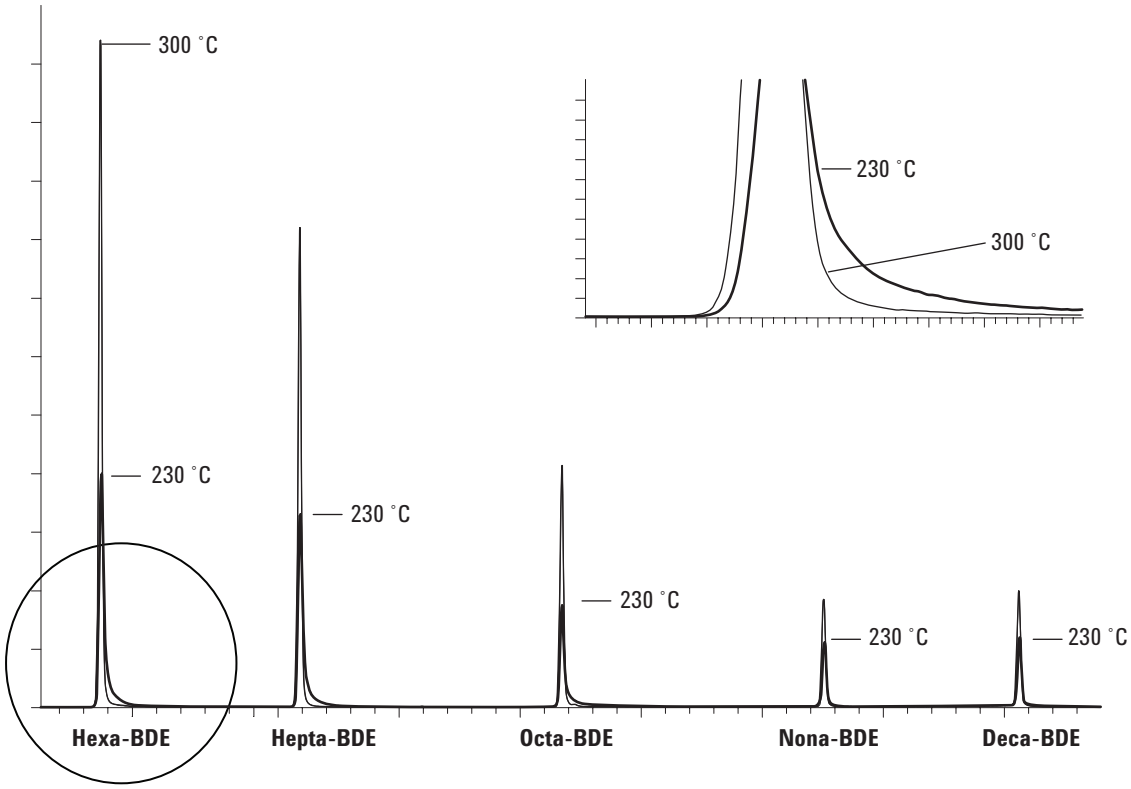

Figure 5. Overlaid RIC SIM acquisitions of five PBDEs at ion source temperatures of 230 °C and 300 °C. Insert is expanded view of hexa-BDE overlays near baseline.

Conclusions

The new 5975 inert MSD has an expanded set of features including mass range. High mass accuracy under standard autotuning is obtained even at the high masses typical of the brominated diphenyl ethers. As users survey higher mass compounds, the heated quadrupole and high temperature capabilities of the 5975 inert MSD will become even more important to rugged and robust analyses in complicated samples.

More details on the other relevant instrumental parameters are available in the eMethod (www.agilent.com/chem/emethods).

Reference

1. C. Tu, and H. Prest, Determination of polybrominated diphenyl ethers in polymeric materials using the 6890 GC/5973N Inert MSD with electron impact ionization. Agilent Technologies, publication 5989-2850EN, www.agilent.com/chem

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Printed in the USA
June 13, 2005
5989-3142EN

Achieving fastest analyses with the Agilent 1200 Series Rapid Resolution LC system and 2.1-mm id columns

Application Note

Michael Frank

Abstract

The need to increase the daily throughputs of LC systems is a constant desire. Now, with the Agilent 1200 Series Rapid Resolution LC system highest throughputs are possible, and in combination with the Agilent ZORBAX RRHT columns and the increased pressure and temperature range of the LC system, excellent chromatographic resolution can be achieved even at run times below one minute.

This Application Note describes the correct set-up of the instrument which is the key for optimal results with narrow bore columns, such as a 2.1 mm x 50 mm column packed with sub two micron particles. Peak capacities in the range of fifty in analysis times as short as 24 seconds and peak widths as narrow as 200 milliseconds are shown. The well-balanced use of all possible module options to achieve shortest cycle times with throughputs far beyond 1500 samples per day is described.

Agilent Technologies

Introduction

Particularly analytical service laboratories in the pharmaceutical industry, responsible for analyzing chemical libraries[1] or performing MS based quantifications of certain ADME-properties and drug metabolism studies of drug candidates[2] are faced with the challenge to increase their throughput, but also to maintain a high chromatographic resolution. In 2003 Agilent Technologies introduced sub two micron particles in their RRHT column series. Because of the small particle size, the chromatographic resolution obtainable with these columns is superior to standard particle sizes such as 3.5 µm or even 5 µm. Due to a unique silica manufacturing process, Agilent ZORBAX RRHT columns show a significantly reduced backpressure, if compared to similar column dimensions of other manufacturers. Excellent chromatographic results are achieved in a very short analysis time with the Agilent 1200 Series Rapid Resolution LC system, which facilitates an increased pressure range and flow rates from 0.05 up to 5 mL/min using column diameters ranging from 2.1-mm id up to 4.6-mm id. This Application Note will focus on 2.1-mm id columns only.
Not only are the run times of the analyses important for high throughput, but also the overhead time. The Agilent 1200 Series Rapid Resolution LC system can be optimized to achieve highest throughputs with exceptionally good overall system performance.

Experimental

An important issue when dealing with narrow bore columns, especially in gradient mode where smallest peak widths can be achieved, is to have small extra column volumes. This also includes any volumes in front of the sampling device, because any volume after the solvent mixing point will increase the time for the gradient composition to reach the column. This results in an increased run time. The Agilent 1200 Series Rapid Resolution LC system can be reconfigured within a few minutes to provide appropriate system volumes for different column ids. Here, the pumps are set-up in the low delay volume configuration with an internal volume of approximately 120 µL. All other modules are optimized for lowest delay volumes by using the low delay volume capillary kit (G1316-68744). Consequently, only capillaries of 0.12 mm id are used beyond the injection valve. In the Agilent 1200 Series thermostatted column compartment SL the newly introduced low dispersion heat exchangers with 1.6 µL internal volume were used. In some experiments, the Agilent 1200 Series Rapid Resolution LC is set up for alternating column regeneration to achieve highest throughput using the ACR-capillary kit (G1316-68721) and 2.1-mm id columns[3]. The high pressure rated 2-position/10-port valve in the thermostatted column compartment was only placed into the flow path if alternating column regeneration was used indeed.

The instrument set-up is as follows (figure 1):

- Agilent 1200 Series binary pump SL with the new Agilent 1200 Series micro vacuum degasser
- Agilent 1200 Series high performance autosampler SL
- Agilent 1200 Series thermostatted column compartment SL, equipped with a high pressure, 2-position/10-port valve, facilitating alternating column regeneration
- Agilent 1200 Series diode-array detector SL with a 2-µL/3-mm cell
- ZORBAX SB C18, 2.1 mm id x 50 mm, 1.8 µm

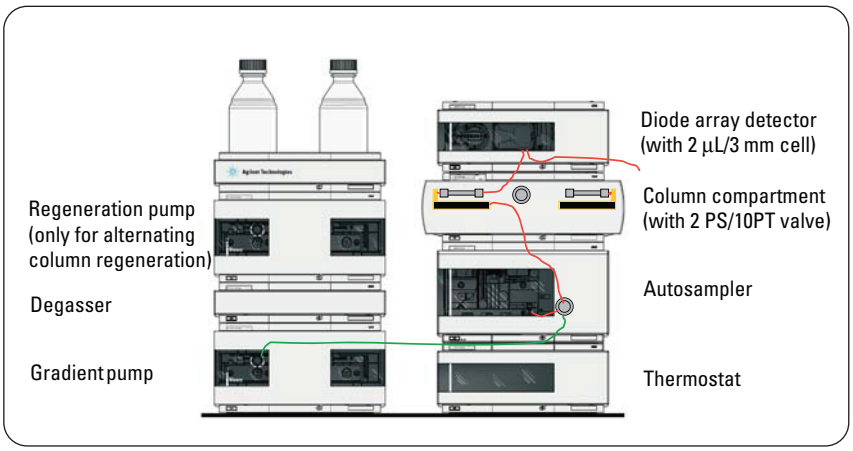

Figure 1
System setup with low delay volume for high speed applications using 2.1-mm id columns with lengths from 20 to 50 mm.

The Agilent 1200 Series binary pump SL is designed to fulfill the demands for high throughput, highest performance, optimum resolution and lowest pump ripple. The pump hardware is significantly different from the standard binary pump. In the Agilent 1200 Series binary pump SL the pressure transducer is separate from the damper which has been modified to have a lower delay volume (pressure dependent ranging from 80-280 µL). In this study the pumps were used in the low delay volume configuration without the mixer and damper in the flow path. In contrast to the standard binary pump the pump heads of the binary pump SL have an additional damping coil (500 µL volume each) to allow damping in the low delay volume configuration. This does not add to the gradient delay volume because it is before the mixing point. Anyhow, pressure ripples are also strongly suppressed by the Electronic Damping Control (EDC). The pressure range of the pump and all other modules is increased to 600 bar.

Only one sample, the so-called "phenone-mix", was used in the course of this study to keep variations low. The sample consists of nine compounds: acetanilid, acetophenone, propiophenone, butyrophenone, benzophenone, valerophenone, hexanophenone, heptanophenone and octanophenone. Unless otherwise stated, the concentration was 0.1 µg/µL for each compound except butyrophenone which was 0.2 µg/µL. The solvent was water-acetonitril 2:1.

Results and discussion

The most frequently sold particle size in chromatographic columns today is 5 µm. Of course, fast and ultra fast LC is also possible with columns packed with particles of these larger diameters – the reduced back pressure is even beneficial to allow higher flow rates. However, resolution will be sacrificed because conditions are usually far on the right side of the van-Deemter-optimum. Here, the big advantage of the RRHT columns with particles of less than 2 µm diameter is proven. The van Deemter optimum is shifted further to the right and the curve is much flatter at the onset because the "resistance of mass transfer" term is diminished (figure 2). In figure 3 the analysis on a 2.1-mm id column with 1.8-µm particles is compared to the linear scaled analysis on the same stationary phase but on 5 µm particles packed in a 4.6-mm id-column. The gain in resolution is obvious – from Rs = 2.1 up to Rs = 3.5 for the critical pair which matches the theoretically expected value of a 1.66 fold increase in resolution. Also note that there is a saving in solvent consumption of 8.6 mL in the "standard" HPLC analysis and only 1.8 mL in the ultra fast HPLC analysis.

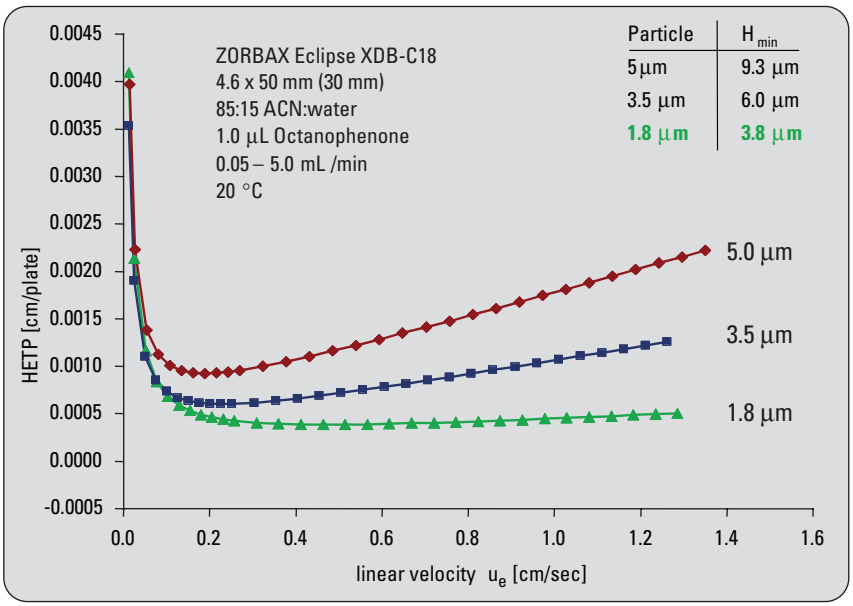

Figure 2
Van Deemter curves of columns packed with 1.8 µm, 3.5 µm and 5.0 µm particles.

For gradient separation the dependencies of the capacity factor can be expressed as:

$$k^* = 0.87 \cdot tg \cdot \frac{F}{Vm \cdot \Delta\%B \cdot S}$$

(tg = gradient time, F = flow rate, Vm = column void volume, △%B = gradient steepness, S = solvent and solute dependent factor)

If the product of the gradient time and flow rate, the so-called gradient volume, is kept constant together with all other parameters, the gradient time might be decreased while the flow rate is increased. Thus, the capacity factors of two compounds will stay constant and if no large alteration of the plate height occurs, the resolution will not change significantly, either. The final point is the big advantage of the sub two micron particles – the van-Deemter curve is nearly flat on the right side of the minimum (figure 2) and flow rates can be increased with only little increase in plate heights. However, the equation is an empirical one and deviations may occur especially under extreme conditions.

With a two-step approach, highest gradient speeds with virtually no loss or only little loss in resolution can be achieved. In the first step, start from a medium temperature and begin to increase the flow rate up to the pressure maximum. Subsequently the temperature should be increased to lower the viscosity of the solvent and then the flow rate is increased again. It may be worthwhile to check the resolution with two identical gradients but with different temperatures to see the influence of the temperature change on the resolution which may be very compound dependent. In figure 4 the result of this approach is shown. A nearly 7-fold increase in separation speed could be achieved with still baseline separation of the critical pair before meeting the pressure and temperature limit (the maximum temperature is a function of flow, temperature, number of controlled Peltier elements and of the heat capacity of the solvent used).

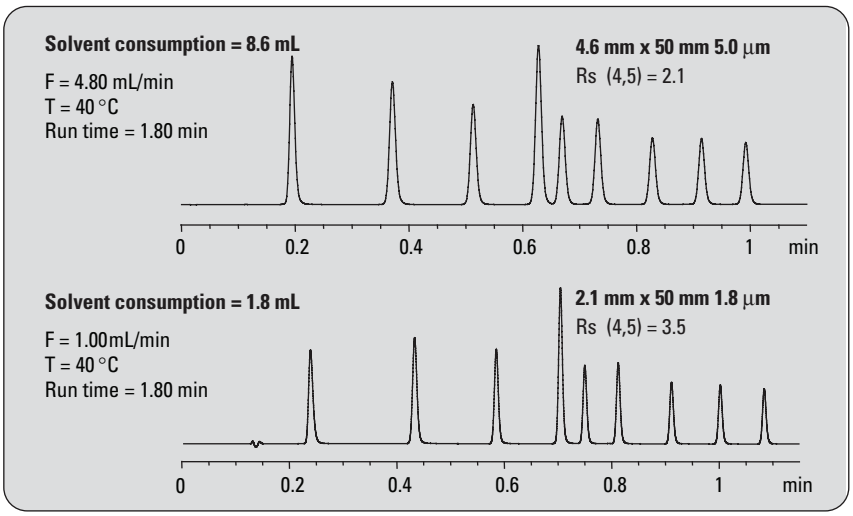

Figure 3
Analysis with 1.8-µm particle column vs. 5.0 µm particle column.

Conditions:	4.6-mm id column used on standard Agilent 1200 system	
Solvent:	A = Water, B = ACN	
Temperature:	40 °C	
Column:	**2.1 mm x 50 mm, 1.8 µm**	**4.6 mm x 50 mm, 5.0 µm**
Flow:	1.0 mL/min	4.8 mL/min (scaled from 2.1 mm col.)
Gradient:	0.00 min 35 %B	0.00 min 35 %B
	0.90 min 95 %B	0.90 min 95 %B
	1.10 min 95 %B	1.10 min 95 %B
	1.11 min 35 % B	1.11 min 35 % B
Stoptime:	1.15 min	1.15 min
Posttime:	0.70 min	0.70 min
Wavelength:	245 nm (8), ref. 450 nm (100)	245 nm (8), ref. 450 nm (80)
Peakwidth:	>0.0025 min (0.05 s res.time), 80 Hz	>0.01 min (>0.2 s), 20 Hz
Injection volume:	1 µL	5 µL (not scaled)

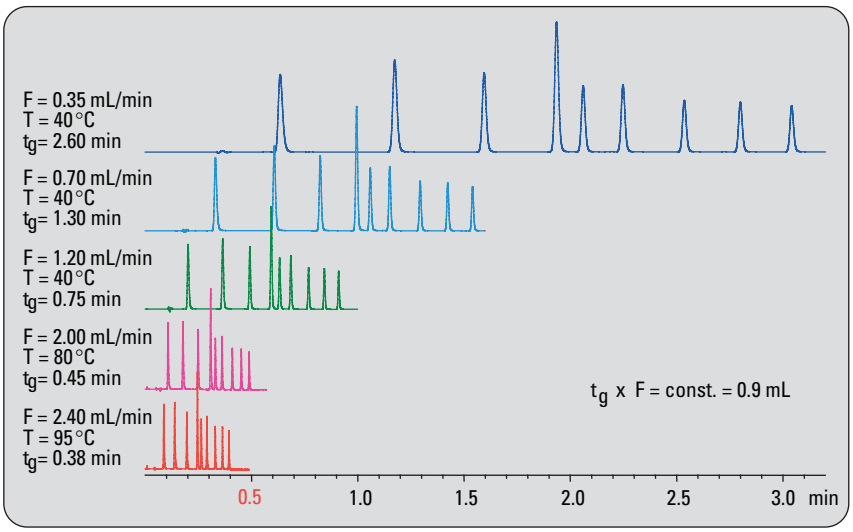

Figure 4
Increasing separation speed by increasing temperature and flow rate while decreasing gradient time.

Conditions:	
Solvent:	A = water, B = ACN
Temp.:	40 °C, 80 °C, 95 °C
Flow:	0.35, 0.70, 1.20, 2.00, 2.40 mL/min
Gradient:	0.00 min 35 %B
	2.60 min 95 %B
	3.20 min 95 %B
	3.21 min 35 %B
	Time values for F = 0.35 mL/min. For all other flow rates times are scaled so that (tg x F) = 0.90 mL
Stop time:	3.20 min
Post time:	2.00 min
Wavelength:	245 nm (8), Ref. 450 nm (100)
Peak width:	>0.0025 min (0.05 s response time), 80 Hz

The last chromatogram is enlarged in figure 5 and reveals the details of this separation. The first peak is eluted after only five seconds and peaks with a width at half height of less than 200 ms are achievable. Within twenty-four seconds nine compounds are separated with a peak capacity in the range of fifty.

Retention time precision at highest analysis speed

High analysis speed is meaningless without precision. One basic performance criteria for HPLC pumps is the precision of gradient formation measured by the precision of retention times of repeated gradients. However, the stability of the column temperature must also be taken into consideration, because temperature fluctuations will also influence the retention times of a given sample. In table 1 and figure 6 the results from the 10-fold repeated analysis of a standard sample are listed and since the deviation between individual runs is so small, the octanophenone peak is enlarged in a separate window. This sample contains compounds that are both not retained and refer to isocratically eluted compounds found at the starting conditions of the gradient, as well as highly unpolar and strongly retained compounds. The analyses

Conditions:

Solvent:	A = Water, B = ACN
Temp.:	40 °C, 80 °C
Flow:	0.35 mL/min, 1.20 mL/min, 2.0 mL/min
Gradient:	0.00 min 35%B
	2.60 min 95%B
	3.20 min 95%B
	3.21 min 35%B
	Time values for F = 0.35 mL/min.
	For all other flow rates times are
	scaled so that (time x flow) = 0.90 mL
Stop time:	3.20 min
Post time:	2.00 min
Injection vol.:	1.0 µL

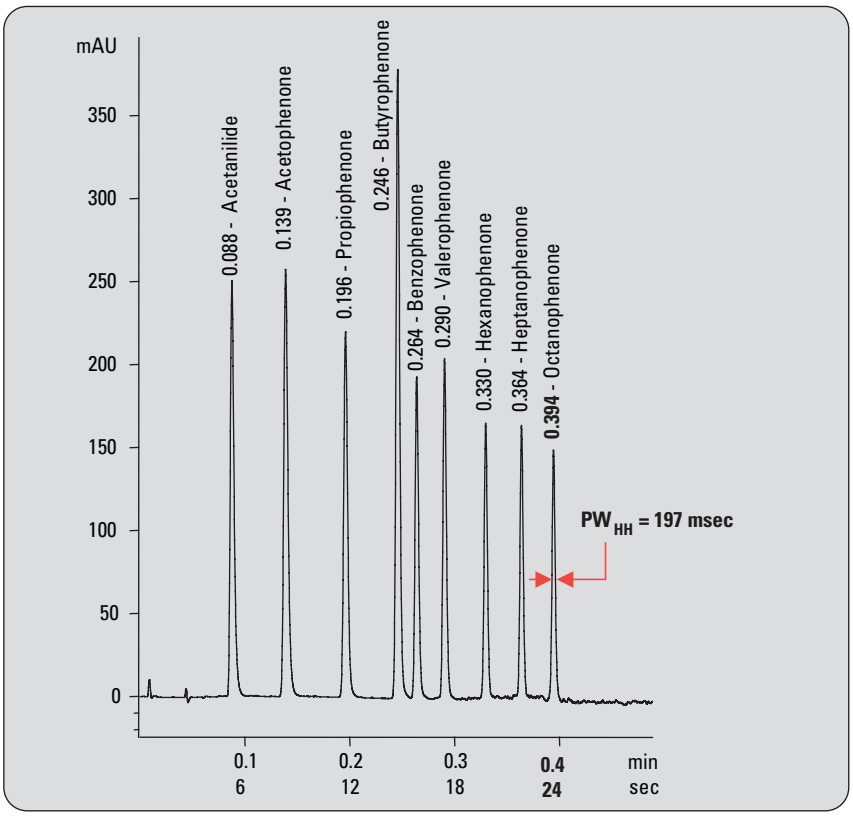

Figure 5
Separation of a nine compound mixture under ultra fast conditions.

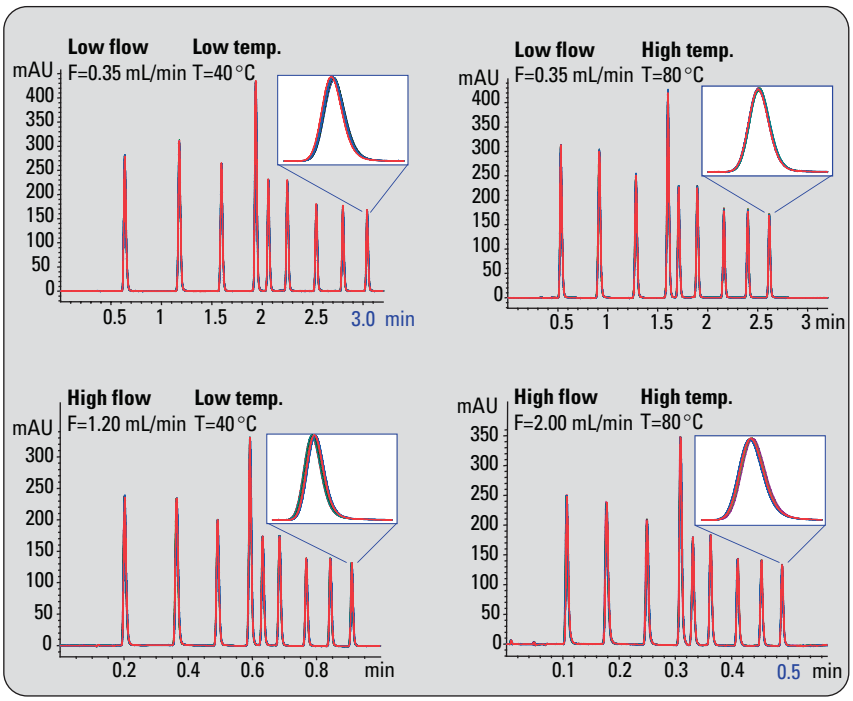

Figure 6
Overlaid chromatograms of the repeated analysis of a 9 compound mixture under various conditions.

were done at high and low flow rates as well as with high and low temperatures as in the examples shown earlier. In all cases the mean retention time precision is below 0.3 % RSD, which was the specification of the Agilent 1100 Series LC system. Of course, the results are also in line with the specifications for the new Agilent 1200 Series Rapid Resolution LC system which is < 0.07 % RSD or < 0.02 min SD, whichever is met first. At these high gradient speeds, the SD criteria are always met. The RSD criteria are also met for both fast-LC gradients of 2.6 min duration (0.35 mL/min flow rate). Even at ultra-fast gradient speeds, the retention time precisions are still below or only slightly higher than 0.1% RSD (table 1).

Improving the cycle-time

Not only is the gradient speed important when dealing with high-throughput analysis but furthermore the over all cycle time of the entire system, which is the time between two consecutive analyses. A good method to measure the cycle time is by using the time stamp the data file is assigned by the operating system of the computer. Clearly, optimizing the cycle time has some drawbacks. For example, extensive needle cleaning procedures are in contradiction with a high sampling speed. Table 2 gives an overview of important parameters influencing the cycle time. Using 1.8-µm particle size columns together with an optimized HPLC system very short run times can be achieved without sacrificing chromatographic resolution. Combining short run times together with low overhead times will result in a high daily throughput. In figure 7 the cycle time and daily throughput is shown for two

	0.35 mL/min, 40°C		0.35 mL/min, 80°C		1.20 mL/min, 40°C		2.00 mL/min, 80°C	
	SD	% RSD	SD	% RSD	SD	% RSD	SD	% RSD
Average	0.00107	0.067	0.00084	0.070	0.00048	0.098	0.00031	0.134

Table 1
Standard deviations (mAU) and %RSD (n=10) of the retention times under different chromatographic conditions in temperature and flow.

Module	Parameter	Effect on cycle time	Other effects
Pump	Low delay volume setting	Reduced retention times, run time can be shortened, reduced cycle time	Increased pressure ripple, slightly increased mixing noise if modifiers such as TFA are used.
Autosampler	Automatic Delay Volume Reduction (ADVR) – activated	Reduced delay volume, reduced retention times, run time can be shortened, reduced cycle time	Increased carry-over
	ADVR activated and Overlapped Injection (OI)	Enables parallel sampling, thus reduces the cycle time independently of the below listed settings (as long as the overall sampling speed does not exceed the gradient and post time)	Increased carry-over
	no OI – Needle Wash	Increased sampling time with increasing wash time	Reduced carry-over with longer needle wash time
	no OI – Equilibration time	Increased sampling time with increased equilibration time	Better injection precision with longer equilibration time
	no OI – Draw/Eject speed	Low speed causes increased sampling time	Low speed results in better injection precision
Column compartment	Alternating column regeneration	Saves column wash-out and equilibration time, reduces cycle time enormously	Additional hardware required, slightly increased extra column volume, slightly different retention times between columns possible
Detector	Pre-run and/or post-run balance	Increased cycle time	Baseline drifts possible if not applied
	Spectral data acquisition with high data rate, small band width and broad wavelength range large data files	Depending on computer power and additional processes running might increase cycle time because of writing speed	Reduced information content if no spectral data acquired or with lower resolution
Software	Data analysis with acquisition	Increased cycle time, depending on computer power and number of peaks	Data analysis has to be done offline is no set
	Save method with data	Slightly increased cycle time	Information is missing if method is not saved
	Execution of pre-run or post-run macros	Increased cycle time, depending on macro	Depending on macro
System	LC controlled over local network between computer and LC (and MS) only	Faster data and method transfer between computer and LC because of reduced net work traffic reduced cycle time	Additional hardware might be necessary (use independent acquisition computer)
	Number of detectors	More detectors produce a higher data amount and lower the data transfer speed, resulting in higher cycle times	More detectors higher information content

Table 2
Influence of various parameters on the overall cycle time.

different methods – both giving virtually the same resolution. The first method (0.45 min gradient) utilizes alternating column regeneration and high temperatures to allow high flow rates and speed optimized settings. A cycle time of 49 s could be achieved, resulting in a theoretical daily throughput of more than 1700 samples per day. The second method (0.90 min gradient) does not use high temperatures or alternating column regeneration and the time saving of some simple and often forgotten method options are shown. By optimizing these parameters the real cycle time gets as close to 8 s to the run time (stop time plus post time) and allows a daily throughput of more than 700 samples per day. By sub-optimal method set up this can easily drop to below 500 samples per day if options like automatic delay volume reduction, overlapped injection or offline data-analysis are not used.

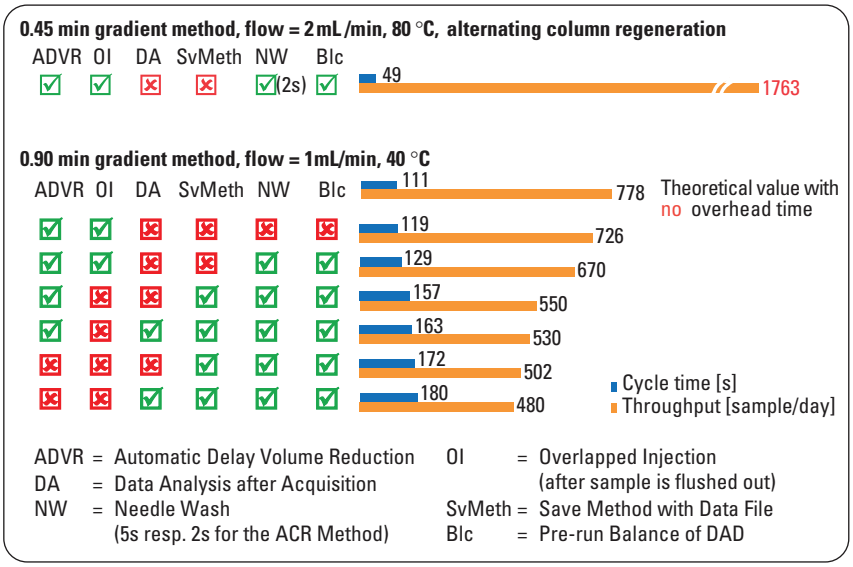

Figure 7
Cycle time and daily throughput optimization.

Chromatographic conditions:

Alternating Column Regeneration Method

Solvent:	A = Water, B = ACN	
Temp.:	80 °C	
Flow:	2.0 mL/min	
ADVR:	Yes	
Gradient:	**Gradient-Pump**	**Regeneration-Pump**
	0.00 min 35 %B	0.00 min 35 %B
	0.45 min 95 %B	0.01 min 95 %B
	0.46 min 35 %B	0.11 min 95 %B
	0.57 min 35 %B	0.12 min 35 %B
Stoptime:	0.57 min	no limit
Posttime:	off	off
Wavelength:	245 nm (8), ref. 450 nm (100)	
Peak width:	> 0.0025 min (0.05 s response time), 80 Hz	
Spectra:	none	
Injection volume:	1.0 µL	
Injector:	Overlapped injection, 2 s needle wash, sample flush-out factor = 10, draw/eject speed = 100 µL/min	
Valve:	next position	

No Alternating Column Regeneration Method

Solvent:	A = Water, B = ACN	
Temp.:	40 °C	
Flow:	1.0 mL/min	
ADVR:	Yes	No
Gradient:	0.00 min 35 %B	0.00 min 35 %B
	0.90 min 95 %B	0.90 min 95 %B
	1.10 min 95 %B	1.10 min 95 %B
	1.11 min 35 %B	1.11 min 35 %B
Stoptime:	1.15 min	*1.40 min (add. 300 µL extra column volume, increased retention times)*
Posttime:	0.70 min	0.70 min
Wavelength:	245 nm (8), ref. 450 nm (100)	
Peak width:	> 0.0025 min (0.05 s response time), 80 Hz	
Spectra:	all, 190-500 nm, BW = 1 nm	
Injection volume:	1.0 µL	
Injector:	See figure 7, 2 s equilibration time	

Conclusion

The Agilent 1200 Series Rapid Resolution LC system is a powerful tool to achieve highest chromatographic resolutions and also highest throughputs. The extended pressure range allows the usage of columns packed with stationary phases with particles sizes below 2 µm, for example, Agilent RRHT columns with particle sizes of 1.8 µm. These columns not only allow an increase in linear flow rates with virtually no loss in resolution but also have an inherently higher resolution compared to 3.5 µm or even 5.0 µm particle sizes. The possibility to switch the pump into its low delay volume configuration allows the use of the entire bandwidth of today's widely used column ids – from 4.6 mm down to 2.1 mm and even 1.0 mm. As illustrated above, the system has uncompromised performance characteristics even at highest gradient speeds.

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Published June 15, 2010
Publication Number 5989-4502EN

Agilent Technologies

Improving the Effectiveness of Method Translation for Fast and High Resolution Separations

Application

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Abstract

The increased availability of sub-2-micron (STM) columns and increased demand for methods friendly to mass spectrometers has led to strong trend toward conversion of existing HPLC methods to smaller diameter and smaller particle size columns. While the conversion is a simple mathematical exercise requiring the scaling flow rates, gradient times and injection volumes, many users observe less than perfect results. Here we look closely at the problem and propose calculations that improve the speed and/or resolution in a more predictable and beneficial way.

Introduction

Methods developed on older columns packed with large 5- or 10-µm particles are often good candidates for modernization by replacing these columns with smaller dimension columns packed with smaller particle sizes. The potential benefits include reduced analysis time and solvent consumption, improved sensitivity and greater compatibility with mass spectrometer ionization sources.

Simplistically, a column of 250-mm length and containing 5-µm particles can be replaced by a 150-mm length column packed with 3-µm particles. If the ratio of length to particle size is equal, the two columns are considered to have equal resolving power. Solvent consumption is reduced by L1/L2, here about 1.6-fold reduction in solvent usage per analysis. If an equal mass of analyte can then be successfully injected, the sensitivity should also increase by 1.6-fold due to reduced dilution of the peak as it travels through a smaller column of equal efficiency.

LC/MS (Liquid Chromatography/Mass Spectrometry) ionization sources, especially the electrospray ionization mode, have demonstrated greater sensitivity at lower flow rates than typically used in normal LC/UV (UltraViolet UV/VIS optical detection) methods, so it may also be advantageous to reduce the internal diameter of a column to allow timely analysis at lower flow rates. The relationship of flow rate between different column diameters is shown in Equation 1.

$$\text{Flow}_{\text{col. 1}} \times \left[\frac{\text{Diam.}_{\text{column2}}}{\text{Diam.}_{\text{column1}}}\right]^2 = \text{Flow}_{\text{col. 2}} \quad \text{(eq. 1)}$$

The combined effect of reduced length and diameter contributes to a reduction in solvent consumption and, again assuming the same analyte mass can be injected on the smaller column, a proportional increase in peak response. We normally scale the injection mass to the size of the column,

though, and a proportional injection volume would be calculated from the ratio of the void volumes of the two columns, multiplied by the injection volume on the original column.

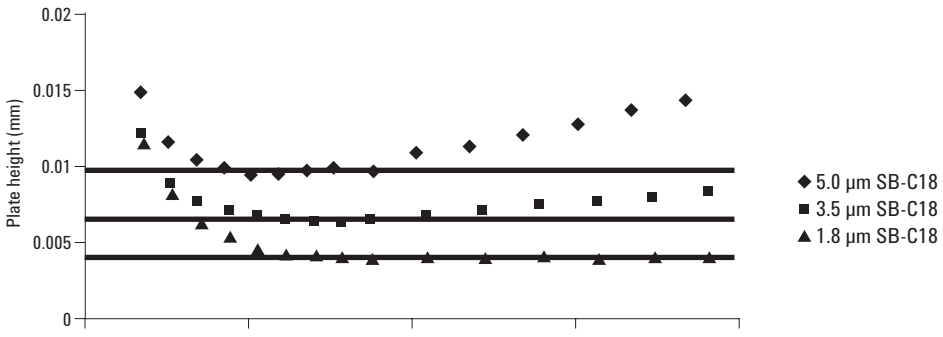
$$\text{Inj. vol.}_{\text{col. 1}} \times \left[\frac{\text{Volume}_{\text{column2}}}{\text{Volume}_{\text{column1}}}\right] = \text{Inj. vol.}_{\text{col. 2}} \quad \text{(eq. 2)}$$

For isocratic separations, the above conditions will normally result in a successful conversion of the method with little or no change in overall resolution. If one wishes to improve the outcome of the method conversion, though, there are several other parameters that should be considered. The first of these parameters is the column efficiency relative to flow rate, or more correctly efficiency to linear velocity, as commonly defined by van Deemter [1] and others, and the second is the often overlooked effect of extracolumn dispersion on the observed or empirical efficiency of the column.

Van Deemter observed and mathematically expressed the relationship of column efficiency to a variety of parameters, but we are most interested here in his observations that there is an optimum linear velocity for any given particle size, in a well-packed HPLC column, and that the optimum linear velocity increases as the particle size decreases. Graphically, this is often represented in van Deemter plots as shown in Figure 1, a modified version of the original plot [2].

In Figure 1 we observe that the linear velocity at which 5-µm materials are most efficient, under the conditions used by the authors, is about 1 mm/sec. For 3.5-µm materials the optimum linear velocity is about 1.7 mm/sec and has a less distinct optimum value, suggesting that 3.5-µm materials would give a more consistent column efficiency over a wider flow range. For the 1.8-µm materials, the minimum plate height, or maximum efficiency, is a broad range beginning at about 2 mm/sec and continuing past the range of the presented data. The practical application of this information is that a reduction in particle size, as discussed earlier, can often be further optimized by increasing the linear velocity which results in a further reduction in analysis time. This increase in elution speed will decrease absolute peak width and may require the user to increase data acquisition rates and reduce signal filtering parameters to ensure that the chromatographic separation is accurately recorded in the acquisition data file.

The second important consideration is the often overlooked effect of extracolumn dispersion on the observed or empirical efficiency of the column. As column volume is reduced, peak elution volumes are proportionately reduced. If smaller particle sizes are also employed there is a further reduction in the expected peak volume. The liquid chromatograph, and particularly the areas where the analytes will traverse, is a collection of various connecting capillaries and fittings which will cause a measurable amount of bandspreading. From the injector to the detector flow cell, the cumulative dispersion that occurs degrades the column performance and results in observed efficiencies that can be far below the values that would be estimated by purely theoretical means. It is fairly typical to see a measured dispersion of 20 to 100 µL in an HPLC system. This has a disproportionate effect on the smallest columns and smallest particle sizes, both of which are expected to yield the smallest



Lin. vel.	mm/sec	1	2	3	4	5
4.6 mm	mL/min	0.7	1.4	2.1	2.8	3.5
3 mm	mL/min	0.3	0.6	0.9	1.2	1.5
2.1 mm	mL/min	0.14	0.29	0.44	0.58	0.73
1 mm	mL/min	0.033	0.066	0.1	0.133	0.166

Figure 1. van Deemter plot with various flow rates and particle sizes.

possible peak volumes. Care must be taken by the user to minimize the extracolumn volume and to reduce, where practical, the number of connecting fittings and the volume of injection valves and detector flow cells.

For gradient elution separations, where the mobile phase composition increases through the initial part of the analysis until the analytes of interest have been eluted from the column, successful method conversion to smaller columns requires that the gradient slope be preserved. While many publications have referred to gradient slope in terms of % change per minute, it is more useful to express it as % change per column volume. In this way, the change in column volume during method conversion can be used to accurately render the new gradient condition. If we think of each line of a gradient table as a segment, we can express the gradient by the following equation:

$$\%\ \text{Gradient slope} = \left[\frac{(\text{End}\% - \text{Start}\%)}{\#\text{Column volumes}} \right] \quad \text{(eq. 3)}$$

Note that the use of % change per column volume rather than % change per minute frees the user to control gradient slope by altering gradient time and/or gradient flow rate. A large value for gradient slope yields very fast gradients with minimal resolution, while lower gradient slopes produce higher resolution at the expense of increased solvent consumption and somewhat reduced sensitivity. Longer analysis time may also result unless the gradient slope is reduced by increasing the flow rate, within acceptable operating pressure ranges, rather than by increasing the gradient time.

Resolution increases with shallow gradients because the effective capacity factor, k*, is increased. Much like in isocratic separations, where the capacity term is called k', a higher value directly increases resolution. The effect is quite dramatic up to a k value of about 5 to 10, after which little improvement is observed. In the subsequent examples, we will see the results associated with the calculations discussed above.

Experimental Conditions

System

Agilent 1200 Series Rapid Resolution LC consisting of:
G1379B micro degasser
G1312B binary pump SL
G1367C autosampler SL, with thermostatic temperature control
G1316B Thermostatted column compartment SL
G1315C UV/VIS diode array detector SL, flow cell as indicated in individual chromatograms
ChemStation 32-bit version B.02.01

Columns

Agilent ZORBAX SB-C18, 4.6 mm × 250 mm, 5 µm
Agilent ZORBAX SB-C18, 3.0 mm × 150 mm, 3.5 µm

Mobile phase conditions

Organic solvent: Acetonitrile
Aqueous solvent: 25 mm phosphoric acid in Milli-Q water

Gradient Conditions

Gradient slope: 7.8% or 2.3% per column volume, as indicated. See individual chromatograms for flow rate and time

Sample

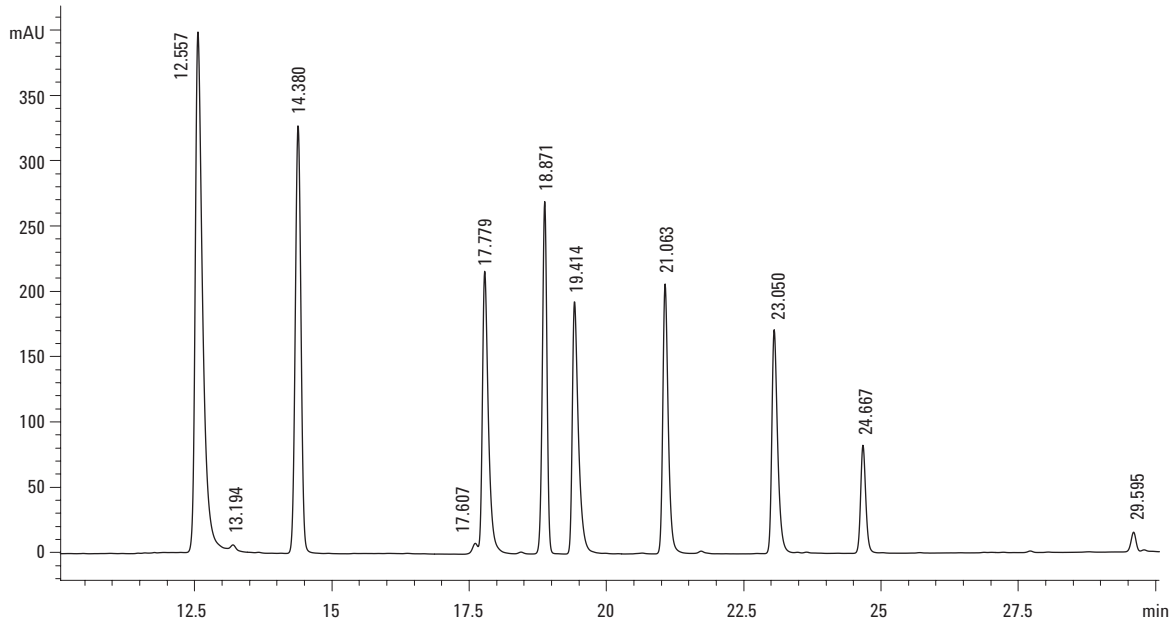
Standard mixture of chlorinated phenoxy acid herbicides, 100 µg/mL in methanol

Results

The separation was initially performed on a standard 4.6 × 250 mm, 5-µm ZORBAX SB-C18 column thermostatted to 25 °C (Figure 2) using conditions referenced in US EPA Method 555. The method was then scaled in flow and time for exact translation to a 3.0 × 150 mm, 3.5-µm column (Figure 3). Solvent consumption is reduced from 60 mL to 15.5 mL per analysis.

The separation was then re-optimized for faster separation with the identical slope, 7.8%, by increasing the flow rate from 0.43 to 1.42 mL/min, and proportionately reducing the gradient time (Figure 4). Finally, increased resolution is demonstrated by keeping the original times used in Figure 3 with the increased flow rate (Figure 5). This yields a gradient with identical time but a reduced slope of 2.3%. The increased resolution of peaks 4 and 5 is readily apparent.

The conditions in Figure 4, 7.8% slope at increased linear velocity on 3.0 × 150 mm, 3.5-µm material, yield a separation with comparable resolution to the original 4.6 × 250 mm method, but with only a 12-minute total analysis time. This is excellent for

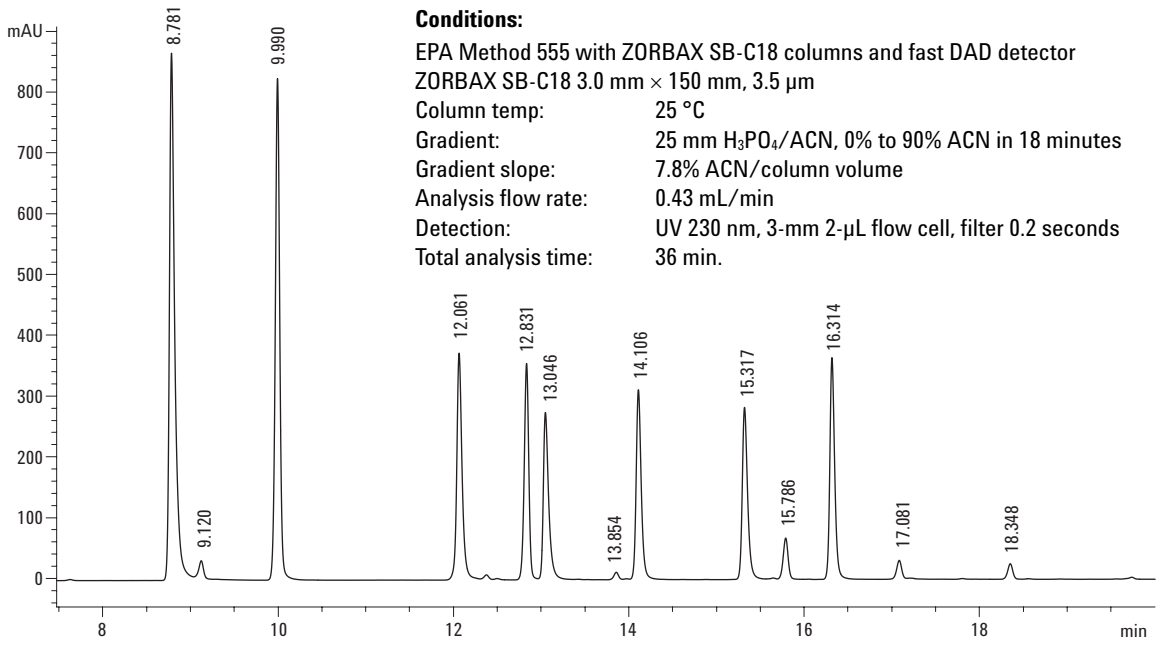


Conditions

EPA Method 555 with ZORBAX SB-C18 columns and fast DAD detector

ZORBAX SB-C18 4.6 mm × 250 mm, 5 µm	
Column temp:	25 °C
Gradient:	10% to 90% ACN vs. 25 mM H_3PO_4
Gradient slope:	7.8% ACN/column volume
Analysis flow rate:	1 mL/min
Group A Compounds	
Total analysis time:	60 min
Detection:	UV 230 nm, 10-mm 13-µL flow cell, filter 2 seconds (default)

Figure 2. Gradient separation of herbicides on 4.6 × 250 mm 5-µm ZORBAX SB-C18.



Conditions:

EPA Method 555 with ZORBAX SB-C18 columns and fast DAD detector

ZORBAX SB-C18 3.0 mm × 150 mm, 3.5 µm	
Column temp:	25 °C
Gradient:	25 mm H_3PO_4/ACN, 0% to 90% ACN in 18 minutes
Gradient slope:	7.8% ACN/column volume
Analysis flow rate:	0.43 mL/min
Detection:	UV 230 nm, 3-mm 2-µL flow cell, filter 0.2 seconds
Total analysis time:	36 min.

Figure 3. Gradient separation of herbicides on 3.0 × 150 mm, 3.5-µm ZORBAX SB-C18.

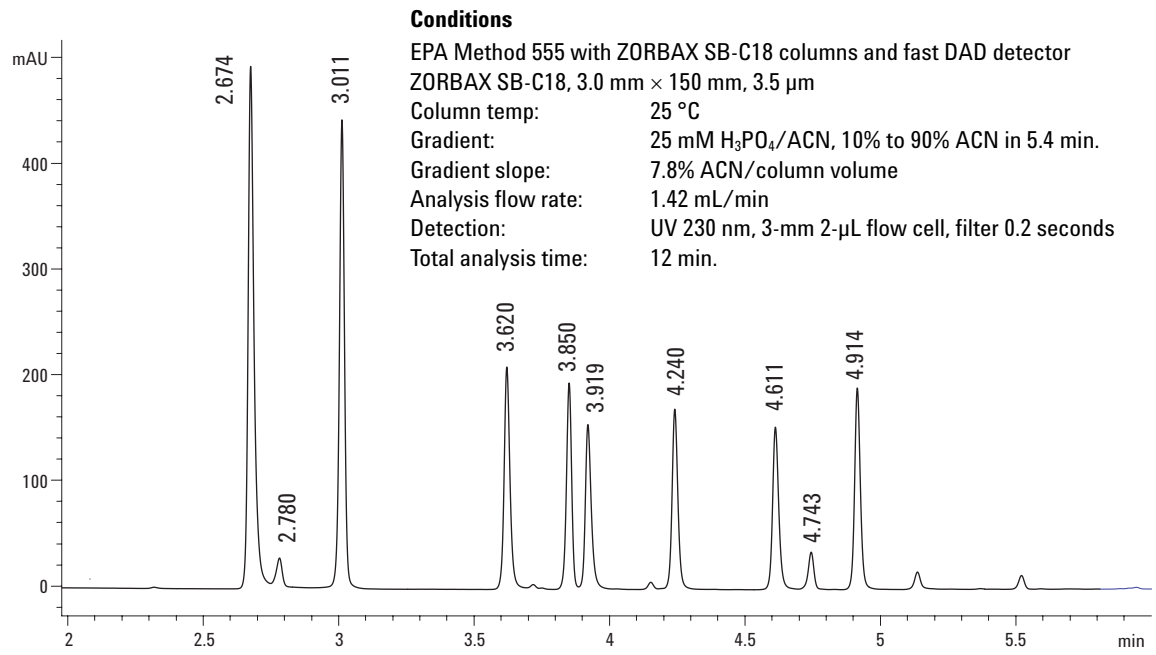


Figure 4. High speed gradient separation of herbicides on 3.0 × 150 mm, 3.5-µm ZORBAX SB-C18.

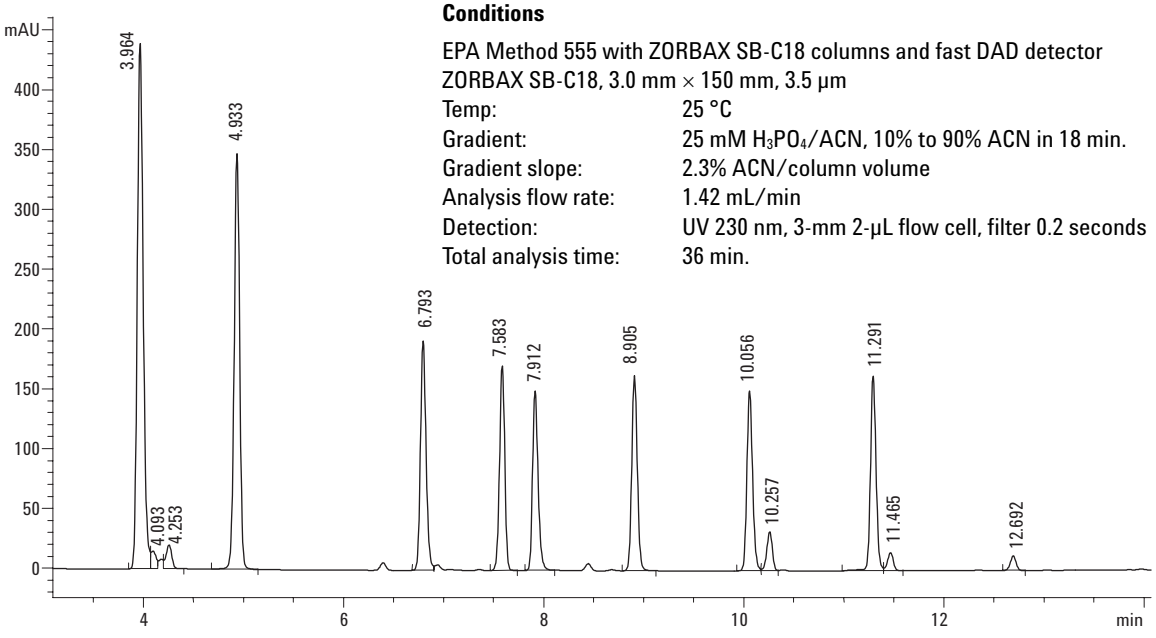


Figure 5. Reduced slope gradient separation of herbicides on 3.0 × 150 mm, 3.5-µm ZORBAX SB-C18.

high throughput screening and quantitation of a large number of samples. Figure 5, with the gradient slope reduced to 2.3%, results in a high-resolution separation with a calculated R value of 3.3 vs. the standard 3.0 × 150 mm separation value of 1.9, for the critical pair seen in Figure 5 at 7.5 to 8 minutes.

In Table 1 the column has been replaced with a low dead volume connecting union in a system fitted with 0.12-mm id capillary tubing at all points of sample contact. A 1-µL injection of dilute actone is made to determine the bandspreading contribution of the system, with various flow cells. Multiple flow cells were tested, and the average result reported, where possible. The elution volume summarizes the total volume of all tubing in the system. While the absolute volume from the 2-µL to the 13-µL flow cells is 11 µL, we observe an increase of 15 to 16 µL because of the larger diameter inlet tubing integral to the larger volume flow cells.

Table 1. Volumetric Measurements of Various Flow Cells

Flow cell	Elution volume (µL)	Half height width (µL)	5 Sigma width (µL)
New SL 2 µL 3 mm	11	5	12
Micro 6 mm 1.7 µL (n = 2)	14	6	18
Semi-micro 6 mm 5 µL (n = 2)	13	6.5	18.5
Standard 10 mm 13 µL	26	11	26
New SL 10 mm 13 µL	27	11	25

Conclusion

Careful analysis of the existing gradient conditions, coupled with an awareness of the need to accurately calculate new flow and gradient conditions can lead to an easy and reliable conversion of existing methods to new faster or higher resolution conditions. In addition, awareness of extracolumn dispersion, especially with small and high resolution columns, will ensure good column efficiency which is critical to a successful translation of the method.

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1. J. J. van Deemter, F. J. Zuiderweg, A. Klinkenberg, Chemical Engineering Science 1956, 5, 271–289
2. The Influence of Sub-Two Micron Particles on HPLC Performance, Agilent Technologies, application note 5989-9251EN, May 2003

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Printed in the USA
August 9, 2006
5989-5177EN

Process Monitoring of Bisphenol-A in Industrial Feedstock using High Throughput HPLC

Application

Process Control

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Abstract

The chromatographic method used to monitor the Bisphenol-A manufacturing process was improved using Agilent RRHT Eclipse XDB-C18 columns. These columns use 1.8-µm particles versus conventional 3.5-µm or 5-µm particles. The improved method allowed seven times faster analyses, improved resolution, and higher sensitivity.

Introduction

Bisphenol-A (Figure 1) is a highly versatile material used to manufacture many modern products. It is also known as 4,4"-Isopropylidenediphenol, 4,4"-(1-Methylethylidene) bisphenol, or simply BPA.

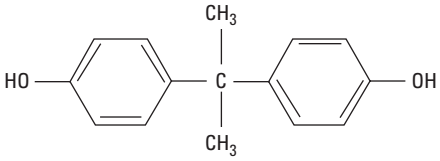

Figure 1. Bisphenol A

Every year, 2.8 million tons of BPA are produced. BPA is a building block for polycarbonate plastic and epoxy resins. Polycarbonate plastic is prized for its scratch resistance, optical clarity, and heat and electrical resistance. Because of these attributes, it is used for eyewear, CD/DVD disks, electronics, and food and drink containers. Epoxy resins are used for protective coatings because of their combination of inertness, chemical resistance, adhesion, and formability. For example, metal food cans are lined to protect taste. Epoxy resins are also used as a component in dental sealants and as a component in dental composites providing an alternative to mercury amalgam in veneers and fillings. Other uses include fungicides, polymer antioxidants, and components in automobiles and appliances.

BPA is produced through an acid-catalyzed condensation reaction of phenol with acetone. During condensation, a number of phenol-based byproducts are also formed. HPLC is used to determine the composition of many of the process streams in a commercial BPA plant.

Here we describe the use of new HPLC column technology for the possible improvement to one of the HPLC methods used in a commercial BPA facility.

Method Optimization and Scalability

The existing HPLC method was proven and robust; however, it was complicated. We sought a similar chromatogram, based on the original method, but using simpler method parameters. Because of the challenge of changing many chromatographic parameters, essentially redeveloping the method, we chose a 4.6 × 50 mm, 1.8-µm Eclipse XDB-C18 column for experiments to reduce the time required. Smaller particles packed in shorter columns increase the speed of analysis and still provide enough efficiency to maintain resolution equivalent to longer columns packed with larger particles. After several trials, we developed a method that produced a chromatogram similar to the original. The short analysis time is a major advantage of Rapid Resolution High Throughput (RRHT) technology. Whereas a handful of experimental runs would take an entire work day using a typical analytical-sized column (50 min/run), the series of runs took about an hour (7.5 min/run), using an RRHT column.

We incrementally scaled up to a 4.6 × 250 mm column. Figure 2 shows an overlay of the sample analyzed by three 4.6-mm id columns of different lengths and particle sizes. Injection volume was also changed proportionally to length. The smaller ZORBAX particles speed up the analysis while maintaining resolution. In fact, resolution increased when using the RRHT columns despite their shorter length.

One reason this method can be easily scaled (up or down) is the uniform spherical Eclipse XDB-C18 packing. It has a proprietary engineered particle size distribution, based on ZORBAX silica with a controlled surface area and pore size. The robust proprietary packing material and proven column manufacturing techniques consistently yield reproducible columns with similar chromatographic performance, independent of the column dimensions.

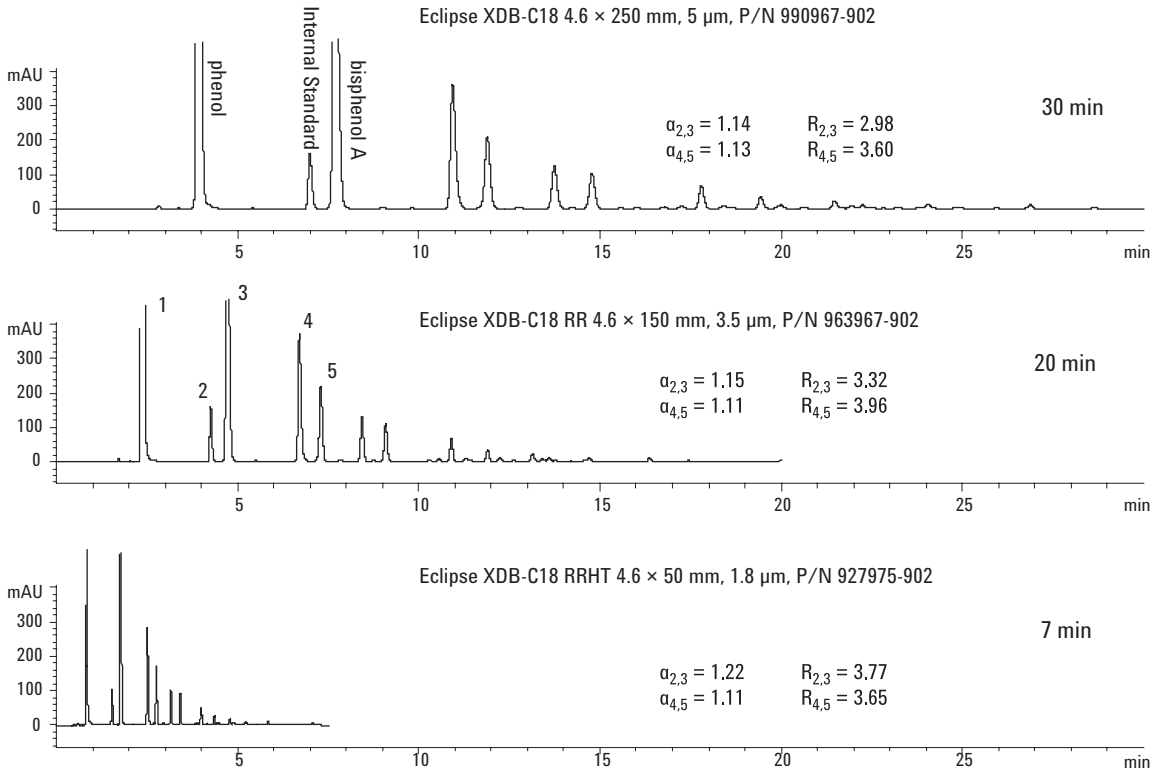

Figure 2. RRHT column configuration increased both speed and resolution.

Particle size does influence resolution. The influence can be noticed when comparing columns of identical dimensions, packed with three different particle sizes. Figure 3 shows the shortened Bisphenol-A analysis using different particle-sized Eclipse XDB-C18 columns. Resolution (Rs) is related to selectivity (α), efficiency (N) and retention (k´):

$$Rs = (1/4)(\alpha-1)\sqrt{N}\,[k'/(1+k')]$$

Factors affecting the selectivity term (stationary phase, mobile phase) and retention term (mobile phase, temperature) are constant for the three chromatograms. The efficiency term is influenced by column length, linear velocity of the mobile phase (both constant), and particle size (varied in Figure 3). N increases as particle size decreases. In Figure 3 the selectivity factors (α) and retention remain about the same, but resolution actually increases. The increase in resolution due to the decrease in particle size highlights the advantage of using smaller particles. The similar selectivity and retention highlight the suitability of ZORBAX Eclipse XDB-C18 columns for scaling methods, especially to more rapid, high-throughput methods.

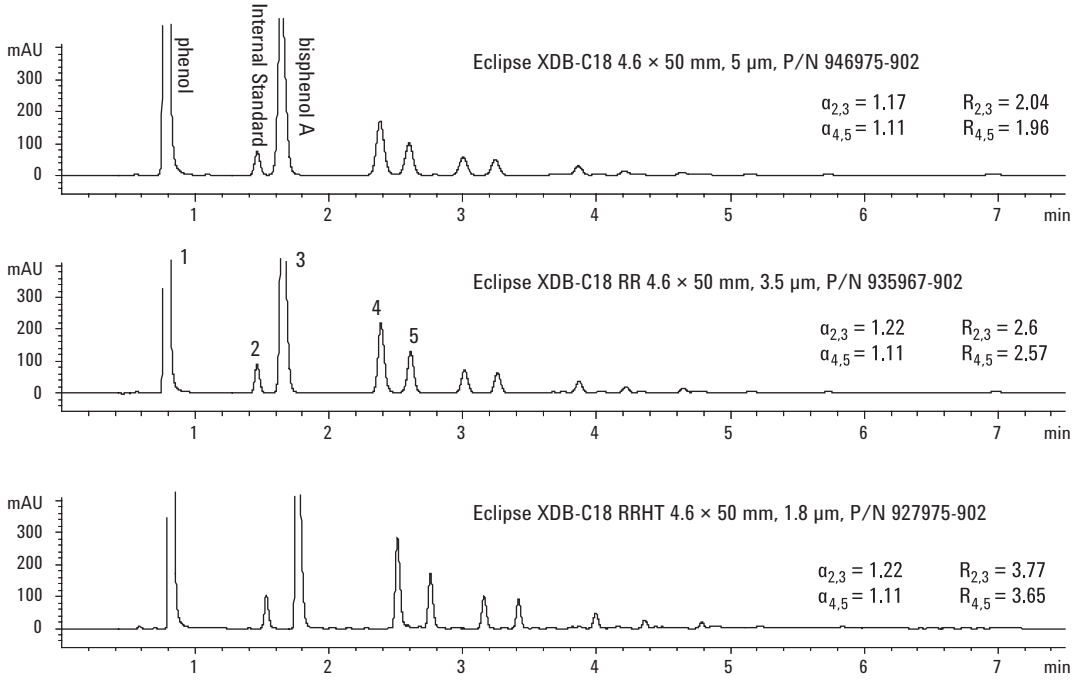

Figure 3. Effect of particle size on resolution and selectivity.

Comparing the Existing Method to the RRHT Method

Figure 4 compares the original BPA separation to the RRHT separation. The top chromatogram is an example of the analysis using the original commercial method, and the bottom is an example of the process sample analyzed with the RRHT method. The method developed with the new column technology clearly increases productivity.

Analysis time is reduced at least six-fold; solvent consumption is reduced about 12.5 times, from 100 mL/analysis to only 7.5 mL/analysis. Interestingly, the peak shape of Bisphenol-A is more symmetrical using Eclipse XDB-C18 as compared to the current C18 column used in the original analysis. The more Gaussian peak shape eluted by the Eclipse XDB-C18 column is important for accurate quantification. Other method improvements such as a simplified gradient and a binary mobile phase are listed in Table 1.

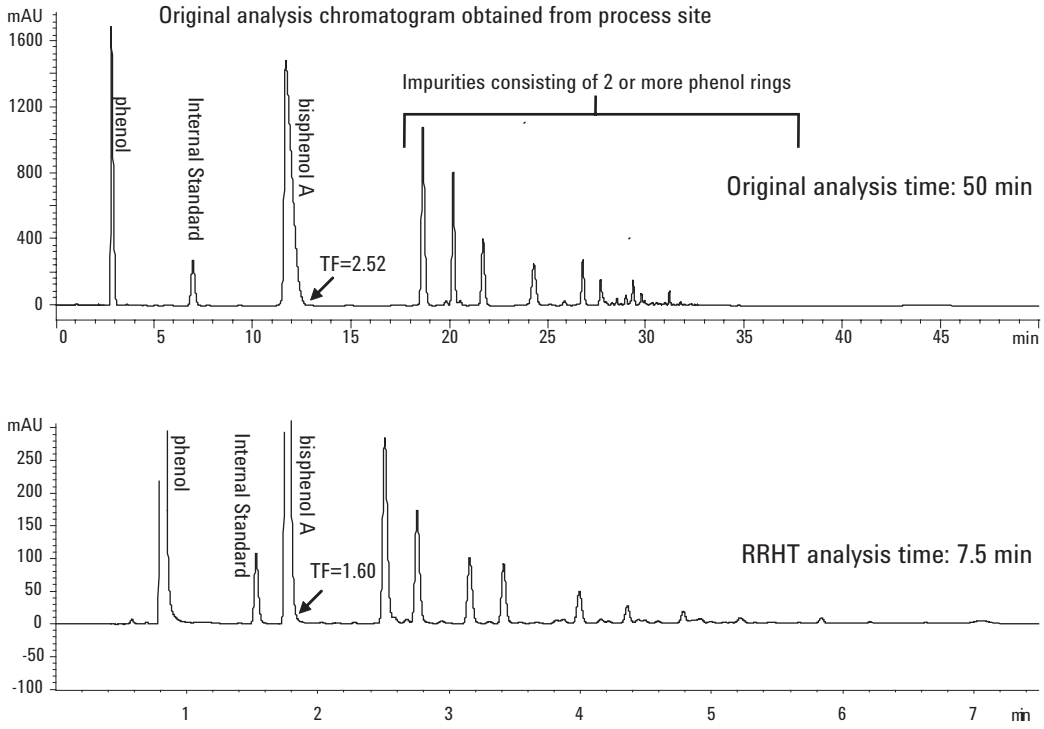

Figure 4. Comparison of methods; original to RRHT.

Table 1. Current and Improved Method Parameters

Original

- Column: Supelco LC-18, 4.6 × 250 mm, 5 µm
- Mobile phase: A: 0.025% H_3PO_4, B: ACN, C: MeOH
- Flow: 2 mL/min
- Temperature: 35 °C
- Sample size: 20 µL
- Gradient: segmented, has isocratic holds

Time	% A:B:C
0	65:25:10
13	65:25:10
18	50:40:10
23	50:40:10
27	30:50:20
32	0:70:30
35	0:70:30
36	0:60:40
40	0:50:50
43	0:20:80
48	65:25:10

RRHT

- Column: ZORBAX XDB-C18, 4.6 × 50 mm, 1.8 µm
- Mobile phase: A: 0.1% formic acid, B: ACN: MeOH (200:800)
- Flow: 1 mL/min
- Temperature: 25 °C
- Sample size: 2 µL
- Gradient: linear, no isocratic holds

Time	% B
0	60
6	95
6.01	60
8	60

Conclusion

Converting an existing method to a high-throughput method is one way to improve lab productivity. Using RRHT columns initially for method development also improves productivity. Eclipse XDB-C18 RRHT columns are a good choice for converting existing C18 methods into high-throughput methods. Smaller particles packed into shorter columns provide comparable resolution to larger particles packed into longer columns in a fraction of the time. RRHT columns are advantageous for gradient method development because gradient re-equilibration is time-consuming and often overlooked in the total analysis time. Methods developed on Agilent RRHT columns can be scaled easily because of the highly uniform particles, bonded phase chemistry, and column manufacturing techniques. An existing method developed on a "traditional analytical-sized" column was easily converted to a high throughput method using an Eclipse XDB-C18 RRHT column. The method was incrementally scaled up to an analytical-sized column, and it performed with predictable results on various column dimensions and particle sizes. The predictability of the results supports Eclipse XDB-C18 RRHT columns' ability to easily improve applications and transfer them into high-throughput and high-resolution applications.

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Printed in the USA
July 26, 2006
5989-5231EN

Agilent Technologies

Improving Productivity and Extending Column Life with Backflush

Application Brief

Chin-Kai Meng

All Industries

A previous application note [1] has shown that multiple GC signals and MS signals can be acquired from a single sample injection. When a 3-way splitter is connected to the end of a column, column effluent can be directed proportionally to two GC detectors as well as the MSD. This multi-signal configuration provides full-scan data for library searching, SIM data for quantitation, and element selective detector data for excellent selectivity and sensitivity from complex matrices.

The system used in this study consists of a 7683ALS, a 7890A GC with split/splitless inlet, 3-way splitter, µECD, dual flame photometric detector (DFPD), and a 5975C MSD. Figure 1 shows four chromatograms from a single injection of a milk extract. The synchronous SIM/scan feature of the 5975C MSD provides data useful for both screening (full scan data) and quantitation (SIM data). DFPD provides both P and S signals without the need to switch light filters.

Noticeably in the full scan TIC in Figure 1, a significant number of matrix peaks were observed after 32 minutes. It is not uncommon to add a "bake-out" oven ramp to clean the column after analyzing complex samples. The bake-out period is used to quickly push the late eluters out of the column to be ready for the next injection. Therefore, it is common to use a higher oven temperature than required for the analysis and an extended bake-out period at the end of a normal

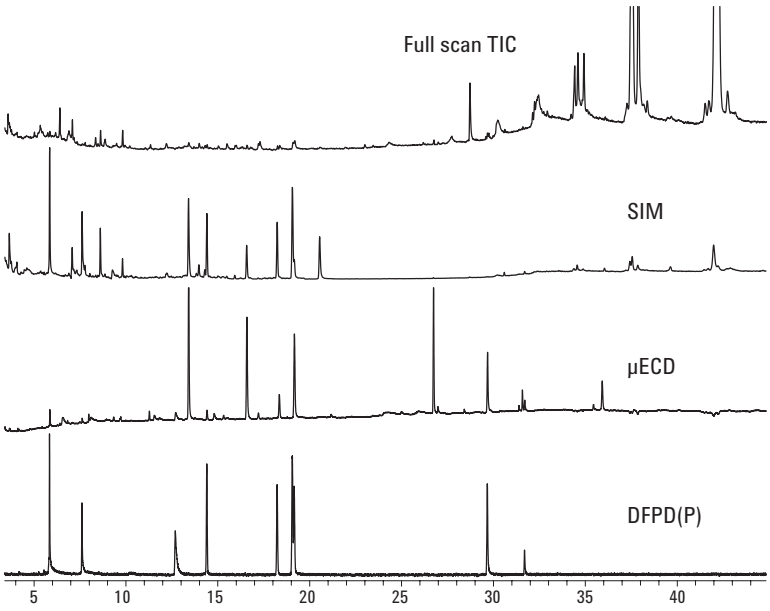

Figure 1. Four chromatograms collected simultaneously from a single injection of a milk extract.

Highlights

- Backflush – a simple technique to remove high boilers from the column faster and at a lower column temperature to cut down analysis time and increase column lifetime.
- The milk extract example shows that a 7-minute 280 °C backflush cleaned the column as well as a 33-minute 320 °C bake-out. The cycle time was reduced by more than 30%.
- Using backflush, excess column bleed and heavy residues will not be introduced into the MSD, thus reducing ion source contamination.

over program to clean out the column, which adds to the cycle time and shortens the column lifetime. Adding the bake-out period to the milk extract analysis, additional matrix peaks were observed even up to 72 minutes, while target compounds already eluted before 42 minutes. This means that 30 minutes were lost in productivity for each injection.

Backflush [2] is a simple technique to drastically decrease the cycle time by reversing the column flow to push the late eluters out of the inlet end of the column. Late eluters stay near the front of the column until the oven temperature is high enough to move them through the column. When the column flow is reversed before the late eluters start to move down the column, these late eluters will take less time and at a lower oven temperature to exit the inlet end of the column.

There are many benefits in using backflush:

- Cycle time is reduced (no bake-out period, cooling down from a lower oven temperature)
- Column bleed is reduced (no high-temperature bake-out needed), resulting longer column life
- Ghost peaks are eliminated (no high boilers carryover into subsequent runs)
- Contamination that goes into the detector is minimized, which is especially valuable for the MSD (less ion source cleaning)

Figure 2 shows three total ion chromatograms from the Agilent 7890A GC/5975C MSD. The top chromatogram is a milk extract analysis with all the target compounds eluted before 42 minutes (over program goes to 280 °C). However, an additional 33-minute bake-out period at 320 °C was needed to move the high boilers out of the column. This bake-out period was almost as long as the required time to elute all target compounds. The middle chromatogram is the same milk extract analysis stopped at 42 minutes with a 7-minute backflush post-run at 280 °C added to the analysis. The bottom chromatogram is a blank run after the backflushing was completed. The blank run shows that the column was very clean after backflushing. The example shows that a 7-minute backflush cleaned the column as well as a 33-minute bake-out.

The milk extract example in Figure 2 illustrates the backflush technique in reducing cycle time and column bleed. The cycle time was reduced by more than 30% and the column was kept at 280 °C, without going to the bake-out temperature of 320 °C. A column effluent splitter or QuickSwap is required to do the backflush.

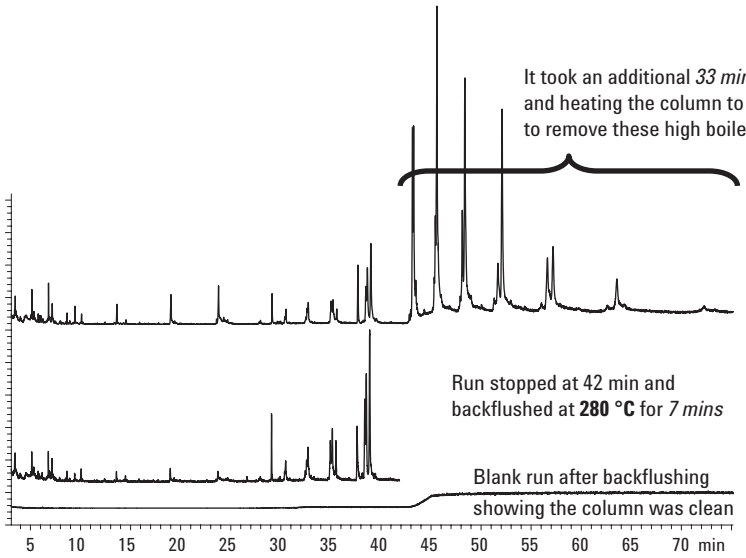

Figure 2. Three total ion chromatograms comparing the results with and without backflush.

References

1. Chin-Kai Meng and Bruce Quimby, "Identifying Pesticides with Full Scan, SIM, μECD, and FPD from a Single Injection," Agilent Application Note, 5989-3299EN, July 2005.
2. Matthew Klee, "Simplified Backflush Using Agilent 6890 GC Post Run Command," Agilent Application Note, 5989-5111EN, June 2006.

Acknowledgement

Milk extract is courtesy of Dr. Steven Lehotay from USDA Agricultural Research Service in Wyndmoor, Pennsylvania, USA.

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Printed in the USA
December 26, 2006
5989-6018EN

A Column-Flow Independent Configuration for QuickSwap

Application

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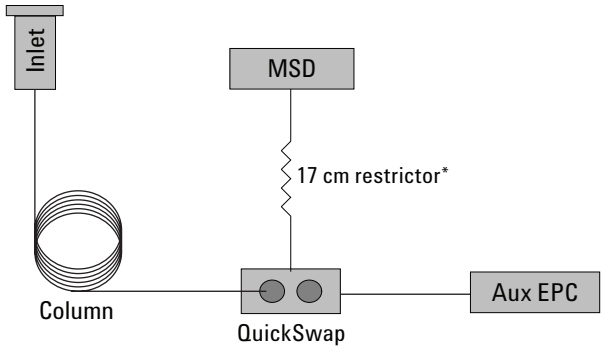
Abstract

A flexible configuration of QuickSwap is presented that allows use of larger id columns, pressure pulse injections, and variable column flow rates without having to change the restrictor or QuickSwap pressure. The split configuration can be set up such that the MSD is run at optimal flow rate. Examples are presented for several different columns and experimental conditions.

Introduction

QuickSwap is a recently introduced Capillary Flow Technology device designed to improve the usability of GC/MSD systems. It allows you to change columns and do inlet maintenance without venting the mass spectrometer. It also facilitates use of the backflush technique. The basic concepts, benefits, and use of QuickSwap are described in several Agilent Technologies publications [1-4] and are illustrated in Figures 1 and 2.

As can be seen from Figure 1, if the column is disconnected from QuickSwap, a flow of inert gas from the Aux EPC will prevent air from entering the MSD.



*QuickSwap restrictor, P, and T are selected for desired flow to MSD, usually the maximum flow that the current application requires.

Figure 1. General concept of QuickSwap.

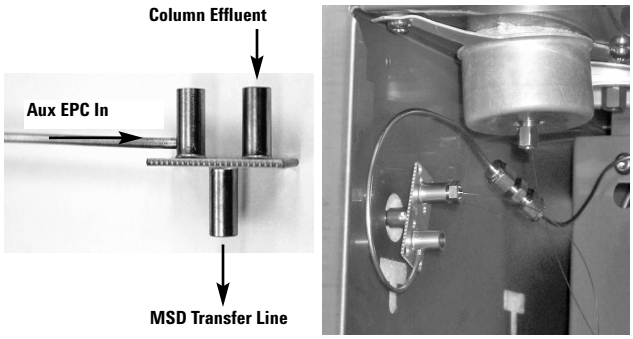


Figure 2. QuickSwap is pictured on the left showing permanent (Aux EPC In) and temporary connections. A picture of a normal QuickSwap installation is shown on the right.

In the standard configuration of QuickSwap, you must determine before installation what the maximum expected flow will be from the analytical capillary column being used. This value is in turn used to select the proper restrictor size (the four available sizes are 92 µm, 100 µm, 110 µm, and 120 µm id), the transfer line temperature, and QuickSwap pressure.

If the flow from the analytical column exceeds that originally planned for, then the pressure at QuickSwap will exceed its setpoint and the GC will go "not ready." This can happen if you do any of the following:

- Do pressure pulse injections, wherein the flow during injection is typically two to three times that during the run
- Increase column flow rate, as you might do when doing a method speed-up with method translation
- Do a retention time locking calibration, where inlet pressure is increased 20% over the nominal pressure
- Change to larger-dimension columns

In these examples, you would need to increase QuickSwap pressure and/or lower restrictor temperature or cool the system and install a new restrictor in order to accommodate the higher flows.

On the other hand, if you were to use a restrictor that allowed excess flow to the MSD, method performance (for example, detection limit and linear dynamic range) might be worse. So, it is important to plan carefully when using the normal QuickSwap configuration to get the right balance in performance and usability.

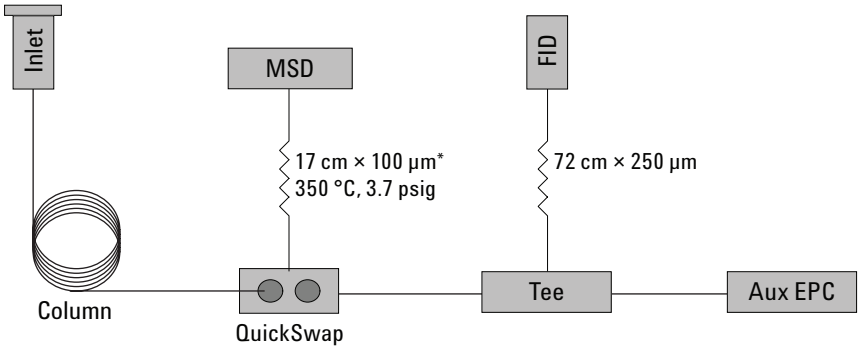
In general, when flow to the MSD changes,

- Tune parameters can change
- Response can change
- S/N and limit of detection can change

An alternate configuration was conceived of that allows the MSD to be run at optimal flow rate and improves flexibility and usability of QuickSwap [QS] in a wider range of potentially useful situations. This configuration incorporates a split between the Aux EPC module and QS and is illustrated in Figure 3.

This configuration has several advantages over the standard configuration. It:

- Simplifies initial setup (restrictor choices)
- Simplifies changes to existing methods



*In this example, the restrictor, transfer line temperature, and QuickSwap pressure were chosen to allow approximately 1 mL/min flow to the MSD—corresponding to its optimal performance regime.

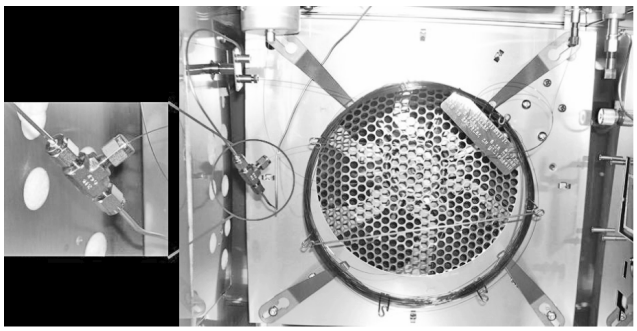
Figure 3. Flexible configuration includes addition of a split vent path on the Aux EPC line leading to QuickSwap.

- Simplifies retention time locking applications with QS
- Allows pressure pulse injections without having to change QS restrictor
- Allows more aggressive backflush conditions than if larger restrictors were used
- Allows method translation and speed up without having to change QS restrictor
- Allows use of medium- and large-bore columns with MSD

In some applications, there are some valid reasons why you might consider larger-bore capillary columns. These include:

- Higher sample capacity (solvent peaks don't tail as much, polar solutes don't front as much)
- Better robustness (better able to handle dirty samples)
- More amenable to large-volume injections—especially the solvent vapor exit version
- Less problematic cool on-column injections (more rugged larger id needles can be used)

However, the problem of higher flow rates associated with larger id columns has limited applications in GC/MS. MSD users are probably aware that there is an optimum flow above which MSD performance degrades. For most MSDs with electron impact sources and standard drawout lenses, optimal performance coincides with a flow rate range of 1 to 1.5 mL/min. Above that, signal and S/N fall approximately linearly with respect to flow rate increases.



Experimental

An 80-ppm mixture of semivolatiles and surrogates was selected based on a validated "fast" USEPA 8270 method [5]. A reference chromatogram is shown in Figure 4.

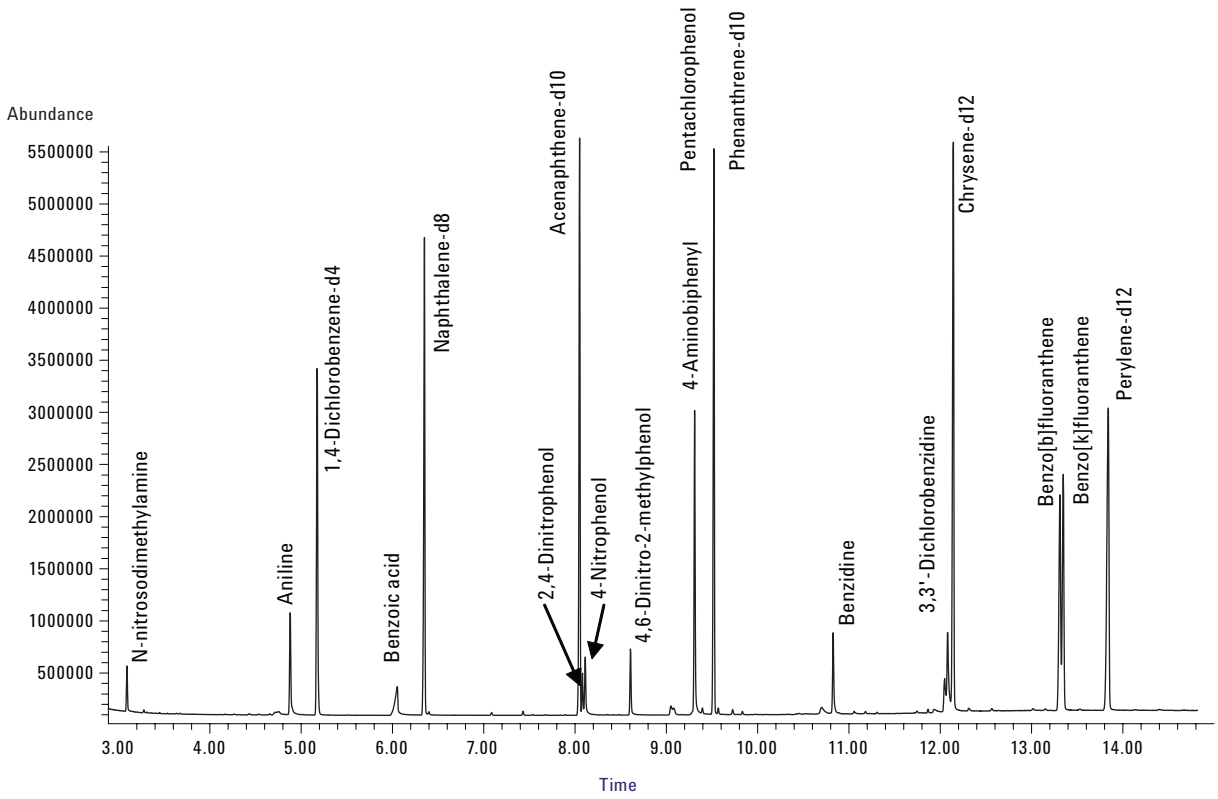


Figure 4. Reference chromatogram for Fast 8270 method.

Restrictor and setpoints were chosen for the flexible split configuration such that approximately 1 mL/min would go to the MSD. Several different combinations of QuickSwap restrictor and setpoints could be used to yield a flow rate in the optimal range for MSD with EI source. These are listed in Table 1.

Table 1. Restrictor and Setpoint Combinations Corresonding to the Optimal Flow Rate Range of the MSD

QuickSwap restrictor id (µm)	QuickSwap pressure (psig)	Transfer line temperature (°C)	Flow to MSD (mL/min)
92 (G3185-60361)	4.0	250	1.0
92	4.0	195	1.2
100 (G3185-60362)	3.7	350	1.0
100	2.7	250	1.2
110 (G3185-60363)	0.5	350	1.0
110	1.4	325	1.2

Referring back to Figure 3, now let's examine the flexible QuickSwap configuration in more detail. In this study, the 1/16-inch Swagelok union connecting the line from QuickSwap to that coming from the Aux EPC was replaced with a stainless steel tee (refer to the parts list). To the third leg of the tee, a restrictor was added leading to a flame ionization detector (FID) to allow monitoring of vented material. In an alternate configuration, one can put the tee outside the oven by cutting the Aux EPC tubing on the top of the GC, and then plumb the restrictor to a separate split vent trap (such as that used to trap vented sample on the split/splitless inlet; refer to the parts list). This configuration is recommended to capture potentially noxious sample components that are vented if an FID is not being used to combust them. The split vent trap cartridge is also easily replaced with a fresh one if and when it is necessary.

The dimensions of the vent restrictor is not as critical as the one used for QuickSwap. The vent flow rate needs to be more than that reasonably expected for the analytical column used and experiments to be conducted. However, there is little downside to using a restrictor with "moderately excessive flow," except that one is wasting clean purge gas from the Aux EPC. In this example, the restrictor was chosen to yield approximately 10 mL/min at the initial oven temp (50 °C) and QuickSwap pressure (3.7 psig).

For experiments where the column flow is less than the 1 mL/min nominal flow to the MSD, makeup gas would be supplied by the Aux EPC to make up the difference and pure purge gas would vent through the FID. In those cases where the column flow exceeds 1 mL/min, the excess would back up the Aux EPC line to the tee, where it will mix with the purge gas and be vented to the FID and detected. In effect, any flow > 1 mL/min is vented while the flow to the MSD remains constant at its optimum.

To test the flexibility of this configuration, several different sizes of columns and several different flow rates were examined using the same semivolatiles sample used earlier. The columns and conditions are listed in Table 2. Again, constant pressure mode conditions were chosen to yield approximately the same void times for the three different columns so that solute retention times would be similar. Later, other flows were tried as were constant flow modes.

Table 2. Conditions for Constant Pressure Mode Experiments (Void times nominally matched at 1.239 min. Conditions: Oven program: 50 °C (1 min) → 350 °C (3 min) @ 20 °C/min; QuickSwap restrictor = 17 cm x 100 µm id at 3.7 psig and 350 °C, yielding 1.0 mL/min flow to MSD; 0.5 µL splitless injection with a 2-min purge delay, inlet at 275 °C)

Dimensions	Head pressure	Initial flow (@ 50 °C)	Ending flow (350 °C)	Relative capacity
20 m x 180 µm	20.5 psig	0.70 mL/min	0.23 mL/min	1 X
30 m x 250 µm	23.4 psig	2.18 mL/min	0.72 mL/min	2.2 X
30 m x 530 µm	7.93 psig	6.85 mL/min	2.26 mL/min	18 X

The results of the comparison are shown in Figure 5. Several points are worth stating.

1. Columns were quickly switched without venting the MSD (a key benefit of QuickSwap).
2. No pump down, retuning, or equilibration time were required prior to applying new pressure setpoints and acquiring data for the different columns.
3. The retention times are approximately the same on each column—a result of determining the setpoints that would yield the same void time.
4. Peak widths, shapes and heights reflect a composite of chromatographic phenomena such as relative stationary phase capacities, column efficiencies, deviation of actual flow from optimal flow, and the amount of post-column split to vent. For example, one might think that the 180-µm id column should have the narrowest peaks (highest efficiency); however, one can see from Table 2 that the flow rate decreases from the optimal flow rate of 0.7 mL/min at the start of the run to well below that at the end. This will cause peaks to be wider than they would be at optimal flow. In contrast, the flow rate of the 250-µm id column starts higher than the 1 mL/min optimal flow but remains at an optimal or faster-than-optimal rate for most of the run. This will cause the peak widths for the 250-µm id column to be narrower than that of the 180-µm id column.
5. The benzoic acid peak (#4) is less distorted on the 530-µm id column as a consequence of the larger column capacity. This is one of the benefits of using larger id columns.
6. The relative elution order is the same for the three columns. This is a consequence of matching void times and using constant pressure mode. This would not be the case when using constant flow mode (see Figure 7).

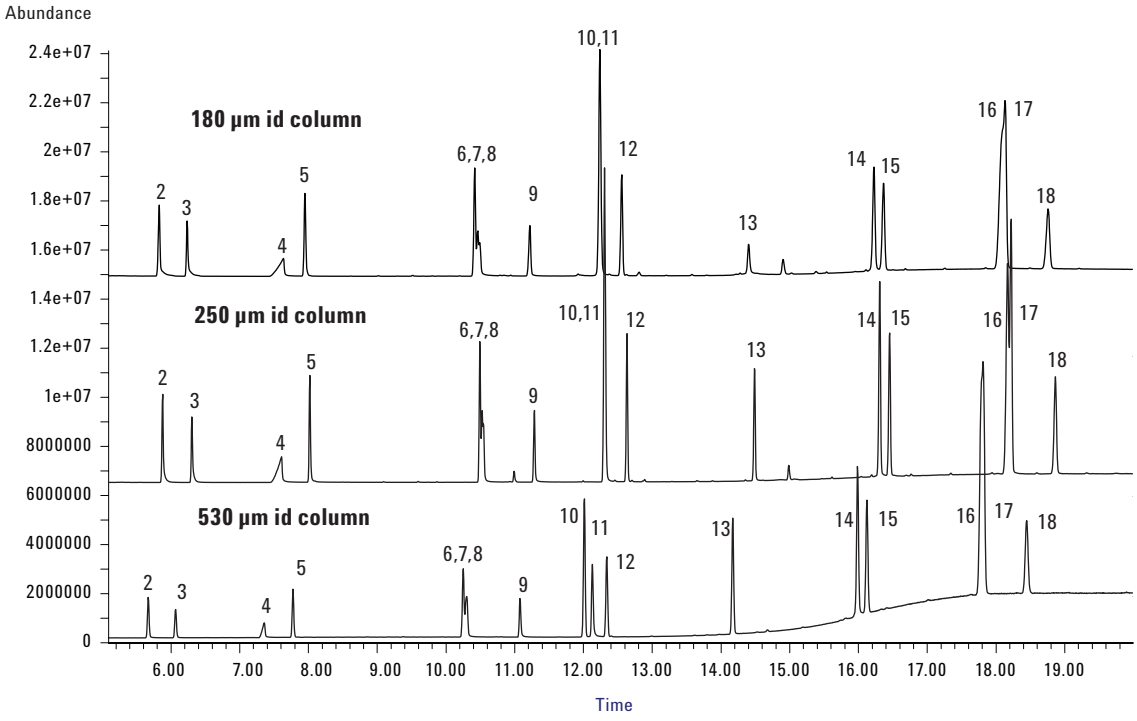


Figure 5. Constant pressure mode analysis with three different column dimensions; 0.5-µL splitless injections of 80-ppm semivolatiles test sample, with flow conditions from Table 2.

As can be seen in Figure 6, the FID signal indicates what was split to the FID when column flow exceeded the 1 mL/min flow to the MSD. At no time does the 180-µm id column flow exceed 1 mL/min, so there is nothing vented and no FID signal. For the 250-µm id column, the flow at initial conditions is > 1 mL/min, and the excess flow is split to the FID, as indicated by a solvent peak. Yet as flow decreases during the run (a normal consequence of constant pressure mode conditions), column effluent all goes to the MSD and FID signal remains flat. For the 530-µm id column, flow is always > 1 mL/min, so some flow is always being vented through the FID. This is easily seen in the inset of Figure 6, where the scale is expanded and peaks can be seen throughout the FID chromatogram.

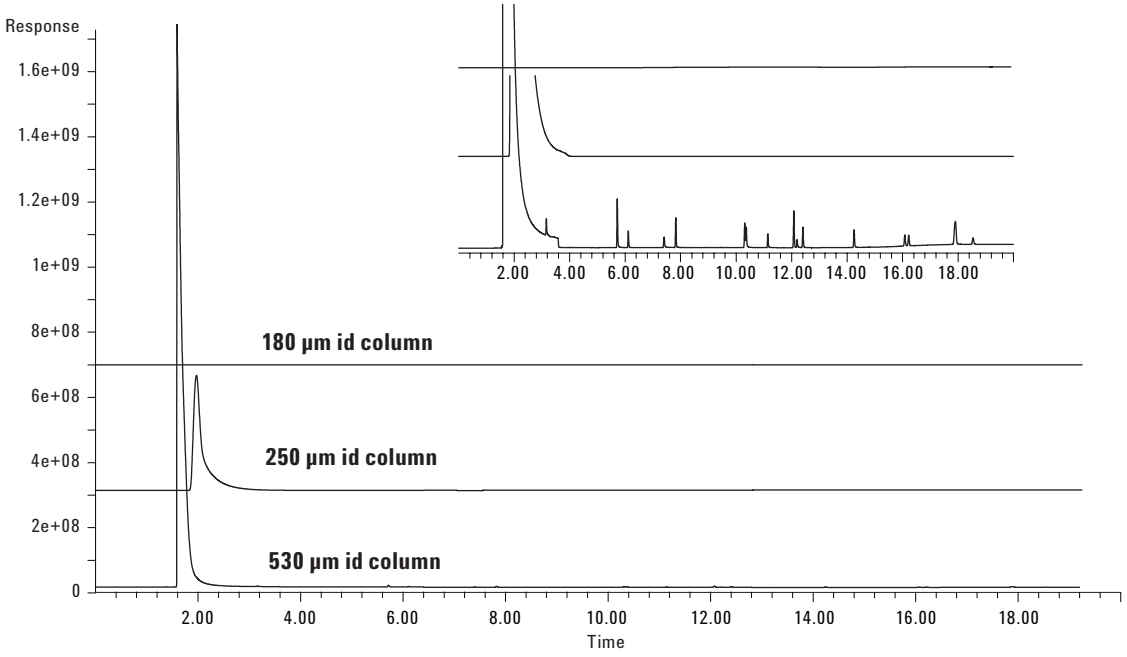


Figure 6. FID signal of vent stream shows what is vented when column flow exceeds flow to MSD.

Table 3. Constant Flow Mode Conditions (Lower flow for each column is its optimal flow, the higher is 2X optimum. Other instrumental paramters were the same as those used for constant pressure mode experiments.)

Dimensions	Outlet flow
20 m X 180 µm	0.72 mL/min
20 m X 180 µm	1.44 mL/min
30 m X 250 µm	2.5 mL/min
30 m X 250 µm	1.0 mL/min
30 m X 530 µm	2.1 mL/min
30 m X 530 µm	7.0 mL/min

Constant flow mode was also evaluated. Conditions for constant flow modes are given in Table 3. Two flow rates were chosen for each column: optimal flow rates (the lower of the two) and 2X optimum.

The MSD TIC for each column at optimal flow rates is shown in Figure 7, with the corresponding FID vent signal in Figure 8. It can clearly be seen that for the 250-µm and 180-mm id columns, no column effluent is split to the FID. Since the flow rate of the 530-µm id column is approximately 2X the flow the MSD, half of the column effluent is split to the FID.

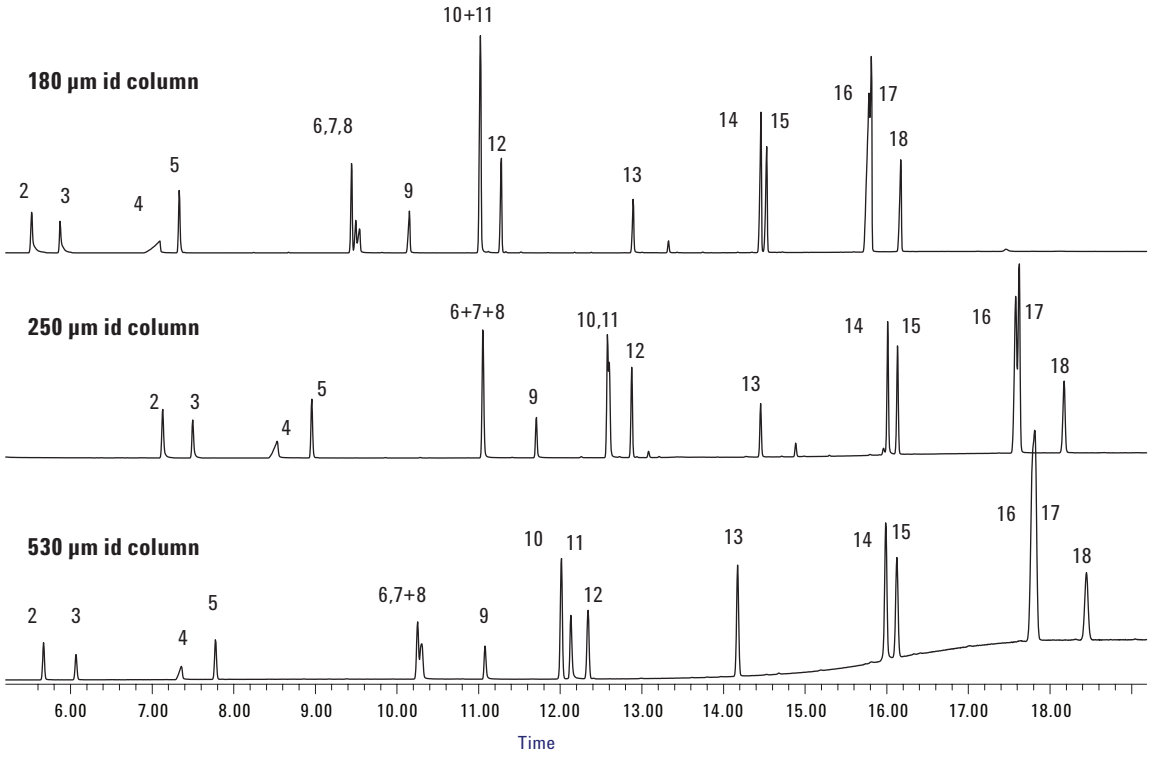


Figure 7. TIC chromatograms for the three columns under optimal constant flow mode conditions.

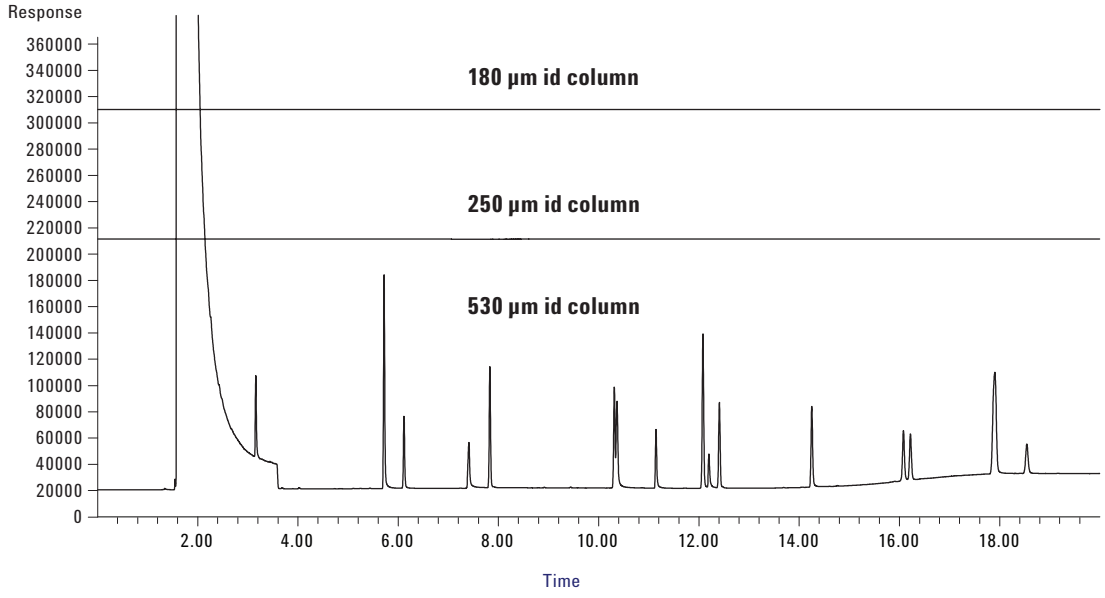


Figure 8. FID vent signal for three columns under optimal flow conditions. Only the 530-µm id column has a flow that exceeds the 1 mL/min flow to the MSD.

Results for the 2X optimal flow conditions are shown in Figures 9 and 10. The flexibility of the QuickSwap split configuration is highlighted here in that no adjustments were made to QuickSwap restrictor size, transfer line temperature, or Aux EPC pressure in order to accommodate all of the flow changes. Only the columns and their individual flow conditions were changed. The QuickSwap split passively accommodated all excess flow.

Notice in Figure 9 that the higher the excess column flow, the less of the sample goes to the MSD (more is split to vent, as seen in Figure 10). The fact that less sample is getting to the MSD might be considered a serious disadvantage for some analyses, but this is tempered by the fact that the larger column has higher sample capacity, so larger sample volumes could be injected without suffering overload (peak distortion). In addition, the larger diameter columns usually generate wider peaks, so a larger value can be selected for MSD sampling (for example, samples = 2^3 or 2^4 instead of 2^2). This will result in higher S/N. So, if one seeks the benefits of larger id columns for MS analysis, one can easily accommodate them with this QuickSwap configuration with only a small compromise.

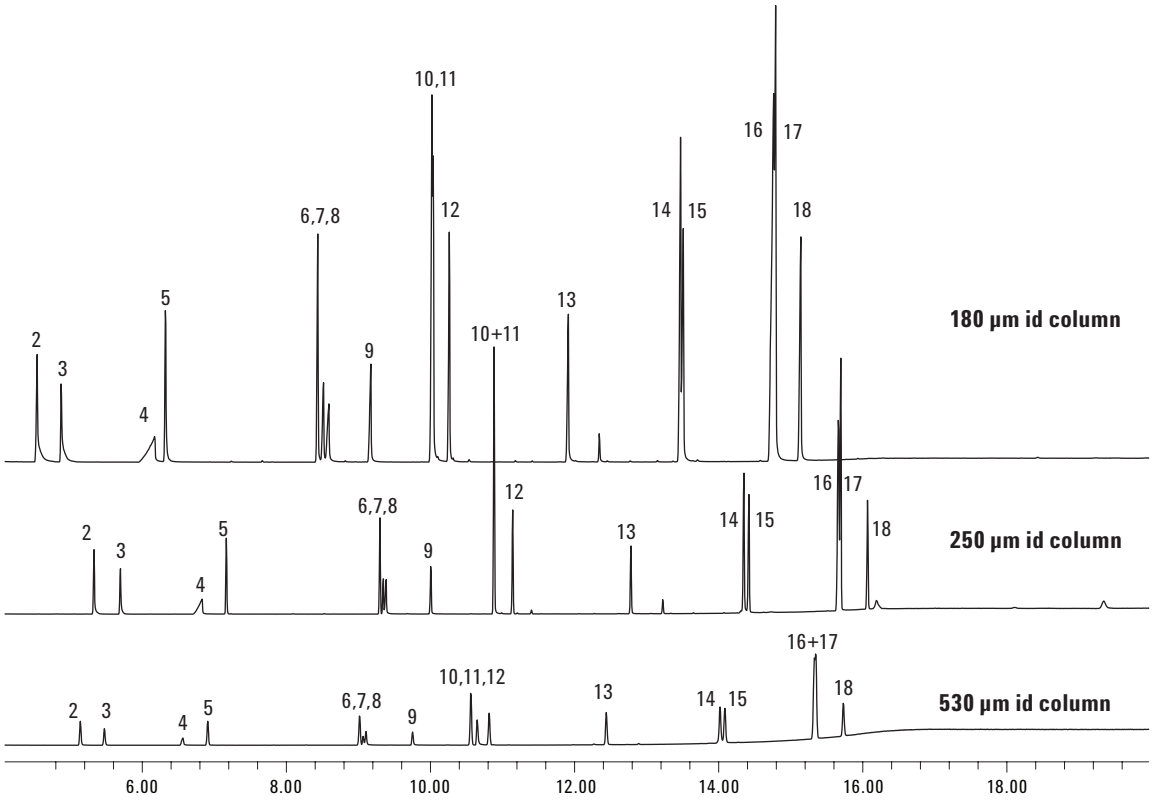


Figure 9. Comparison of MSD TIC chromatograms for three columns run at 2X optimal constant flow mode. Scale is constant for the three, showing the absolute amount of sample reaching the MSD.

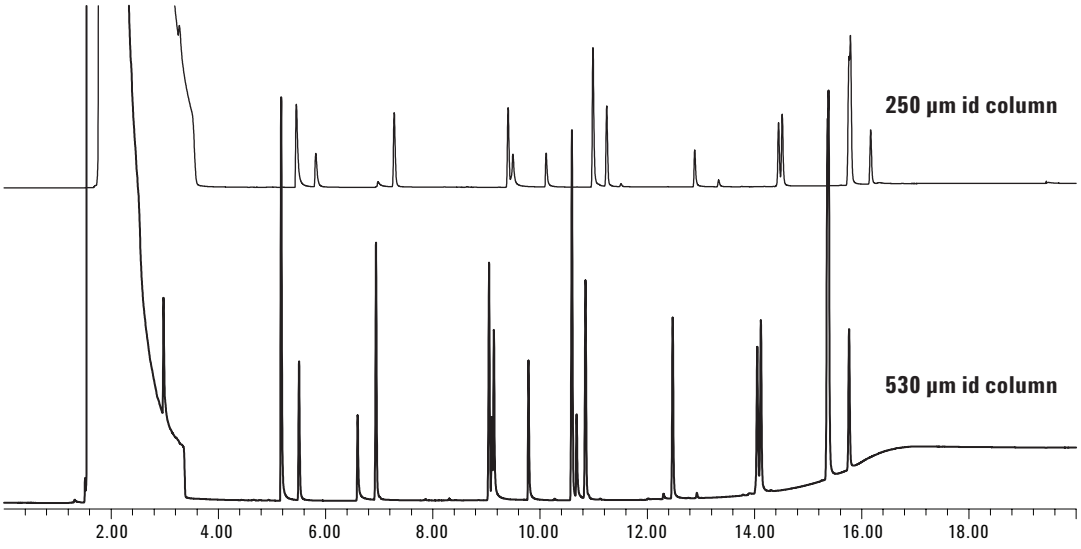


Figure 10. FID vent signals for the two largest columns operated at 2X optimal constant flow rate conditions.

Pressure-pulse injection is often used to minimize the time labile samples stay in the inlet and to avoid inlet overload when large volume sample injections. With this technique, pressures are typically two to three times the starting pressure of the standard analysis. As such, the flow through the column is increased significantly. In the standard QuickSwap configuration, this higher flow can exceed the ability of the chosen QuickSwap restrictor to handle at the selected QuickSwap (Aux EPC) pressure. When this happens, pressure exceeds the setpoint, the GC goes "not ready," and automated injection does not proceed. With the flexible split configuration for QuickSwap described herein, the extra flow during pressure pulse injection is vented, so there is no issue with maintaining setpoint.

A pressure pulse injection was done with the 250-µm id column to verify that the split configuration would accommodate the extra flow. The pulse pressure was 50 psi (approximately two times the standard pressure) for 1 min, after which the pressure returned to 23.41 psig for the remainder of the run. For the standard run, the pressure was 23.41 psig for the whole time. No other changes were made to experimental conditions.

Figure 11 compares MSD TIC chromatograms for the standard and pulsed-pressure experiments. One can see a slightly earlier retention time for the first couple of peaks in the pressure pulse experiment (this is typical due to the higher initial column flows). Other than that, the chromatograms are indistinguishable.

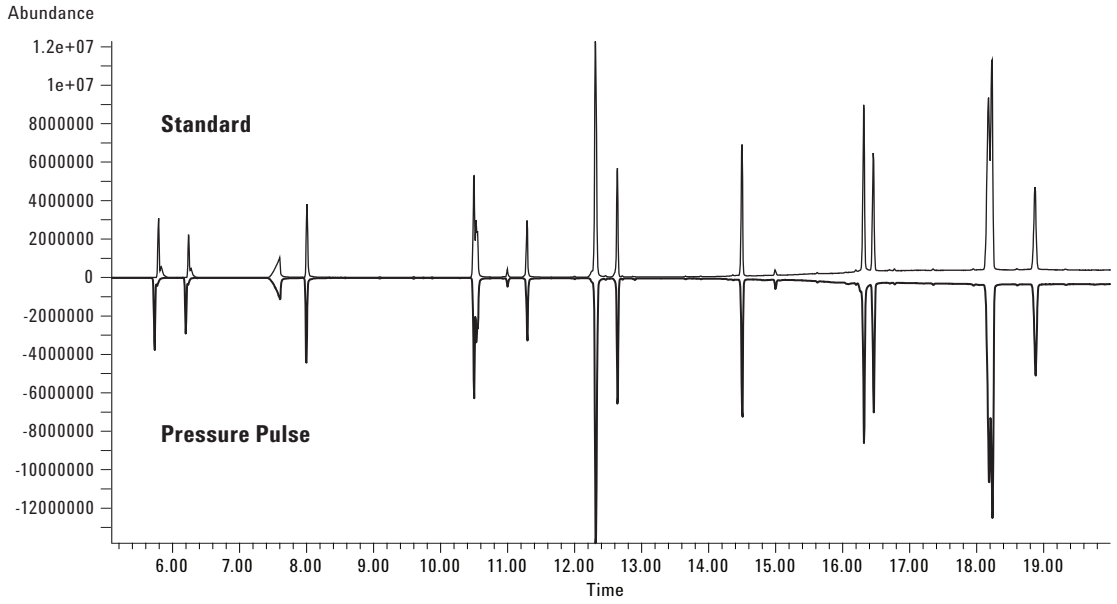


Figure 11. Comparison of standard and pressure-pulse injection modes. No adjustment of QuickSwap pressure was required for the pressure-pulse mode—a benefit of using QuickSwap split configuration.

As can be seen from the FID vent signal, (Figure 12), more solvent is vented in the pressure-pulse injection than in the standard because of the higher initial flow. Yet for the analytical portion of the run after completion of the pressure pulse period (1 min), the column flows are the same in the two cases and decrease to near or below 1 mL/min. As a result, there is no excess column flow to split to the FID and the FID baseline is flat.

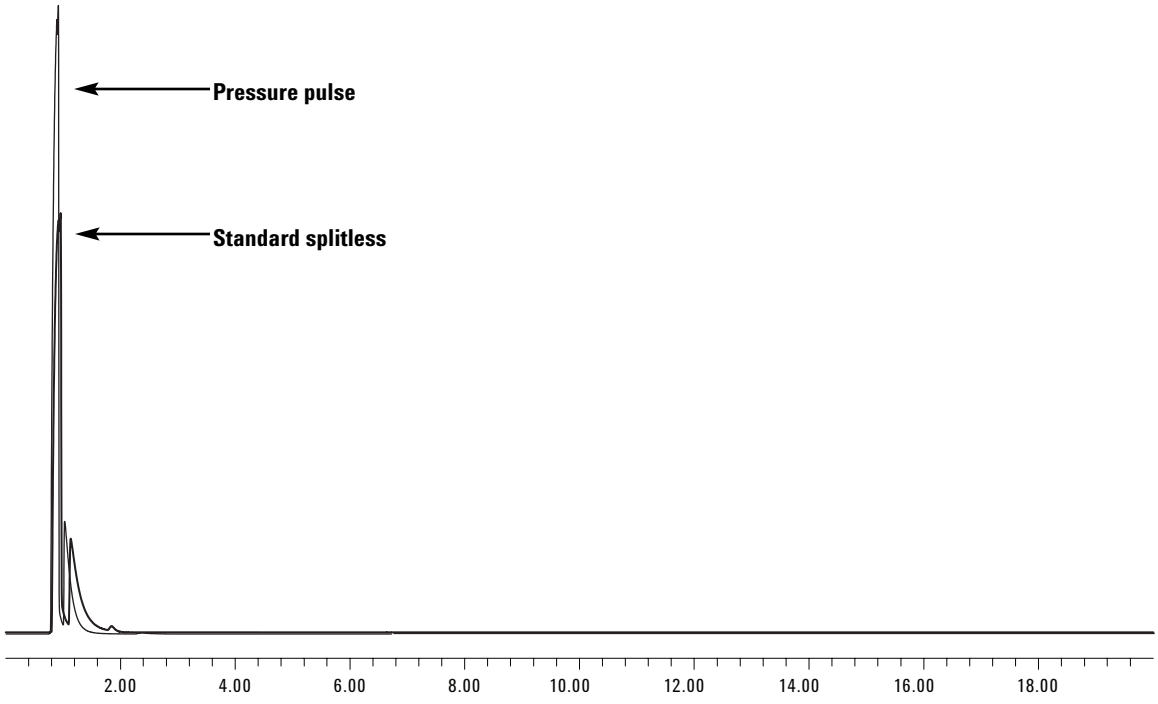


Figure 12. FID vent signal for pressure-pulse injection versus standard splitless injection.

Conclusions

The QuickSwap split configuration provides a flexible and simple alternative to the standard configuration. The split configuration can benefit MSD users who change columns frequently, seek the benefits of using larger id columns, and/or use pressure pulse injection. The configuration allows the MSD to run at optimal flow conditions while accommodating a wide range of column flows.

References

1. "How QuickSwap Works," f03002.pdf.
2. "Agilent G3185B QuickSwap Accessory Installation and Setup," Agilent publication number G3185-90100.
3. "Agilent G3185B QuickSwap Accessory Reference Manual," Agilent publication number G3185-90101.
4. "Simplified Backflush Using Agilent 6890 GC," Agilent publication number 5989-5111EN.
5. "Fast USEPA 8270 Semivolatiles Analysis Using the 6890N/5975 Inert GC/MSD," Agilent publication number 5989-2981EN.

Parts List

Part	Description	Part number
QuickSwap	Kit	G3185B
QuickSwap restrictors	92 µm	G3185-60361
	100 µm	G3185-60362
	110 µm	G3185-60363
1/16" tee	Regular	0100-0782
	ZDV	0100-0969
SilTite 1/16" ferrules	For connecting 1/16" SS lines	G2855-2055
Deactivated FS	250-µm id FID vent restrictor	160-2255-5
Split vent trap	Kit—vent alternative to FID	G1544-0124
1/16" straight union		0100-0124
SilTite ferrules for capillary	250 µm	5188-5361
column connections	320 µm	5188-5362
	530 µm	5188-5363
20 m X 180 mm X 0.36 mm	DB-5.625	121-5622
30 m X 250 mm X 0.5 mm	DB-5MS	122-5536
30 m X 530 mm X 1 mm	DB-5	125-503J

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Printed in the USA
May 15, 2007
5989-6702EN

Agilent Technologies

Increased Identification of Impurities in Octyl Dimethyl p-Aminobenzoic Acid Using the Agilent 6140 High Throughput LC/MS

Application

Chemical Analysis

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Abstract

The 6140 Single Quadrupole LC/MS High Throughput Mass Spectrometer is used to analyze octyl dimethyl para-aminobenzoic acid (OD-PABA) for the presence of impurities. The Agilent 1200 Series Rapid Resolution Liquid Chromatography (RRLC) system is used for the chromatographic separation of the compound from its impurities on a 3.0 mm id C18 column with a 1.8 µm-particle size. The LC/MS interface used in this work is a G1948B electrospray ionization source (ESI) in positive ion mode. While many compounds can be analyzed at the standard scan rate of 5400 amu/sec, one impurity is only clearly seen at the scan speed of 10,000 amu/sec, which is a unique capability of the 6140 system. This impurity is identified as p-dimethylbenzoic acid, a known degradate of octyl-dimethyl-p-aminobenzoic acid (OD-PABA).

Introduction

Para-aminobenzoic acid (PABA) has historically been used as an ultraviolet filter ingredient in sunscreen formulations. As its use can increase the risk of skin cancer, a derivative in the form of OD-PABA is currently and more commonly used. However, as PABA may be formed as a degradate of OD-PABA, it is important to monitor its potential presence in neat standards of OD-PABA. As a commercial product, the purity of OD-PABA is important to manufacturers, not only for the purpose of safety, but for economics as well. In this work we investigate the capability of the Agilent 6140 Single Quadrupole Mass Spectrometer to detect impurities that are seen above 0.1% of the OD-PABA absorbance level in UV.

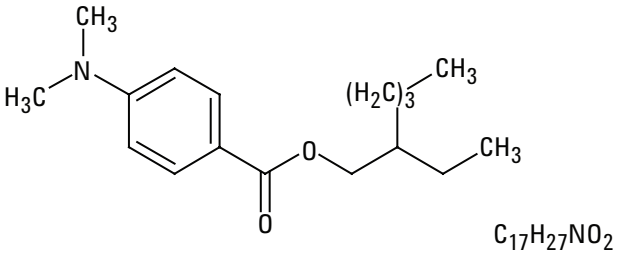


Figure 1. Octyl-dimethyl-p-aminobenzoic acid (OD-PABA).

The structure of the OD-PABA compound analyzed in this work is shown in Figure 1.

Experimental

Sample Preparation

The OD-PABA is obtained at a concentration of 1 mg/mL in methanol. Injection volumes of 5 µL at this concentration are made into the LC/MS system.

LC/MS Method Details

LC Conditions

Agilent 1200 Series binary pump SL, wellplate sampler, thermostatted column compartment

Column:	Agilent ZORBAX SB-C18, 3 × 30 mm, 1.8 µm (p/n 824975-302)
Column temp:	45 °C
Mobile phase:	A = 0.1% formic acid in water B = 0.1% formic acid in acetonitrile
Flow rate:	1.0 mL/min
Injection volumn:	5 µL

Gradient:	Time (min)	%B
	0	25
	7	75

Stop time:	7 min
Post-run time:	2 min

UV Conditions

Sample: 320 nm; Bw, 5 nm; reference off

MS Conditions

Mode:	Positive ESI using the Agilent G1948B ionization source
Nebulizer:	60 psig
Drying gas flow:	12 L/min
Drying gas temp:	350 °C
V_{cap}:	3000 V
MS Scan:	*m/z* 100–450

Cycle times (sec/cycle), 0.09 in Standard Fast Scan mode; 0.04 in Ultra Fast Scan mode

Table 1. Integration Results of Three Significant Peaks Found in OD-PABA Chromatogram of Figure 2

Peak #	Time (min)	Area	Height	Area %
1	0.707	76.4	44.1	0.4
2	5.184	176.6	50	0.925
3	6.005	18847.7	2911.3	98.676

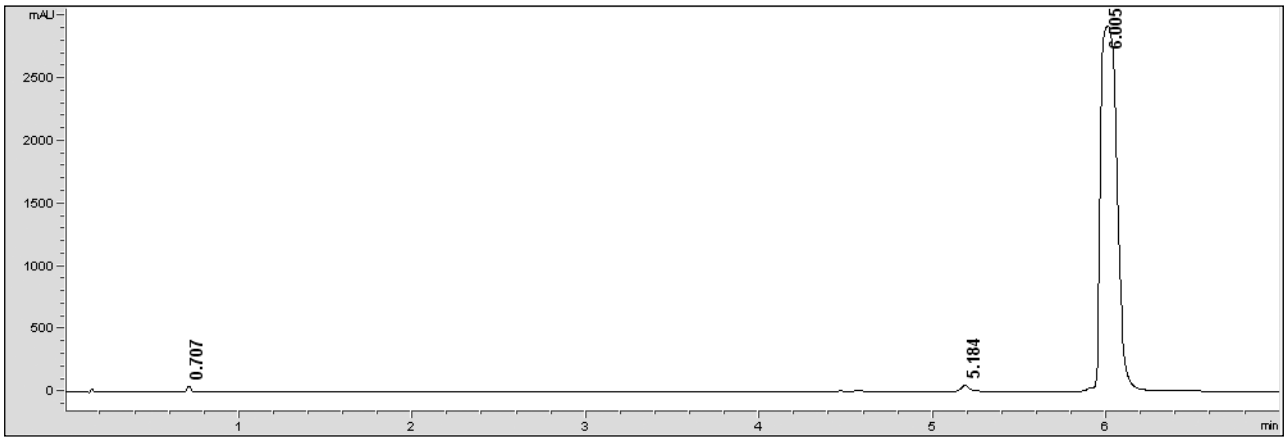


Figure 2. UV chromatogram of OD-PABA at 320 nm absorbance.

Results and Discussion

The UV absorbance of OD-PABA is shown at a retention time of 6.005 minutes in Figure 2. The tabulated integration results for each of the peaks shown are given in Table 1.

The highest data acquisition speed in the Standard Fast Scan mode (5,400 amu/sec) is 0.09 sec/cycle. The total ion chromatogram (TIC) corresponding to this mode is shown in Figure 3A. The Ultra Fast Scan mode (10,000 amu/sec) is only available in the Agilent 6140 mass spectrometer and has a corresponding cycle time of 0.04 sec/cycle. The total ion chromatogram corresponding to the Ultra Fast Scan mode is shown in Figure 3B.

While the Standard Fast Scan is adequate for detecting the peaks at 5.184 and 6.005 minutes in the UV chromatogram, and a few more peaks are detected as well (4.379, 4.478, 4.594, and 5.616 min in the TIC of Figure 3A), the peak at 0.707 minutes in the UV chromatogram is much more easily seen in the Ultra Fast Scan mode of Figure 3B. This is because the earlier eluting peak at 0.707 minutes has a relatively narrower peak width so that the scan speed must be higher to adequately detect signal in such a narrow window of time.

It should be noted that while the faster scan speed in Ultra Fast Scan mode results in the acquisition of more data points across the ion chromatogram, the variation in amount of signal from scan to scan is larger because the amount of time involved with collecting signal is reduced. When less ions are collected during each cycle, the variation in signal from one cycle to the next is larger. As a result, Figure 3B shows more variation of the baseline signal in comparison to Figure 3A.

In Figure 4 an overlay of the two TICs is shown with the region around the 0.707 min peak (0.732 min in the MS) expanded. With more data points acquired in the Ultra Fast Scan mode of the 6140 Single Quadrupole, the impurity peak is more readily seen.

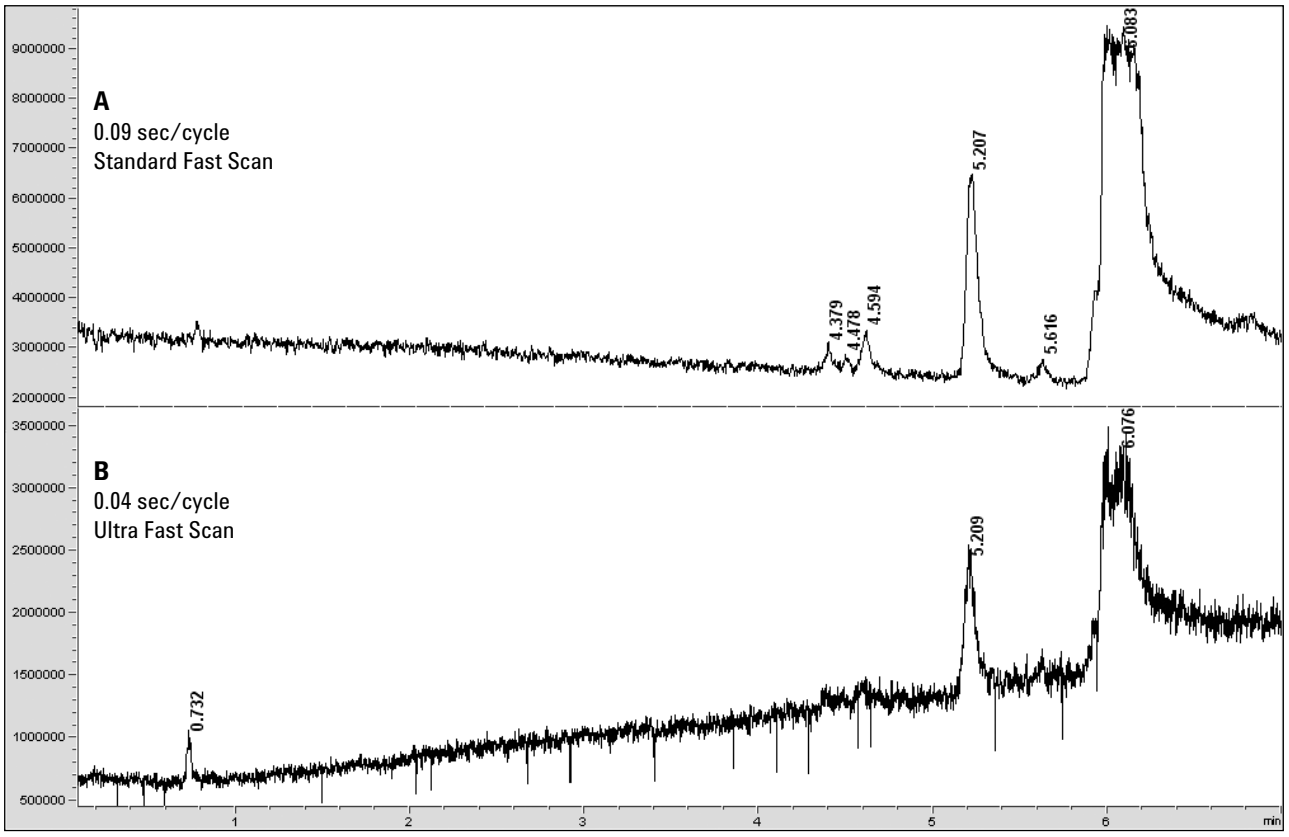


Figure 3. The total ion chromatograms (TICs) corresponding to the highest acquisition speed of the Standard Fast Scan mode (A) and the Ultra Fast Scan mode (B).

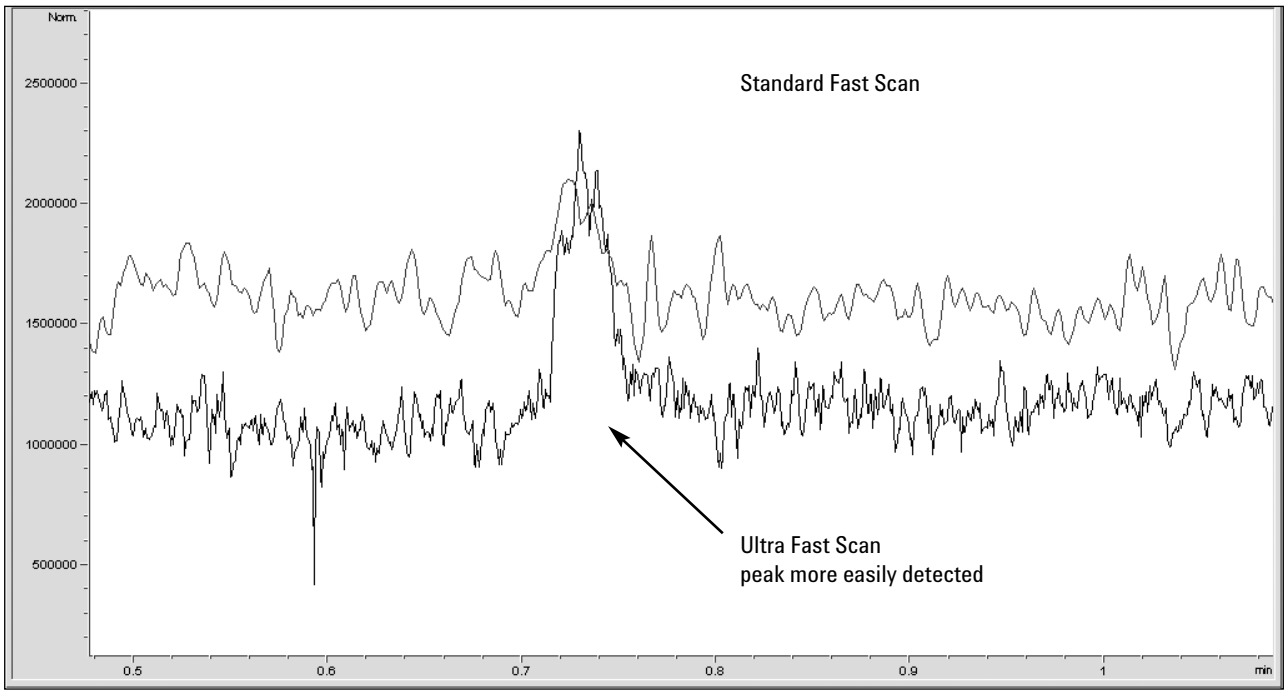


Figure 4. An overlay of the TICs in the expanded region around the peak seen at 0.707 min in the UV chromatogram (Figure 2). The peak is more easily detected in the Ultra Fast Scan mode.

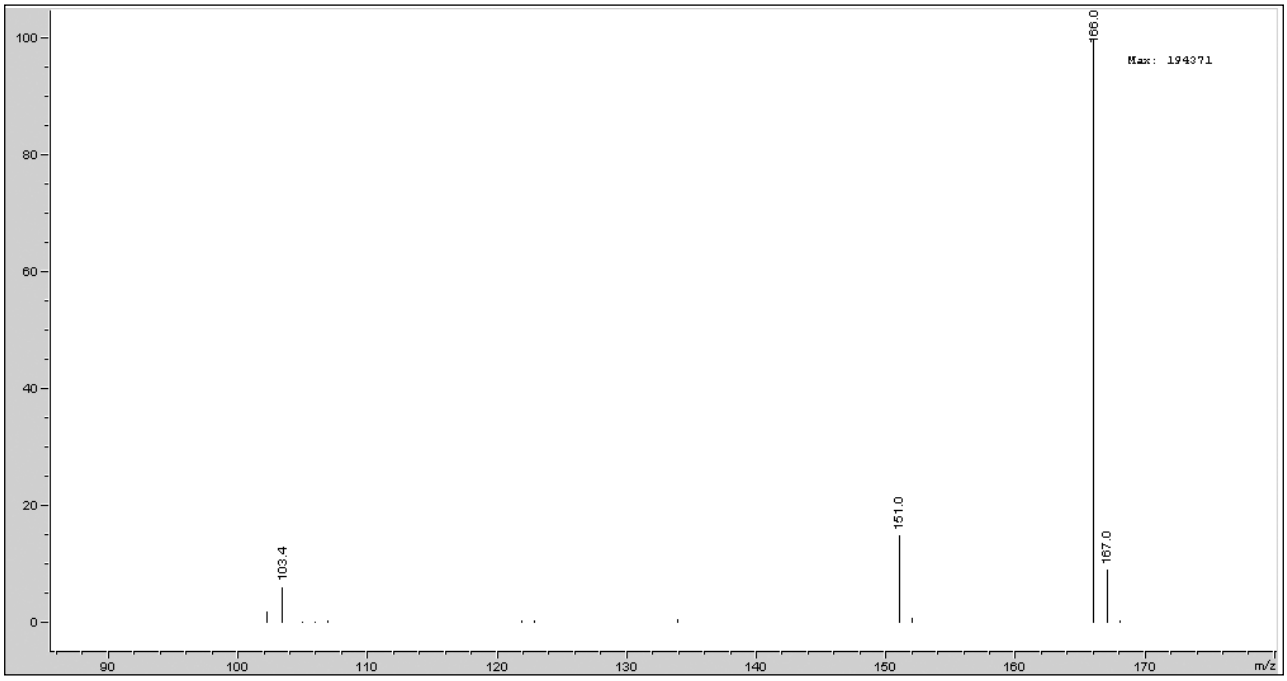
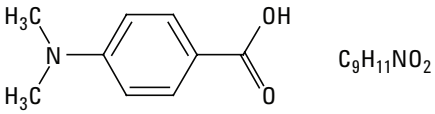


Figure 5. Background subtracted averaged spectrum in Ultra Fast Scan mode of peak at ion chromatogram peak at 0.732 minutes (0.707 minutes in UV).

According to the integration results in Table 1, the peak at 0.707 minutes of the UV chromatogram has a percent relative area of 0.4 % and should be considered an impurity requiring further investigation. A background subtracted spectrum of this



$C_9H_{11}NO_2$

Figure 6. Structure of p-dimethyaminobenzoic acid, which has a protonated ion mass $[M + H]^+$ of 166.0 in positive ion mode using electrospray.

peak is derived from the Ultra Fast Scan mode acquisition and shown in Figure 5.

The *m/z* 166.0 peak clearly dominates the spectrum of Figure 5. A possible structure corresponding to this *m/z* value is shown in Figure 6. This structure corresponds to p-dimethylaminobenzoic acid, which is a known degradate of OD-PABA.

Conclusions

Detection of impurities is enhanced at higher acquisition speeds in mass spectrometry. This work demonstrates the usefulness of the Ultra Fast Scan mode (10,000 amu/sec) in detecting a relatively narrow peak impurity, eluting early (0.707 minutes) in the analysis of the OD-PABA neat standard. The peak, which clearly surpasses the 0.1% area cutoff in the UV chromatogram, is easily detected in the Ultra Fast Scan mode of the Agilent 6140 Single Quadrupole Mass Spectrometer. Upon analysis of the background subtracted averaged spectrum under this peak, an *m/z* 166 ion is clearly observed and believed to be p-dimethylaminobenzoic acid, a known degradate of the OD-PABA compound.

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For more details concerning this application, please contact Michael Zumwalt at Agilent Technologies, Inc.

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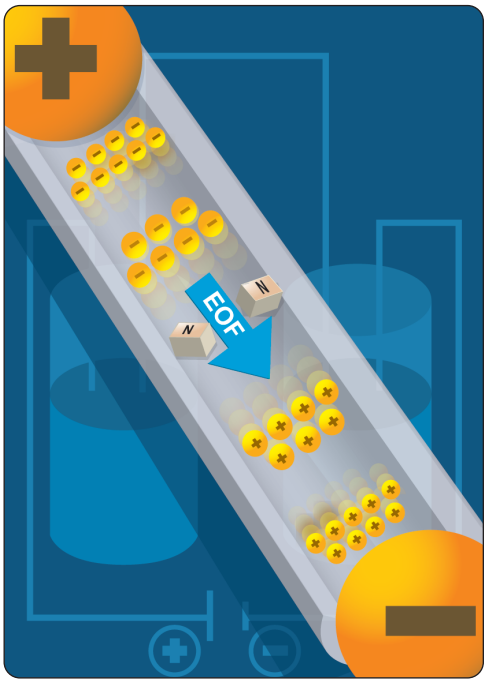
Printed in the USA
January 11, 2008
5989-6953EN

Agilent Technologies

Monitoring of electroless plating baths by capillary electrophoresis

Application Note

Chemical



Authors

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Abstract

Electroless plating is mainly used for the plating of non-metals, for example, ceramics and plastics, and allows the plating of complex shaped parts with a uniform film-thickness. In addition to metal cations, the bath solutions contain additives such as reducing agents (which drive the plating reaction) and organic acids (as buffering and/or metal complexing agents). Inorganic anions are also present as counter-ions of the plating metals. These ions can easily be monitored using capillary electrophoresis (CE) with indirect UV detection.

Agilent Technologies

Experimental

Anion analysis was performed using the Agilent Capillary Electrophoresis system equipped with diode array detection and Agilent ChemStation software. The analysis uses the Agilent Plating Bath Analysis Kit (Agilent part number 5064-8228).

Prior to first use, a new capillary was flushed with run buffer for 15 minutes (at 1 bar). Between analyses the capillary was flushed for 4 minutes from an extra buffer vial into waste. Buffer vials were replaced after 10 runs when using 2 mL-vials and after 5 runs when using 1 mL-vials. Sample preparation consisted simply of dilution with water.

Equipment

- Agilent Capillary Electrophoresis system
- Agilent ChemStation
- Agilent Plating Bath Analysis Kit

Results and discussion

Figure 1 shows the analysis of two different plating baths. Electroless nickel-plating baths contain nickel sulfate or nickel chloride, together with hypophosphite as the reducing agent. Formate, present in the electroless copper-plating bath, is an oxidation product of formaldehyde, which is used as a reducing agent. The assay was linear over the range 10–100 ppm with $r^2 > 0.999$. The method detection limit was 1–2 ppm. For the analysis of the electroless nickel-plating bath repeatability (n = 8) was < 0.1 % RSD for migration times and < 4.5 % RSD for peak area. The assay also allows the analysis of iron (II) and iron (III) in electro-plating with direct UV detection at 230 nm (data not shown).

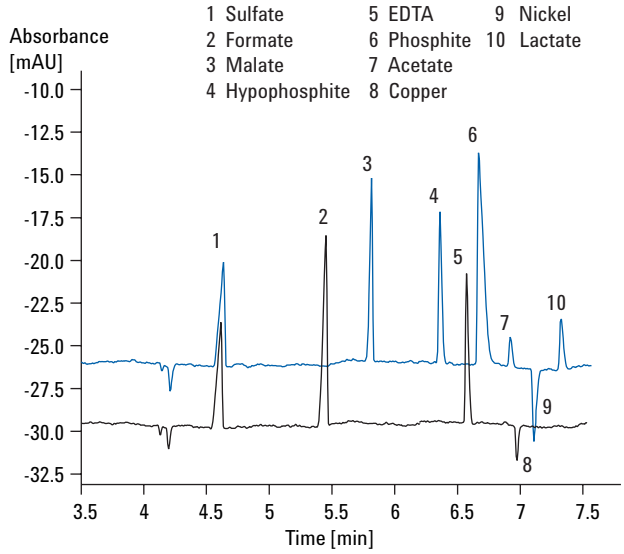

Figure 1
Analysis of electroless nickel- or copper-plating baths.

Chromatographic conditions

Sample:	Electroless nickel-and copper-plating bath, 1:500 diluted with water
Injection:	8 seconds at 50 mbar
Capillary:	Fused silica capillary, total length 80.5 cm, effective length 72 cm, internal diameter 50 µm (Agilent part number G1600-62211)
Buffer:	Agilent Plating Bath Analysis Buffer (Agilent part number 5064-8236)
Voltage:	-25 kV
Temperature:	20 °C
Detection:	Signal 350/20 nm, reference 275/10 nm

In the plating bath industry, the monitoring of additives in bath solutions or waste is essential for quality control, cost saving and environmental concerns. Electroless plating bath samples have presented a number of challenges to ion chromatography. CE, in contrast, allows a quick determination of all major components with only minor sample preparation.

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Published November 1, 2008
Publication Number 5989-9812EN

Agilent Technologies

Analysis of Suspected Flavor and Fragrance Allergens in Perfumes Using Two-Dimensional GC with Independent Column Temperature Control Using an LTM Oven Module

Application Note

Food and Flavors

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Abstract

Several different analytical methods based on GC/MS are used for the determination of flavor and fragrance allergens in raw materials and cosmetic products in accordance with EU Directive 2003/15/EC. For complex perfume samples with possible coelution of target compounds with other solutes, two-dimensional GC with heartcutting is preferred.

In this application note, a multidimensional capillary GC method is presented coupling Deans switch heartcutting with GC/MS and a low thermal mass (LTM) column module for optimal separation and quantitation of regulated allergens in complex samples. The method was applied to a perfume sample containing several regulated allergens. By using an LTM column module, the temperature of the second column could be controlled independently from the primary column in the main GC oven. Allergens were heartcut to the LTM at 50 °C, where they were focused and then later separated in an independent temperature program, resulting in optimum selectivity and better resolution of target compounds from sample matrix.

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Introduction

Recent European regulation requires allergen compounds to be monitored in fragranced products [1]. The target compounds include some common organic compounds such as limonene, citral, and cinnamic aldehyde. These compounds are often detected in natural products but can cause irritation to sensitive skin. According to the regulation, cosmetic products should therefore be labeled if the allergens are present above specified concentrations (10 ppm in "leave-on" and 100 ppm in "rinse-off" products). Consequently, effective methods are needed for qualitative and quantitative determination of the targeted compounds in these complex matrices.

The official target compound list includes 24 compounds. Some of the solutes consist of more than one chemical identity. Citral consists of two isomers: neral (Z citral) and geranial (E citral). Lyral also contains two isomers: (3- and 4-(4-hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde). Farnesol consists of at least four possible isomers, of which the Z,E (farnesol 1) and E,E isomer (farnesol 2) are the predominant compounds observed. In addition, some related compounds, such as phenylacetaldehyde, estragole, methyl 2-nonynoate, and methyleugenol are also monitored [2]. In total, 31 target compounds are analyzed. The list of solutes is given in Table 1 and the first dimension separation is shown in Figure 1.

Table 1. Target Allergen List in Order of Elution on the Agilent J&W HP-5MS Column

Peak number	Compound
1	Limonene
2	Benzyl alcohol
3	Phenyl acetaldehyde
4	Linalool
5	Estragol
6	Methyl 2-octynoate (= folione)
7	Citronellol
8	Neral
9	Geraniol
10	Geranial
11	Cinnamaldehyde
12	Anisyl alcohol
13	Hydroxy citronellal
14	Methyl 2-nonynoate (methyl octane carbonate)
15	Cinnamic alcohol
16	Eugenol
17	Methyleugenol
18	Coumarin
19	Isoeugenol
20	Alpha isomethyl ionone
21	Lilial (BMHCA)
22	Amyl cinnamaldehyde
23	Lyral 1
24	Lyral 2
25	Amyl cinnamyl alcohol
26	Farnesol 1
27	Farnesol 2
28	Hexyl cinnamaldehyde
29	Benzyl benzoate
30	Benzyl salicylate
31	Benzyl cinnamate

The range of matrices in which the target compounds have to be measured is very broad and includes natural essential oils, synthetic mixtures of flavor and fragrance compounds, natural product extracts, and finished products, such as soaps, gels, shower gels, lipsticks, and other cosmetic products. Moreover, the range of concentrations of the fragrance compounds in these matrices is very wide (from high ppb to percent). It is clear that to analyze all target compounds in all classes of matrices using one single method would be impossible. Therefore we have proposed classifying the different matrices into four classes [3]. For each class, dedicated analytical methods have been developed and validated. Direct injection of a diluted sample and analysis by one-dimensional GC/MS either in scan mode [4] or selected ion monitoring (SIM) mode is effective for samples that contain solutes that elute on an apolar column between decane (retention index 1000) and docosane (retention index 2200), providing that the sample complexity and analyte concentration range are not high, and that no nonvolatile matrix compounds are present [2]. One such method was developed using an Agilent J&W HP-5MS (apolar) column. The conditions and corresponding retention time locked information [5] and a complete allergens deconvolution reporting software (DRS) database with peak deconvolution are available from the Agilent Technologies Web site (www.agilent.com).

For highly complex samples (> 100 solutes) containing only volatile and semivolatile solutes, or for samples with a very broad concentration range of components (for example: very low concentrations of target compounds in a very high concentration of matrix compounds), a single-dimension GC separation is not effective. For these, the added power of two-dimensional capillary GC (GC/GC, 2D GC) has been shown to be helpful [3]. Using multiple heartcuts from a primary apolar column, target compounds can be isolated and resolved from interfering sample components on a polar secondary column, making accurate quantification possible even in cases where MS deconvolution of one-dimensional GC/MS data fails.

In this paper, the application of capillary flow technology Deans switching is demonstrated for the 2D GC analysis of a complex perfume sample. For even more method flexibility and separation power, the second-dimension column was housed in a low thermal mass (LTM) oven module for independent control of the column temperature. With this configuration, multiple heartcuts could be focused on the cooler secondary column and then released with an independent temperature program, which could be independently optimized for best separation of target compounds from complex sample matrix.

Experimental

The perfume sample was diluted to 5% (50 mg/mL) in acetone. Standard solutions were prepared from pure compounds at 100 ng/µL in acetone.

The analyses were performed on a 7890A GC/5975 MSD combination. The GC was equipped with an SSL inlet, FID detector, a capillary flow technologies based Deans switching system (p/n G2855B), a PCM flow module (option #309), and an LTM system controller bundle (p/n G6579A).

As illustrated in Figure 2, the primary column was installed in the GC oven and configured from the split/splitless inlet to the Deans switch. "Long leads" were requested when ordering the column for the LTM so that the inlet end could be connected directly to the Deans switch. The outlet of the column was cut close to the column module and connected to the MSD via uncoated but deactivated fused silica (FS) tubing using an Agilent Ultimate Union (p/n G3182-61580). This configuration results in better method translation of conditions than when the long lead is left on the outlet end of the column because this 1 m extends into the GC column oven and becomes an isothermal (third) separation zone that broadens peaks and can alter the relative retention and resolution achieved at the exit of the LTM module. A restrictor (uncoated but deactivated retention gap) was also connected between the second output of the Deans switch and a monitoring FID. The conditions are summarized in Table 2.

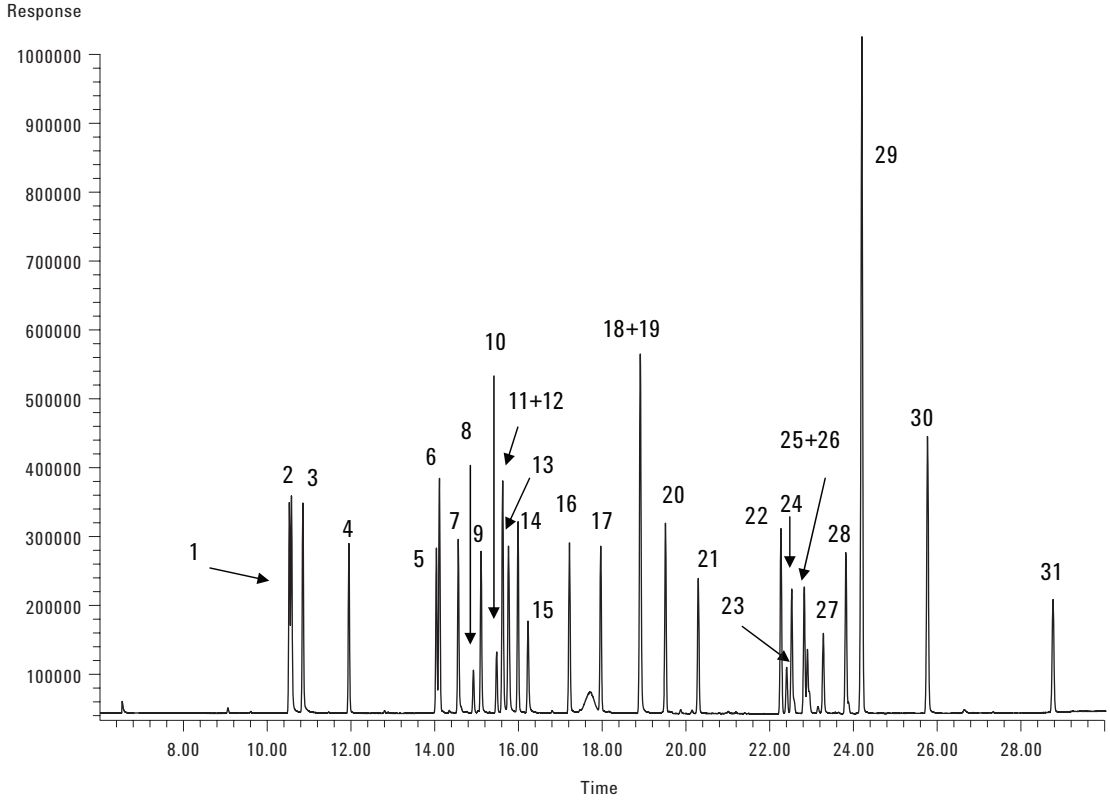

Figure 1. Separation of flavor and fragrance allergen test mixture (100 ppm) on the first dimension column (Agilent J&W HP-5MS) and FID detection. Peak identification is given in Table 1.

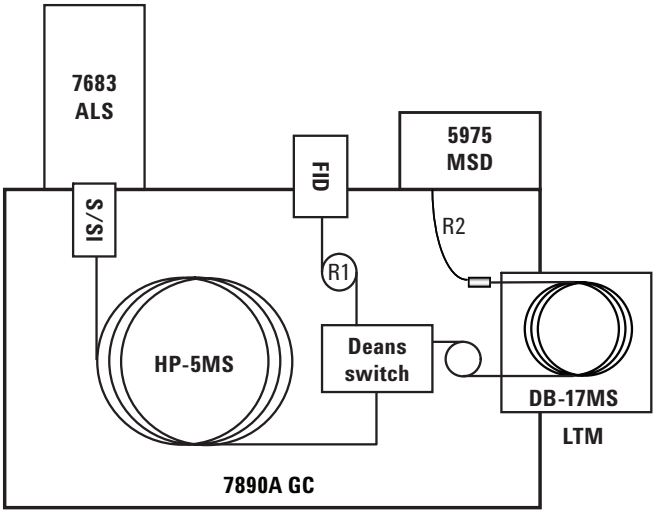

Figure 2. System configuration.

Results and Discussion

First, a standard mixture containing all target compounds at 100 ng/µL was analyzed. No heartcutting was used. The resulting chromatogram from the separation on the J&W HP-5MS column on the monitor FID is given in Figure 2. A good separation was obtained. Some coeluting pairs can effectively be resolved by mass spectral deconvolution (specific ions), as is done with DRS methods.

Next, the perfume sample was run under the same conditions. The chromatogram from the monitor FID detector shown in Figure 3A shows that the perfume is very complex, making determination of target compounds difficult. Some target solutes, such as linalool (peak 4) and alpha-isomethyl ionone (peak 20) are clearly resolved and can be determined. However, the elution window between 22 and 24.5 min, is quite complex. In this window, several target allergens elute,

Table 2. Analytical Conditions

Injection	1.0 µL
Inlet	S/SI, 250 °C, split ratio = 1:25
Column 1 (Carrier gas = He)	30 m × 0.25 mm id × 0.25 µm Agilent J&W HP-5MS, p/n 19091S-433 Flow = 1.4 mL/min; constant flow mode (185 kPa at 50 °C) Inlet = SSL; outlet = PCM1
Column 2 (LTM) (Carrier gas = He) Flow (PCM1)	30 m × 0.25 mm id × 0.25 µm Agilent J&W DB-17ms, p/n 122-4732LTM with "long leads" (1 m at each end not wrapped) 2 mL/min constant flow mode (120 kPa at 50 °C) for first experiment, 120 kPa (1 min) → 256 kPa (28 min) at 4.35 kPa/min for second experiment
Restrictors	R1 = 63 cm × 100 µm id deactivated FS (cut from, for example, p/n 160-1010-5) R2 = 1 m × 250 µm id deactivated FS (p/n 160-2255-1)
GC oven temperature	50 °C (1 min) → 300 °C (27.75 min) at 8 °C/min Total run time = 60 min
LTM oven	50°C (25 min, after last heartcut) → 250 °C (1 min) at 6 °C/min (Total run time = 60 min)
FID monitor detector	300 °C, 30 mL/min H_2, 400 mL/min air
Deans switch heartcutting	Initially OFF Cut 1: ON at 10.2 min, OFF at 11.0 min Cut 2: ON at 15.3 min, OFF at 16.4 min Cut 3: ON at 22.0 min, OFF at 24.5 min
MS data acquisition	Autotune, scan mode, 41–300 u, samples = 2^2
MSD transfer line	300 °C
MS solvent delay	5 min
MS temperatures	Source = 300 °C, quad = 150 °C

including amyl cinnamaldehyde, lyral (two isomers), amyl cinnamyl alcohol (with a related impurity), farnesol (two isomers), hexyl cinnamaldehyde, and benzyl benzoate. Within the same window, interfering perfume constituents such as methyl dihydrojasmonate, ionones, and sesquiterpenes elute. Most of these have mass spectra with strong fragmentation, resulting in many nonspecific low mass ions, interfering significantly with target ion spectra and ion ratios. Traditional selective detection and quantification using SIM data or deconvolved scan data from DRS that are effective with simpler samples would therefore be problematic with this sample.

For example, confirming the presence of lyral in this sample was difficult with the simpler approach. With GC-SIM-MS, it was not possible to accurately quantify lyral, and its qualifier ions did not fall within the specified range. Review of the scan data clearly showed the presence of coeluting interferences.

Next, the sample was rerun with three heartcuts, including the problematic region between 22 and 24.5 minutes, which were heartcut to the second column. Propylene glycol, used as "keeper" in some perfumes, is a potential interferent in the first window that contains limonene, benzylalcohol, and phenylacetaldehyde. Quantification and identification of hydroxycitronellal in the second heartcut window is another component that, in the presence of interferences, is sometimes problematic to quantify using standard methods. The chromatogram obtained on the monitor detector is shown in Figure 3B, wherein the three heartcut windows show up as flat sections in the baseline.

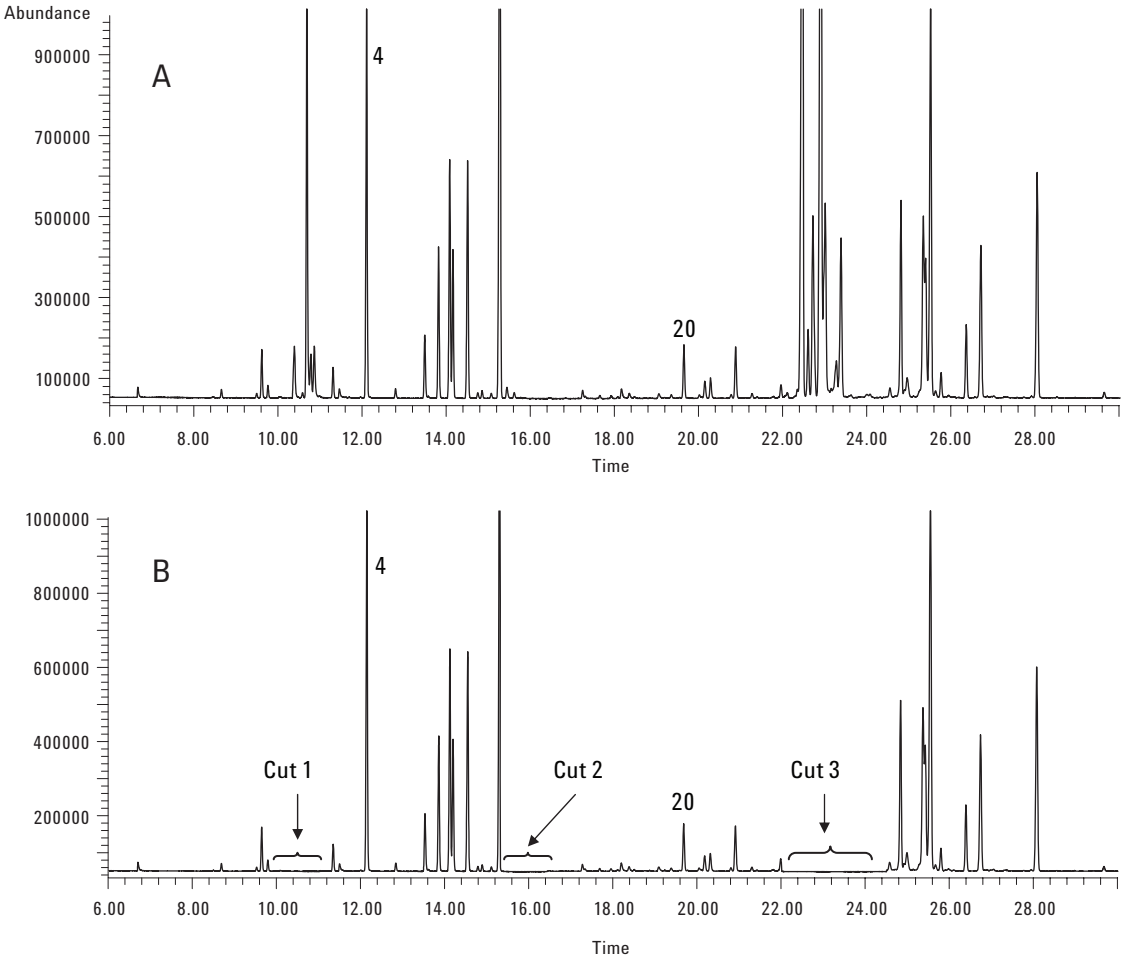

Figure 3. A) Separation of a perfume sample on the first-dimension column (Agilent J&W HP-5MS) using FID detection without heartcutting. Peaks: 4. Linalool; 20. Alpha-isomethyl ionone. B) Separation of a perfume sample on the first-dimension column (Agilent J&W HP-5MS) using FID detection with heartcutting (fractions: 10.2–11.0, 15.3–16.4, and 22.0–24.5 min).

The TIC chromatogram obtained after separation on the second-dimension column of the lyral fraction (heartcut 3) is shown in Figure 4A. First the analysis was performed using the same temperature program for the second column as for the first column (LTM program = 7890A oven program), emulating what would happen if the secondary column were housed in the GC oven (traditional configuration 2D GC). At least eight peaks were detected. The lyral isomers elute at 25.4 and 25.5 minutes. The second isomer, however, coelutes with another solute, and confirmation and quantification are not possible. The elution temperature of the lyral isomers in this case was around 240 °C. Both retention and selectivity at this temperature are low.

The experiment was repeated, this time with the J&W DB-17ms secondary column kept at 50 °C until the last heartcut was completed, and then the temperature was increased (at 6 °C/minute). Using this approach, the solutes are first focused at the head of the LTM column, and then elute at lower temperature (200 °C) during the temperature ramp, allowing both retention and selectivity to play more important roles. An added benefit is that the peak widths are narrowed due to the focusing, which improves peak resolution. Under these conditions, the isomers elute at 49.25 and 49.4 minutes and can be quantified without interference. The chromatogram of heartcut fraction 3 (22 to 24.5 minutes from column 1) is shown in Figure 4B. In contrast to Figure 4A, at least 20 peaks spanning a wide concentration range are clearly resolved. The presence of lyral isomers in the sample could thereby be confirmed and accurately quantified.

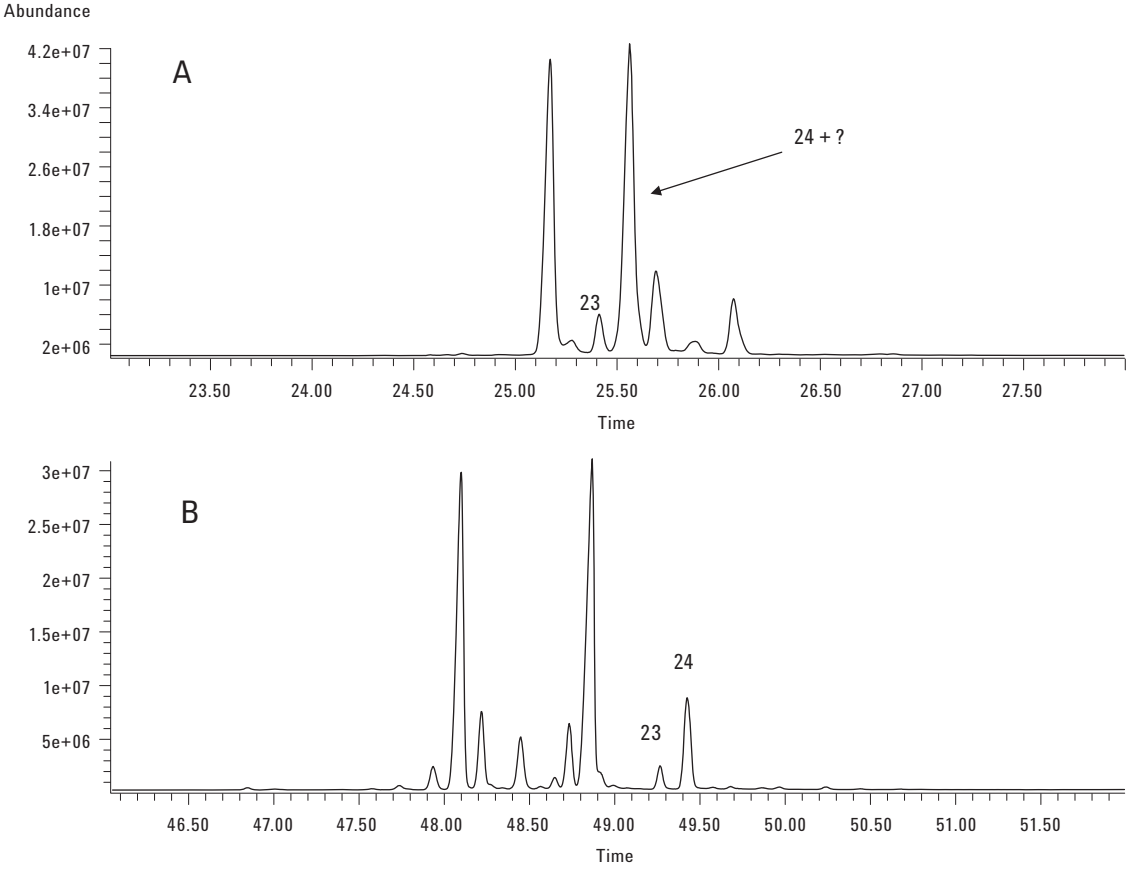

Figure 4. A) Separation of fraction 3 on the second-dimension column (Agilent J&W DB-17ms) using MS detection. Column 1 temperature = column 2 temperature: 50 °C (1 min) → 270 °C at 8 °C/min. Peaks: 23. Lyral 1; 24. Lyral 2. B) Separation of fraction 3 on the second-dimension column (Agilent J&W DB-17ms) using MS detection. Column 2 temperature: 50 °C (25 min) → 250 °C at 6 °C/min. Peaks: 23. Lyral 1; 24. Lyral 2.

By comparing the chromatograms in Figure 4, it is obvious that the independent temperature control of the second column in a 2D GC greatly increases the ability to optimize selectivity and resolution. This point was also demonstrated in the analysis of enantiomers using a chiral second-dimension column [6].

In addition to perfume samples, the approach presented herein can also be used for the determination of flavor and fragrance allergens in finished products. In these applications, any nonvolatile or late-eluting matrix compounds could be backflushed from the first-dimension column, as discussed in a manner similar to that described in an earlier application note [7].

Conclusions

Two-dimensional GC using Deans switch heartcutting in combination with MS can be used for the determination of flavor and fragrance allergens in complex perfume and cosmetic samples. Using LTM technology, the second dimension column temperature can be optimized independently from the primary column, resulting in better selectivity and resolution of target solutes from matrix interferences. Addition of an LTM module is more cost-effective, less cumbersome to configure, and takes up less space than if using a second GC as the independent zone.

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Printed in the USA
February 12, 2009
5990-3576EN

Agilent Technologies

Achieving Lower Detection Limits Easily with the Agilent Multimode Inlet (MMI)

Application Note

All Industries

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Abstract

This application note discusses three injection techniques: hot splitless, cold splitless, and solvent vent mode available on the Multimode Inlet. The cold splitless and solvent vent mode injections allow analysts to achieve a lower detection limit by making large volume injections (LVI). A total ion chromatogram overlay of 40-ppb pesticide standards from 2-µL hot splitless, 10-µL cold splitless and 25-µL solvent vent illustrates the improvement in signal-to-noise ratios using LVI.

Agilent Technologies

Introduction

A growing number of analysts are exploring large volume injection (LVI) techniques to improve existing analyses. With traditional liquid injection techniques in capillary gas chromatography, most inlets and columns can only handle 1 – 2 µL at a time. Attempts to increase the injection volume can lead to broadened and distorted analyte peaks, large and long solvent peak tails, and saturated or damaged detectors.

The purpose of increasing the injection volume is normally to improve detection limits in trace analysis. By introducing more of the sample to the system, the mass of analyte reaching the detector will be proportionally increased, resulting in larger peak areas and peak heights. If the baseline noise is constant, larger peak heights mean greater signal to noise ratios and lower system detection limits. An additional benefit of LVI is the ability to reduce the amount of sample originally processed. By injecting 10 – 100 times more volume of processed sample and concentrating it in the inlet, the sample preparation can start with 10 – 100 times smaller sample volume and still achieve the same mass of analyte on column. Another advantage of using LVI (solvent vent) is the decrease in solvent that actually reaches the detector. Usually, only 10 – 30% of the injection solvent actually enters the column and makes it to the detector.

LVI can be applied to injection volumes ranging from a few microliters up to 1 mL or more. In most LVI approaches, the sample solvent is evaporated and removed from the inlet system before the analytes are transferred to the separation column. In this way, LVI is similar to nitrogen evaporation or rotary evaporation of the solvent, with the added benefit of being performed in the GC inlet rather than in a fume hood. Analytes that would be lost during nitrogen evaporation may be retained in the inlet and successfully analyzed via LVI. Furthermore, the LVI process can be automated and is reproducible. As in the other evaporation techniques, the LVI approach is a function of the solvent type, the inlet temperature, the vent flow of evaporation gas, and the analyte boiling point. In addition, the inlet pressure during evaporation and the inlet liner have an impact on the rate of solvent removal and analyte recovery. These parameters will be discussed in this application note.

Experimental

MMI Operational Modes

The Agilent Multimode Inlet (MMI) uses the same liners and consumables as a standard split/splitless inlet, making it compatible with existing hot split and splitless methods. Its operational modes include: Hot Split/Splitless (also in pulsed mode), Cold Split/Splitless (also in pulsed mode), Solvent Vent and Direct mode.

Hot Splitless (for 1 – 3 µL injections)

For most analysts considering LVI, their current methods are using hot splitless injection. This proven and reliable sample introduction technique has worked well for almost 40 years; however, it does present some challenges to the sample integrity and to the method developer. First, the inlet must be hot enough to flash vaporize the solvent and analytes so that the resulting vapor cloud can be transferred to the column. The inlet liner volume must be sufficiently large to contain this vapor cloud. If the liner volume is too small, the vaporized sample can overflow the liner and reach reactive surfaces, leading to analyte loss. In addition, the pressure wave generated by the vaporized sample can push back against the incoming carrier gas and enter sensitive pressure and flow control systems. Using the Agilent pressure/flow calculator [1], a 1-µL injection of acetone into an inlet at 240 °C and 14.5 psig expands to 288 µL of gas. Most inlet liners for standard split/splitless inlets have a nominal volume of 1 mL. An increase of injection volume to only 3.5 µL under these conditions creates a vapor cloud of 1 mL which could easily overflow the inlet liner.

Hot splitless injection also creates a challenging environment for thermally unstable or labile analytes. Compounds such as the organochlorine pesticides DDT and endrin can rearrange to form breakdown compounds. This process is accelerated with the inlet temperatures normally used to analyze them. Effective chemical deactivation of the liner can minimize analyte breakdown. However, high inlet temperatures can decrease the lifetime of deactivated liners.

Another challenge created by hot splitless injection is the opportunity for needle fractionation or analyte discrimination. The needle temperature increases as the sample is being transferred from the syringe to the inlet because the needle is in contact with the septum. The rise in needle temperature can cause the solvent to "boil" away and deposit high boiling analytes inside the needle. To avoid this fractionation problem, some analysts load a solvent plug into the syringe first and then draw up the desired sample volume (available in 7693A Automatic Liquid Sampler). The thought is that the solvent plug will wash any deposits into the inlet. An effective way to address this problem is to make a high speed injection. This minimizes the time the needle is in contact with the septum and the time the sample touches the needle. Even with these issues, hot splitless injection is a well-accepted technique. An alternative technique, such as cold splitless can address these concerns and improve the analysis results.

Cold Splitless (for 1 – 10 µL injections)

MMI's versatile temperature programmability allows it to perform cold split and splitless analyses. In cold splitless mode, the MMI is cooled to a temperature below the normal boiling point of the sample solvent so that when the sample is injected, no vaporization takes place. The injection is simply a liquid transfer from the syringe to the inlet. Once the syringe is removed from the inlet, the inlet is heated to vaporize the sample and transfer it to the column. The solvent vaporizes first and moves to column, allowing analyte focusing to take place as in normal hot splitless injections. The analytes subsequently vaporize and move to the column. The main advantage is that the analytes vaporize at the lowest possible inlet temperature, rather than at a constant high temperature. This minimizes thermal degradation while still allowing a wide range of analytes to vaporize. Cold splitless operations also do not thermally stress the liner as harshly as hot splitless does, prolonging its usable life. Cold splitless can also extend the amount of sample that can be injected in some cases. If a slow inlet temperature program is used, the solvent can be vaporized slowly and will not overflow the liner volume. As long as the analytes can be refocused on the column, slow inlet temperature programs cause no detrimental effects to the chromatography.

Solvent Vent (for 5 – 1000 µL injections)

The solvent vent mode is the method which enables MMI to do LVI of more than 5 µL. In solvent vent mode, the inlet is kept at a low initial temperature during sample injection. Pneumatically, the inlet is in split mode with a low inlet pressure. The flow of gas through the inlet liner and out to vent removes the evaporating solvent. The sample is injected slowly so that the incoming liquid is deposited on the liner wall and the solvent evaporates at a similar rate. Once the entire sample has been injected, the inlet switches to a splitless mode for analyte transfer. The inlet is then heated to vaporize the concentrated sample and any remaining solvent and the vapor is transferred to the column. After a sufficient period to ensure the sample transfer, the inlet is then switched to a purge mode to allow any remaining material in the inlet liner to be vented. During the sample injection and solvent venting period, the GC oven has been held at an appropriate temperature to allow the solvent to refocus the analytes on the column. When this refocusing is complete, the oven is then programmed to perform the separation.

LVI Method Development

An effective procedure for developing an LVI method on a MMI is to run the existing method first to determine peak areas for a small volume injection. Such results serve as a baseline for evaluating the LVI method performance. The next step is to switch to the solvent vent mode with a slightly larger injection volume (for example, 2 to 5 times larger). By comparing the resulting peak areas and accounting for the increased injection volume, the analyte recovery can be calculated and conditions can be further optimized.

Backflush

A traditional bakeout step for removing late eluters can be very time consuming for samples with complicated matrices, even as long as the analysis time. Capillary flow devices (in this case, a purged ultimate union) provide backflush [2, 3] capability. "Backflush" is a term used for the reversal of flow through a column such that sample components in the column are forced back out the inlet end of the column. By reversing column flow immediately after the last compound of interest has eluted, the long bake-out time for highly retained components can be eliminated. Therefore, the column bleed and ghost peaks are minimized, the column will last longer, and the MS ion source will require less frequent cleaning. The split vent trap may require replacement more frequently than usual.

Instrument Parameters

GC	Agilent 7890A
MS	Agilent 5975C MSD
Column	HP-5MS UI, 15 m × 0.25 mm × 0.25 µm (19091S-431UI), from inlet to purged union
MMI	Constant pressure (~18 psi), chlorpyrifos-methyl RT locked to 8.297 min, 2 psi at post run for backflush
MMI liner	Double taper deactivated, Helix (5188-5398)
Septum purge	3 mL/min
Purged Union	4 psi; 70 psi at post run for backflush
Restrictor	0.7 m × 0.15 mm deactivated fused silica tubing (from purged union to MSD)
Syringes	10 µL, for splitless injections (5181-3354) 50 µL, for solvent vent mode (5183-0318)
ALS	Agilent 7693A
MS parameters	
Solvent delay	2.5 min
Gain factor	1
Mass range	44–550
Threshold	0
Samples	2
Tune file	atune.u

Oven

Initial temperature	70 °C
Initial hold time	1 min
Rate 1	50 °C/min
Temperature 1	150 °C
Hold time	0 min
Rate 2	6 °C/min
Temperature 2	200 °C
Hold time	0 min
Rate 3	16 °C/min
Temperature 3	280 °C
Hold time	5 min
Total runtime	20.933 min
Post run	5 min (for backflush)
Oven post run temp	280 °C

Sample: 40-ppb pesticide standards in acetone (for a list of compounds, see Figure 5).

Multimode Inlet (MMI)

Parameter	Hot Splitless	Cold Splitless	Solvent Vent
Initial temperature	280 °C	30 °C	35 °C
Initial time	–	0.01 min	0.35 min
Rate 1	–	700 C/min	700 °C/min
Final temperature	–	320 °C	320 °C
Vent flow	–	–	150 mL/min
Vent pressure	–	–	5 psig
Vent time	–	–	0.33 min (from
	–	–	calculator, Figure 3)
Purge time	0.75 min	1.25 min	1.5 min
Purge flow	50 mL/min	50 mL/min	50 mL/min
Injection volume	2 µL	10 µL	25 µL
Injection speed	Fast	Fast	75 µL/min (from
	–	–	calculator, Figure 3)
Cryo	–	On (liquid CO_2)	On (liquid CO_2)
Cryo fault detection	–	On	On
Cryo use temperature	–	125 °C	125 °C
Time out detection	–	On (15 min)	On (15 min)

The parameters for the 25-µL Solvent Vent injection were determined with the Solvent Elimination Calculator integrated in the ChemStation. This calculator was designed to help determine reasonable starting conditions for LVI methods. When the MMI is put into the PTV Solvent Vent mode, an additional button appears in the inlet screen, shown in Figure 1.

In the first screen of the Solvent Elimination Calculator (Figure 2), the sample solvent and desired injection volume are selected and entered. The calculator "knows" the syringe currently installed and will only allow 50% of that volume to be injected at once. Larger injection volumes can be entered into the calculator but the injection volume will not be downloadable. The calculator also requests the boiling point of the earliest eluting analyte, as this allows the initial inlet temperature to be selected. If the boiling point is unknown, the temperature should be left at 150 °C as this will work for a wide range of analytes.

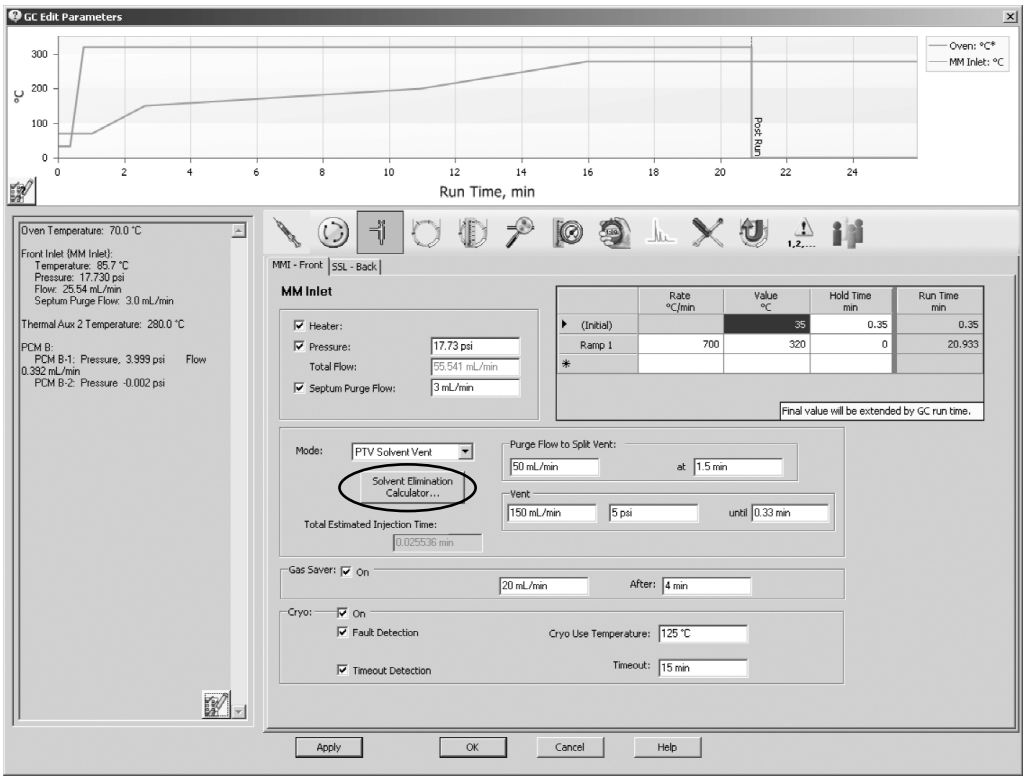

Figure 1. Multimode Inlet "Solvent Elimination Calculator" imbedded in ChemStation for easy method development.

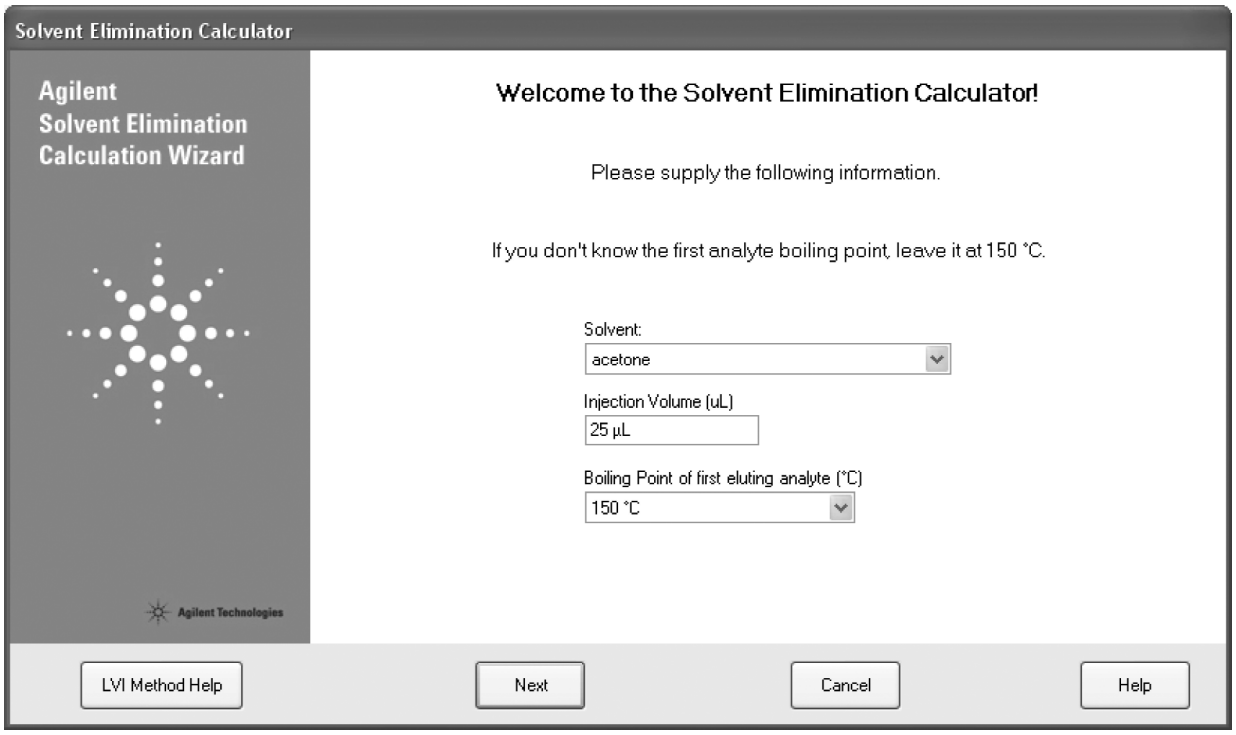

Figure 2. Select solvent of choice and enter the injection volume to start the calculation.

Figure 3 shows the calculation screen. The calculator uses an initial set of inlet conditions to determine the solvent elimination rate according to fundamental theory [4]. This "Elimination Rate" does not account for other factors (for example, local cooling due to solvent evaporation) specific to LVI and is normally faster than that determined from practical experience. The "Suggested Injection Rate" does consider these factors and is designed to leave a small amount of solvent in the liner at the end of the venting period. This solvent serves as a liquid "trap" for the more volatile analytes and promotes their recovery. The "Suggested Vent Time" is determined by dividing the injection volume by the "Suggested Injection Rate."

Several variables for determining elimination rate can be set by the user in the lower portion of the window. A small change in inlet temperature has a significant impact on elimination rate. Vent flow has a linear effect such that a decrease by a factor of two in vent flow gives an equal decrease in elimination rate. As the vent pressure decreases, the elimination rate increases. Bear in mind that the vent pressure also impacts the amount of solvent that reaches the column during venting. As the vent pressure is increased, more solvent is loaded onto the column before the analytes are transferred. Finally, the type of solvent, specifically its normal boiling point, has a substantial impact on the elimination rate.

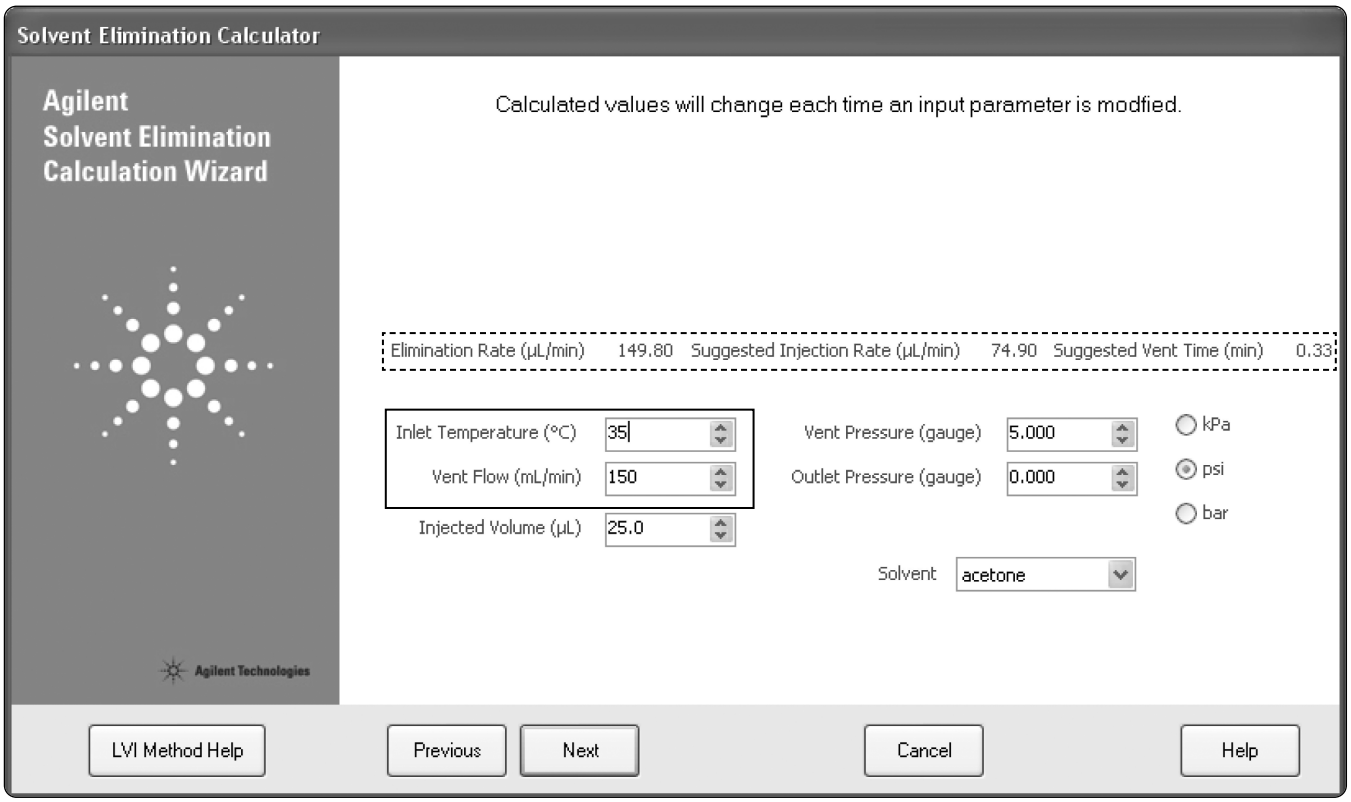

Figure 3. The calculator calculates the injection rate and vent time according to the selected inlet temperature and vent flow.

The download screen in Figure 4 shows all of the method changes that are downloaded to the edit parameters screen. The check boxes allow the user to accept (by checking) or reject any of these parameters. The oven initial temperature and hold times are not automatically checked in case the current method requires these values to be unchanged (for example, a Retention Time Locked method).

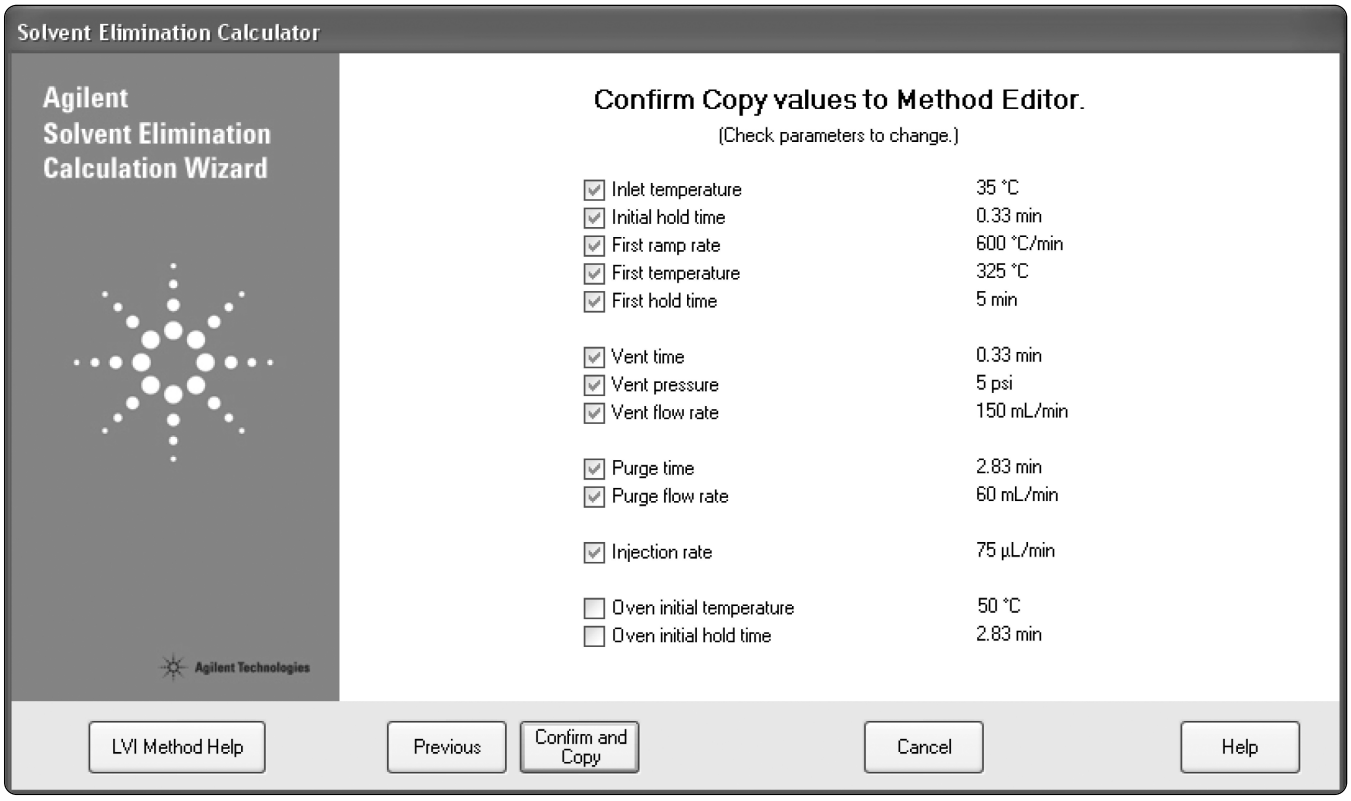

Figure 4. Confirm values suggested by the Calculator and download to ChemStation.

Results and Discussion

Figure 5 compares the responses of a 40-ppb standard solution from three injection modes.

The bottom total ion chromatogram (TIC) is a typical 2-µL hot splitless injection. Some of the 40-ppb pesticides are barely visible (80 pg each on column). The middle TIC is from a 10-µL cold splitless injection. The MMI starting temperature was 30 °C. In this TIC, the on column amount for each analyte is 400 pg. Lastly, the top TIC is from a 25-µL solvent vent injection with MMI starting temperature at 35 °C. In this TIC, the signal-to-noise ratio is significantly better than the TIC from hot splitless injection (bottom TIC), as noted in the Introduction section. The peak shape and resolution are maintained, even with the 25-µL injection volume. This implies that the solvent was mostly eliminated during the injection.

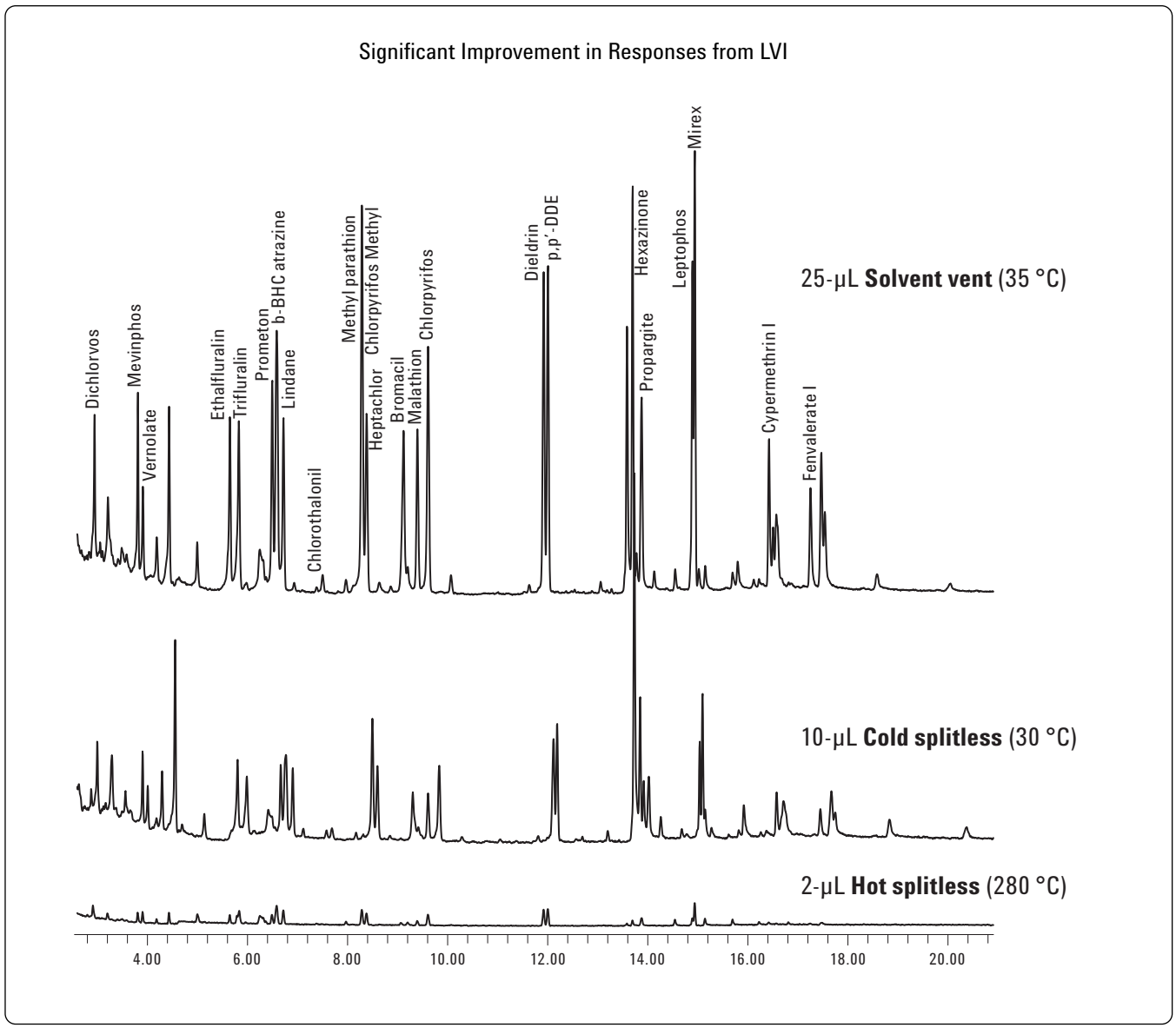

Figure 5. Overlay of total ion chromatograms (TICs) from three injection modes, plotted on the same scale.

Conclusion

The new Agilent Multimode Inlet (MMI) has the same form factor and uses the same consumables (for example, liners, o-rings and septa) as the existing split/splitless inlet, allowing existing hot splitless methods to be replicated. In addition, the temperature programmability permits both cold splitless and large volume injection (LVI) methods for improved detection limits. An integrated Solvent Elimination Calculator provides a complete set of initial conditions for easy LVI method development. The application results show a significant signal-to-noise improvement (lower detection limits) comparing the 25-µL solvent vent injection to the 2-µL hot splitless injection.

References

1. Agilent Pressure/Flow Calculator Included in the Instrument Utility DVD, available with each gas chromatograph and MMI accessory kit.
2. Chin-Kai Meng, "Improving Productivity and Extending Column Life with Backflush, "Agilent Technologies publication, 5989-6018EN, December 2006.
3. Matthew Klee, "Simplified Backflush Using Agilent 6890 GC Post Run Command," Agilent Technologies publication, 5989-5111EN, June 2006.
4. J. Stanieski and J. Rijks, *Journal of Chromatography* 623 (1992) 105-113.

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Printed in the USA
June 18, 2009
5990-4169EN

Agilent Technologies

Screening Impurities in Fine Chemicals Using the Agilent 1290 Infinity LC System

Application Note

Fine Chemical

Authors

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Abstract

The Agilent 1290 Infinity LC System with ultra violet/visible (UV/VIS) Diode Array detection (DAD) is used to analyze octyl-dimethyl-p-aminobenzoic acid for the presence of impurities. The system is used for the chromatographic separation of the compound from its impurities on 3.0 and 2.1 mm id C18 columns, of various lengths, with 1.8-um packing materials prepared in 600-bar (9000 psi) or special 1200-bar (18,000 psi) configurations. The ability of the 1290 Infinity LC System to operate with long, high resolution columns under conditions of rapid analysis is demonstrated with low viscosity acetonitrile (ACN) and higher viscosity methanol (MeOH) solvent conditions.

Agilent Technologies

Introduction

The analysis of impurities in starting materials, intermediates and finished products intended for a wide range of final uses is essential for ensuring product quality, performance, and consumer safety. The general conditions for successful analysis of impurities by high-performance liquid chromatography (HPLC) include gradient elution and multi-wavelength monitoring of the overall separation and may benefit from other detectors including evaporative light scattering (ELSD) and mass spectrometers (MS). Because impurity determination is the primary goal, one needs to ensure that mobile phase, vials, and HPLC components are free of minor impurities that might lead to confusing results during the analysis. Careful preparation of diluent blanks and blanks that might represent contamination sources due to additional sample preparation, such as filtration, are also appropriate. The analysis sequence is likely to include runs of the production material, solvent or diluent blank runs. It is also typical to include limit standards prepared by diluting the primary component to the lowest level where detection of impurities might be required. Finally, it is generally essential to include an authentic high purity reference standard.

Para-aminobenzoic acid (PABA) has historically been used as an ultra-violet filter ingredient in sunscreen formulations. As its use can increase the risk of skin cancer a derivative in the form of octyl-dimethyl-p-aminobenzoic acid (OD-PABA), is currently and more commonly used. However, PABA may be formed as a degradate of OD-PABA, so it is important to monitor its potential presence in samples of OD-PABA. As a commercial product, the purity of OD-PABA is important to manufacturers, for the purposes of safety and economics. In this work we investigate the capability of the Agilent 1290 Infinity LC system (UHPLC system with 1200 bar pressure limit) to detect impurities in OD-PABA samples with UV/VIS Diode Array detection.

The structure of the OD-PABA compound analyzed in this work is shown in Figure 1.

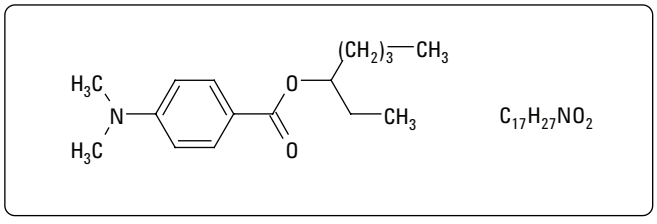

Figure 1. Octyl-dimethyl-p-aminobenzoic acid (OD-PABA).

Experimental

Sample Preparation

The primary OD-PABA solution was prepared at a concentration of 1 mg/mL in 2-propanol and subsequently diluted to lower concentrations as needed. Injection volumes of 0.2–2 µL were made into the LC/DAD system.

LC Method Details

LC Conditions

Agilent 1290 Infinity LC system binary pump G4220A,
Agilent 1290 Infinity LC system autosampler G4226A
Agilent Thermostatted Column Compartment G1316C with switching valve
Agilent 1290 Infinity system diode array UV/VIS detector G4212A with 10 mm path fiber optic flow cell

Columns:	(See individual figures for specific usage) Agilent ZORBAX SB-C18 RRHT, 3 mm × 50 mm, 1.8 µm 600 bar p/n 827975-302 Agilent ZORBAX SB-C18 RRHD, 2.1 mm × 100 mm, 1.8 µm 1200 bar, p/n 858700-902 Agilent ZORBAX SB-C18 RRHD, 2.1 mm × 150 mm, 1.8 µm 1200 bar, p/n 859700-902
Column temp:	40 °C
Mobile phase:	A = HPLC grade water B = Acetonitrile (ACN) or methanol (MeOH) (See individual figures)
Flow rate:	See individual figures
Gradient:	Gradient: the gradient conditions were either 40% to 90% ACN or 50% to 100% MeOH. The gradient slope was maintained at 3.5% organic phase increase per column volume, altering gradient time and flow rate accordingly. This is based on calculations using a modification of the Agilent Method Translator. [1]

UV Conditions

Monitoring 210, 254, 280 and 320 nm, bandwidth 4 nm, reference wavelength off

Results and Discussion

The UV response of OD-PABA, with four wavelengths monitored, is shown at a retention time of 2 min in Figure 2. Multi-wavelength monitoring of the separation provides a simple way to account for multiple impurities and assist in the selection of a final wavelength condition that can maximize sensitivity for all detected analytes.

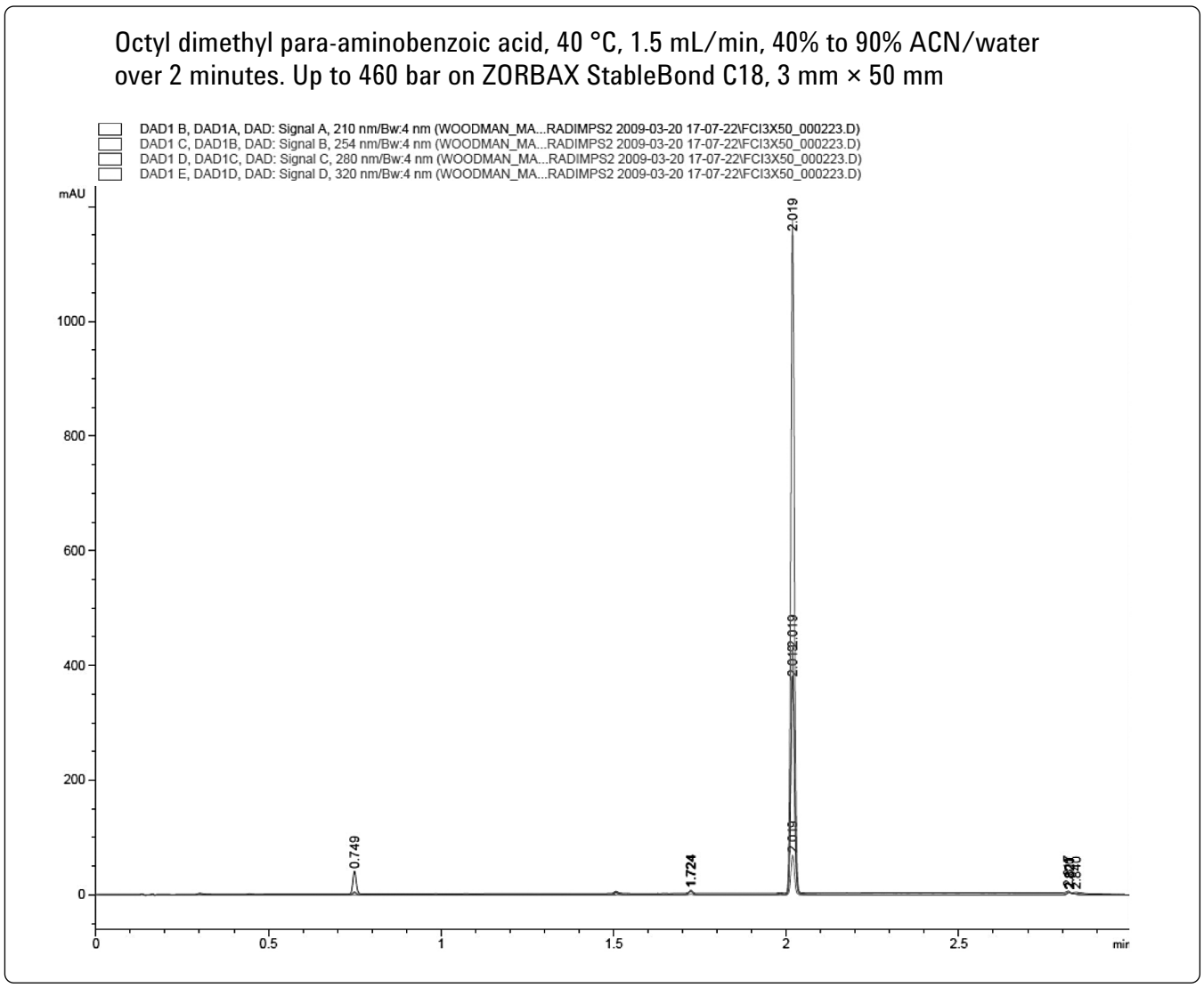

Figure 2. Multi-wavelength UV chromatogram of OD-PABA production material on a 3 mm × 50 mm ZORBAX Rapid Resolution High Throughput (RRHT) column. The chromatogram demonstrates the typical difficulties encountered with this type of separation, which are a need for wide dynamic range detection and sensitive impurity measurement. The peak at 0.75 minutes is confirmed by retention time matching and UV spectra to be PABA, the primary impurity in the mixture.

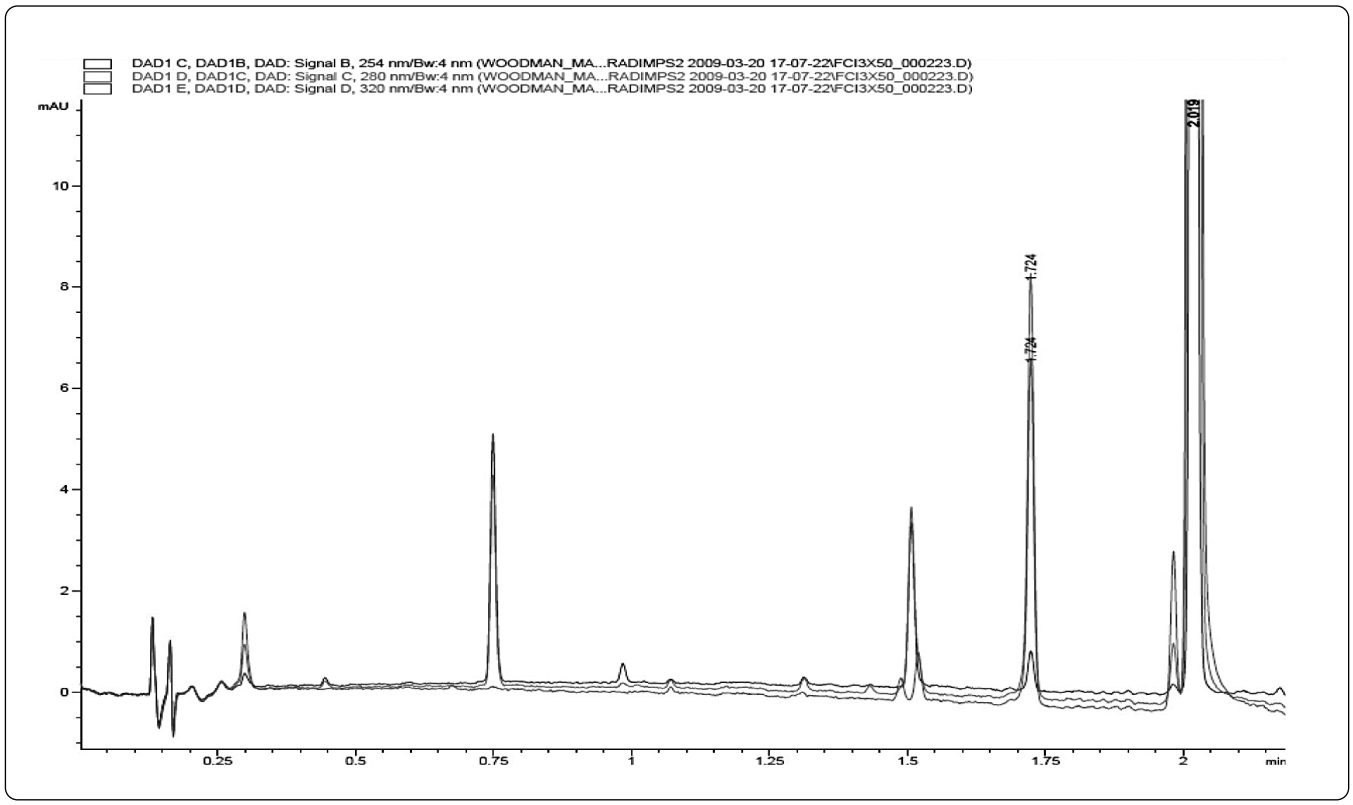

Figure 3. An expanded presentation of the chromatogram shown in Figure 2 based on the 3 mm × 50 mm gradient separation.

In Figure 3 the expanded multi-wavelength chromatogram allows us to see close detail and shows the number of impurities, as well as several areas where chromatographic resolution is clearly inadequate for individual component measurement. Despite the small particle size used in this column, the relatively short length limits the total resolution. As we move to longer column dimensions we will often reduce column diameter to reduce overall solvent consumption at the same time.

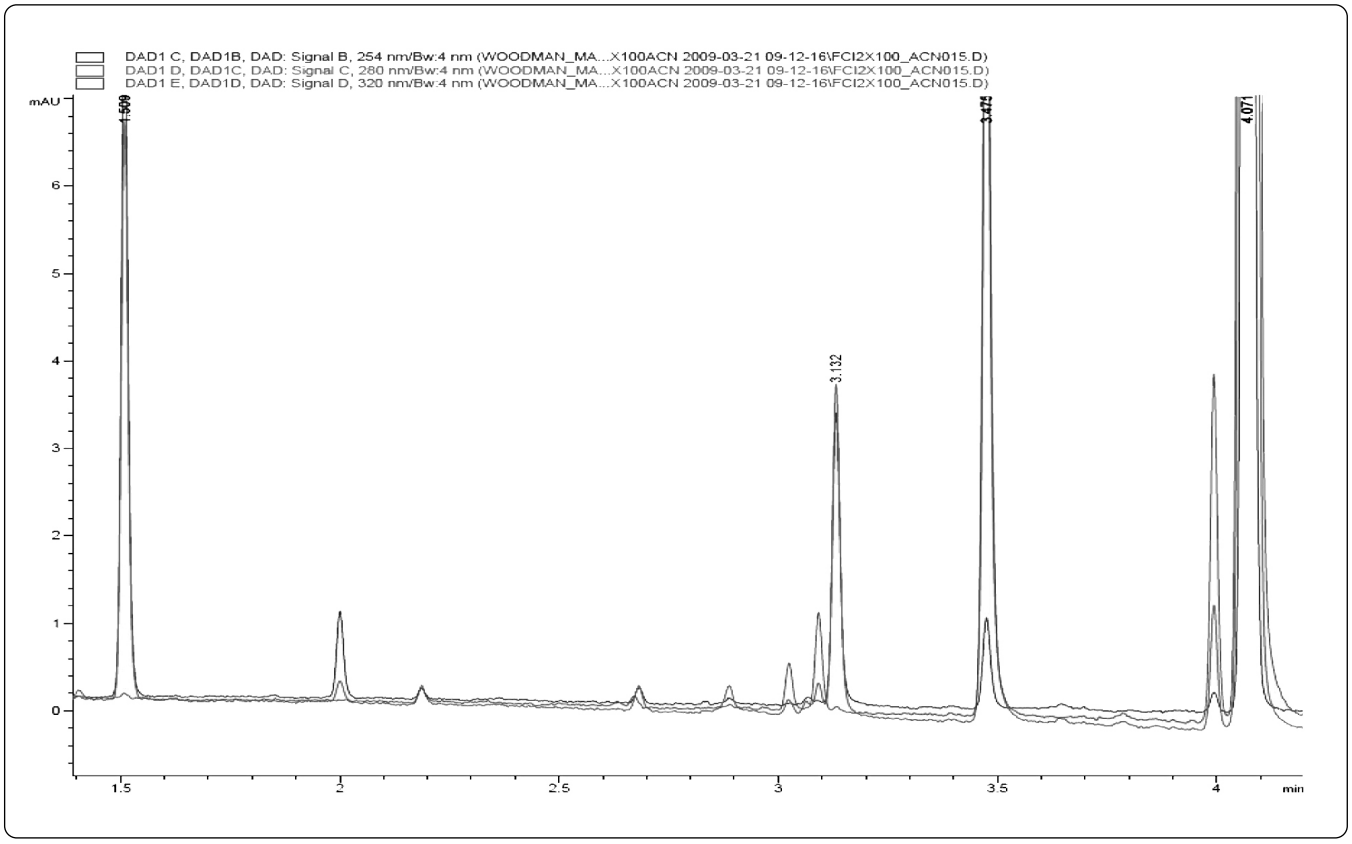

Figure 4. Analysis of the standard material on a 2.1 mm × 100 mm Agilent ZORBAX StableBond C18 column prepared for operation at 1200 bar pressure limit. Acetonitrile water gradient, 0.74 mL per minute, gradient time 4.0 minutes.

In Figure 4, we see that increasing the length of the column has resulted in a significant increase in the resolution of some of the observed components. To further increase resolution it would be practical to explore longer columns or explore alternative mobile phase or column chemistries.

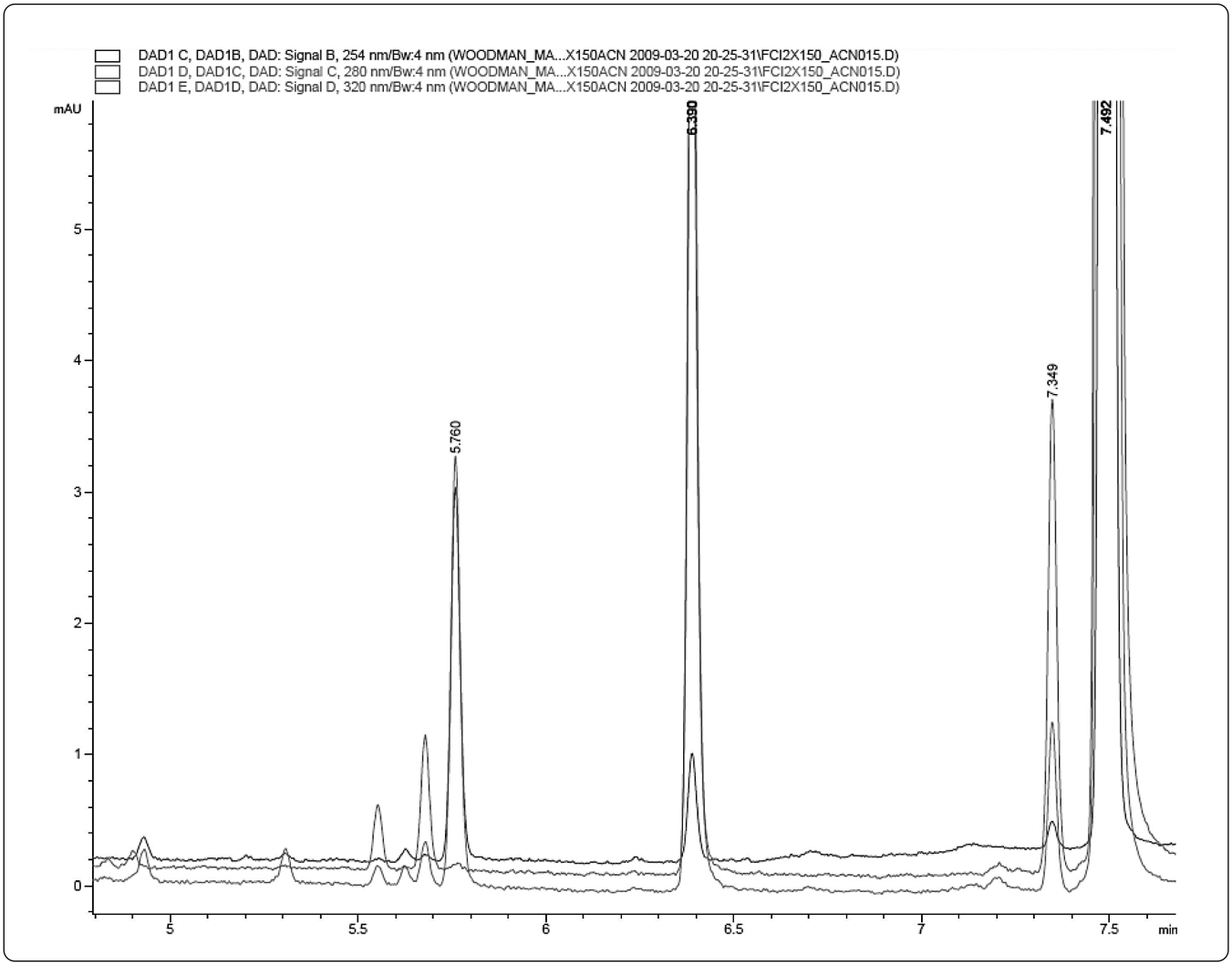

Figure 5. An expanded view of the acetonitrile separation using the same gradient slope on a 2.1 mm × 150 mm column rated for 1200 bar operating pressure. Agilent ZORBAX StableBond C18, 1.8 µm.

The increased column length clearly gives more resolution, however the increased back pressure also limits the flow rate if one is to operate in a conservative range of operating pressure. The Agilent 1290 Infinity LC system and associated ZORBAX Rapid Resolution High Definition (RRHD) chemistries are capable of operating pressures up to 1200 bar, approximately 18,000 psi. To ensure robust and rugged system operation many users typically specify the upper pressure limit for a method at a value less than 80% of the rated operating pressure.

Octyl dimethyl para-aminobenzoic acid, 0.52 mL/min, 50% to 100% MeOH/water over 5.7 minutes. Up to 845 bar on Agilent ZORBAX StableBond C18, 2.1 mm × 100 mm, 1.8 µm.

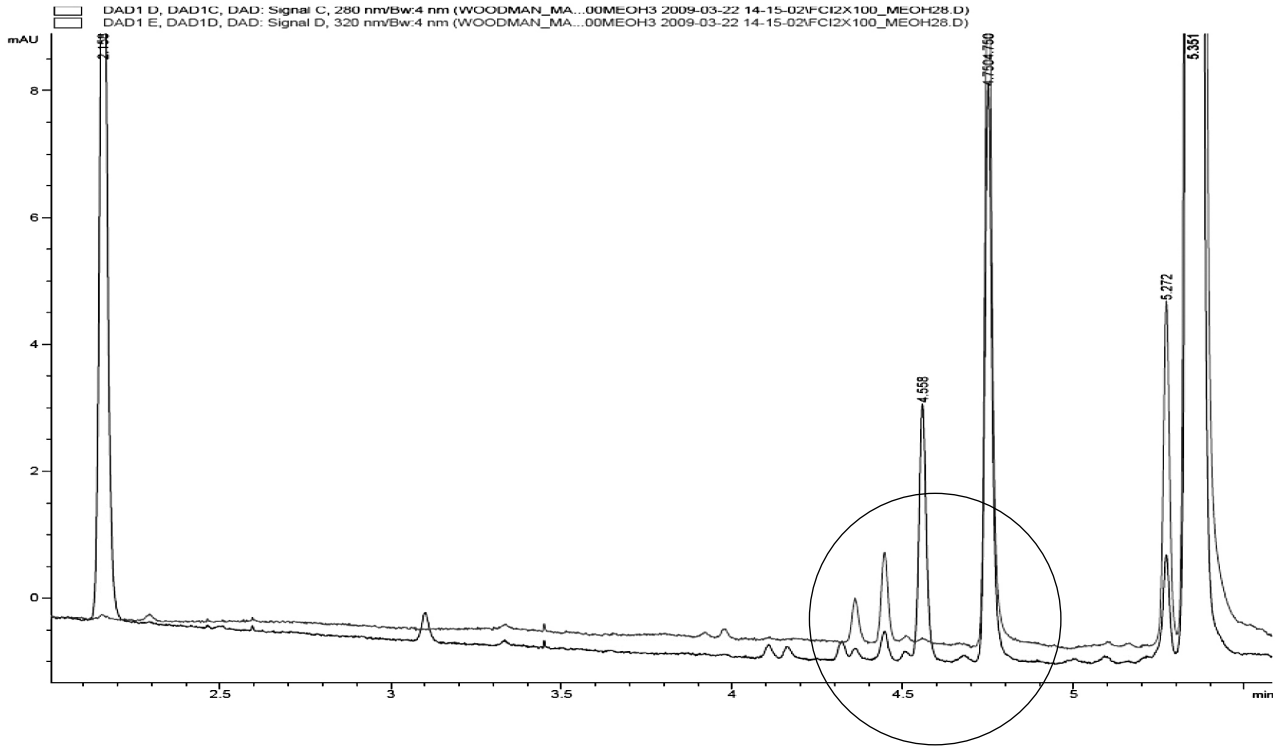

Figure 6. Separation of the sample mixture on a 2.1 mm × 100 mm Agilent ZORBAX StableBond C18, using methanol as the organic phase. Flow rate 0.52 mL/min gradient time 5.7 min, for a gradient of 5% to 100% methanol

When considering the fundamental components of the resolution equation we are all quite familiar with the concepts of capacity, selectivity, and efficiency. Increasing the column length, like decreasing the particle size of the packing material, will increase the efficiency of the overall separation. Because the increase in efficiency yields a relatively low return in terms of resolution, users often need to ensure that the capacity factor is optimized by exploring alternative chemical variables that could promote increased selectivity in the separation.

In Figure 6 we see the dramatic results achieved by changing the separation conditions from using acetonitrile as the organic phase to methanol. If this separation was highly dependent on monitoring the separation at very low wavelengths one might find the UV cutoff of the methanol, 205 nm, to be problematic. In this example, however, the highly conjugated structures of the parents and related impurity structures allow sensitive detection at wavelengths well above the UV cutoff of common organic solvents used in reversed phase chromatography. In about the same amount of analysis time as the example in Figure 5, we achieve significantly higher selectivity leading to more resolved impurities while reducing overall solvent consumption and eliminating the need for expensive acetonitrile as the organic phase.

Octyl dimethyl para-aminobenzoic acid, 0.52 mL/min, 50% to 100% MeOH/water over 5.7 minutes. Up to 845 bar on Agilent ZORBAX StableBond C18, 2.1 mm × 100 mm, 1.8 µm, 40 °C.

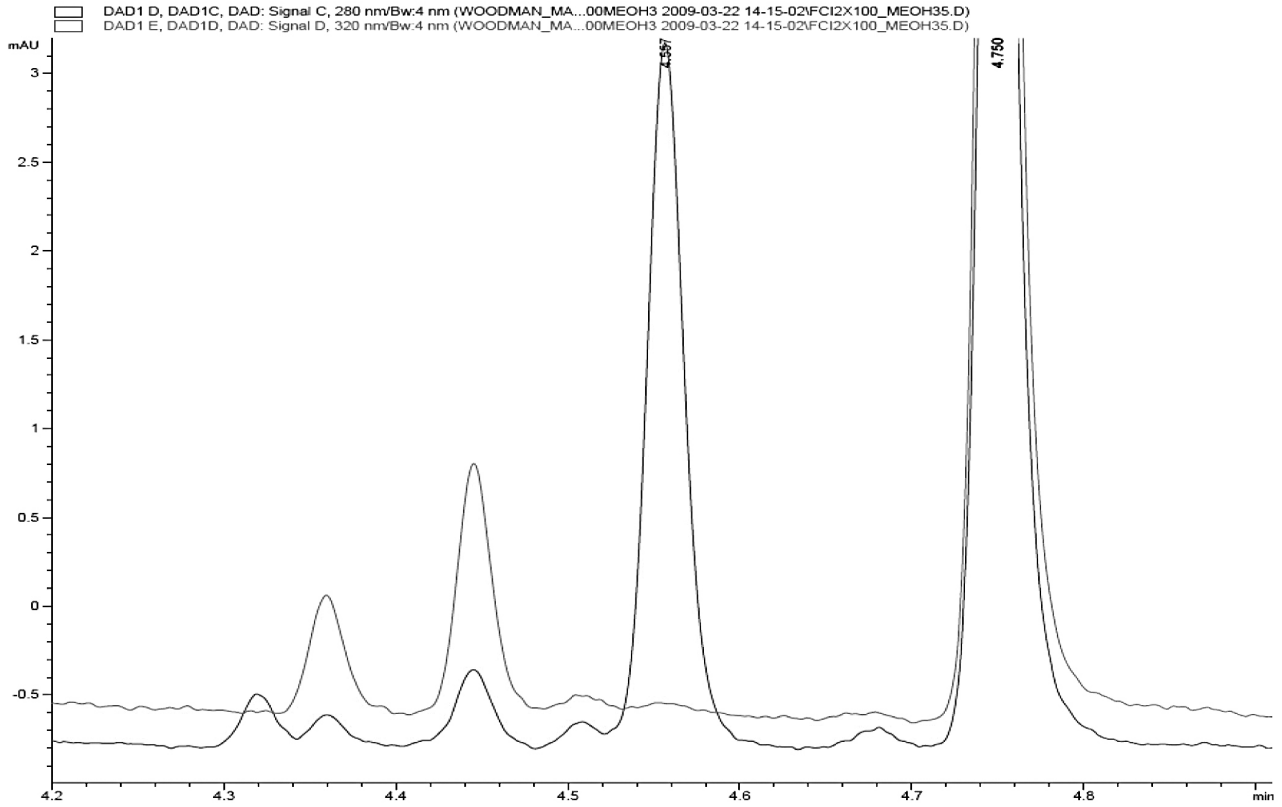

Figure 7. An expanded view of the small region of the chromatogram in Figure 6 showing a significant number of low concentration impurities. Conditions as in Figure 6. Estimated impurity concentrations for the smallest peaks in this figure are less than 0.02%.

Conclusions

The detection of low-level impurities in synthetic materials and highly refined natural products is of critical importance to the ultimate utility of these substances. Rapid analysis by HPLC using high-resolution columns and appropriately chosen organic phases ensures consistent results with rapid analysis turnaround time. Using the Agilent 1290 Infinity LC system, we were able to easily demonstrate UHPLC capabilities well within the operating range of the designed system. Higher throughput could still be achieved with this system by increasing flow rate and simultaneously reducing the gradient segment time to reproduce the gradient slope in a shorter total analysis time.

For More Information

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References

1. http://www.chem.agilent.com/en-US/products/instruments/lc/pages/gp60931.aspx)

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Printed in the USA
August 3, 2009
5990-4293EN

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Determination of Formic Acid in Acetic Acid for Industrial Use by Agilent 7820A GC

Application Brief

Wenmin Liu, Chunxiao Wang

HPI

With rising prices of crude oil and a future shortage of oil and gas resources, people are relying on the development of the coal chemical industry.

Acetic acid is an important intermediate in coal chemical synthesis. It is used in the production of polyethylene, cellulose acetate, and polyvinyl, as well as synthetic fibres and fabrics. The production of acetic acid will remain high over the next three years. In China, it is estimated that the production capacity of alcohol-to-acetic acid would be 730,000 tons per year in 2010.

The purity of acetic acid determinates the quality of the final synthetic products. Formic acid is one of the main impurities in acetic acid. Many analytical methods for the analysis of formic acid in acetic acid have been developed using gas chromatography. For example, in the GB/T 1628.5-2000 method, packed column and manual sample injection is used with poor separation and repeatability which impacts the quantification of formic acid.

In this application brief, a new analytical method was developed on a new Agilent GC platform, the Agilent 7820A GC System. The GC was configured with a micro thermal conductivity detector (μTCD) which provides an easy to use method for the determination of formic acid in acetic acid. To achieve a better separation, an Agilent J&W DB-FFAP (30 m × 320 μm, 0.25 μm) capillary column was used as the analytical column.

Highlights

- The Agilent 7820A GC coupled with a μTCD provides a simple method for analysis of formic acid in acetic acid.
- ALS and EPC ensure good repeatabiltiy and ease of use which makes the 7820GC appropriate for routine analysis in QA/QC labs.
- Using a capillary column as the analytical column ensures better separation of formic acid in acetic acid compared to the China GB method.

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Experimental

Analytical conditions

Inlet	150 °C, Split ratio: 10:1
Injection volume	1 µL
Column	Agilent J&W DB-FFAP, 30 m × 320 µm, 0.25 µm (p/n 123-3232)
Carrier gas	He, Constant flow: 1.5 mL/min
Oven	80 °C (3 min) 8 °C/min 150 °C (5 min)
Detector	µTCD: 200 °C, Reference gas: 15 mL/min, Makeup gas: 6.5 mL/min FID: 300 °C, H_2: 30 mL/min; Air: 350 mL/min; Makeup flow (N_2): 60 mL/min
Autosampler	Agilent 7693A automatic liquid sampler
Data analysis system	EZChrom Elite Compact

Results

Figure 1 shows the chromatogram of the analysis of formic acid in acetic acid at 1% (weight to weight) on the Agilent 7820A GC. From the chromatogram it could be seen that formic acid elutes after acetic acid on the Agilent J&W DB-FFAP column, which is confirmed by the Agilent 6890 GC and the Agilent 5975C series GC/MSD. In this experiment, a flame ionization detector (FID) was also used to confirm the detection of formic acid. The results are shown in Figure 2.

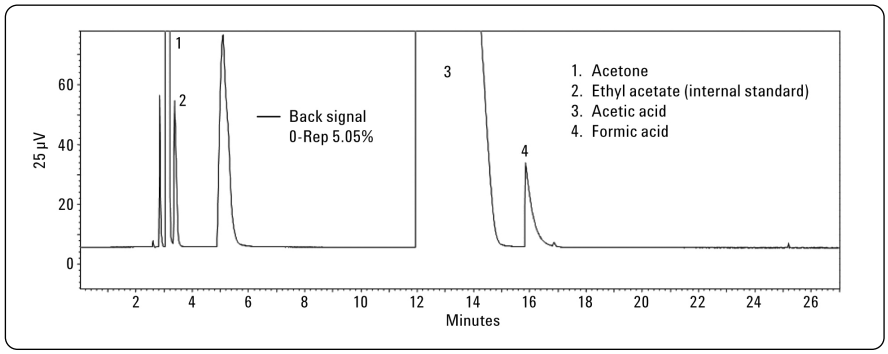

Figure 1. Chromatogram of formic acid analysis in acetic acid on the TCD channel.

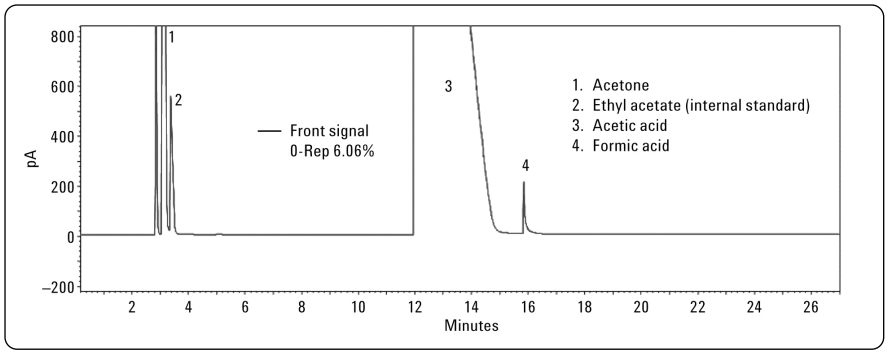

Figure 2. Chromatogram of formic acid analysis in acetic acid on the FID channel.

Agilent μTCD is a proprietary designed single-filament flow switching detector. This design eliminates the need for a reference column, by exposing the filament to column effluent and reference flows at a frequency of 5 Hz. There is no other reference column that assures a stable baseline even with a ramped temperature program. Compared to the typical TCD, the smaller volume cell of μTCD provides higher sensitivity.

The detection limits of formic acid on μTCD was tested using a series of diluted formic acid samples. The method has a signal-to-noise of 6.8 for a formic acid concentration of 0.05 wt%. A method precision of 1.75% RSD for five injections was also calculated for the 0.05 wt% concentration. These excellent results were attributed to the Electronic Pressure Control (EPC) and precision auto sampler that are the key features of the Agilent 7820A GC.

In this method, ethyl acetate was used as the internal standard according to the GB/T 1628.5-2000. Concentrations of 0.1%, 0.5%, 1%, 10% (weight to weight) formic acid in acetic acid standard solution were made with ethyl acetate as an internal standard. The results show that from 0.1% to 10% (weight to weight), formic acid response to concentration was linear with an $r^2 = 0.9917$.

Conclusions

The Agilent 7820A GC coupled with a μTCD provides a simple method for analysis of formic acid in acetic acid. Use of a capillary column ensures good separation of impurities and acetic acid in both high concentrations and low concentrations. The stable and sensitive μTCD is a good choice for formic acid analysis compared to an FID which has a relatively low response. The 7693A autosampler with a capacity of 16 sample vials and 7820A GC EPC control ensure good repeatability and ease of operation, which is suitable for the fast growing coal to chemical industry and routine analysis labs where feedstock and intermediate quality control is important. The data processing system, EZChrom Elite Compact software specially designed for Agilent 7820 GC, is easy to use and provides commonly used report templates.

References

1. GB/T 1628.5-2000. Determination of formic acid in acetic acid by gas chromatography.

Wenmin Liu, Chunxiao Wang are application chemist based at Agilent Technologies, Shanghai, China.

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Printed in the USA
August 25, 2009
5990-4456EN

Agilent Technologies

Analysis of impurities in fine chemical octyl-dimethyl-4-aminobenzoate using the Agilent 1290 Infinity LC and ZORBAX RRHT and RRHD 1.8 µm columns

Application Note

Fine Chemicals

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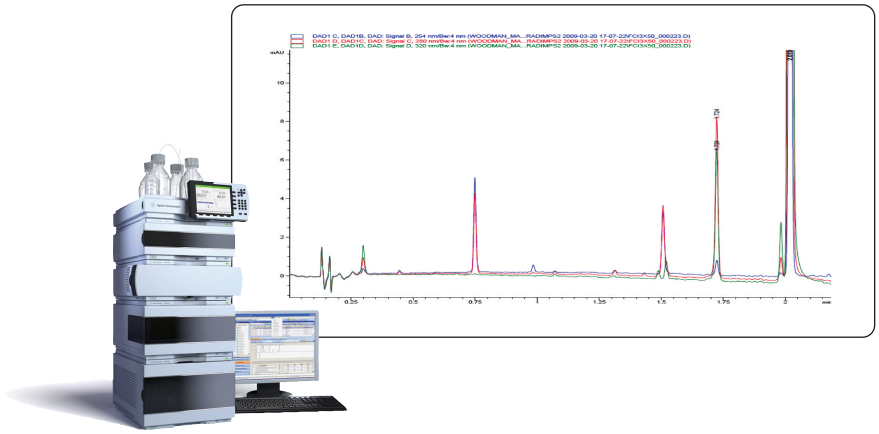
USA



Abstract

The Agilent 1290 Infinity LC has significant capabilities for a wide range of HPLC and UHPLC applications. It exhibits a broader power range (that is, the combination of pressure and flow capabilities) than any other commercially available system and has the flexibility to operate with a wide range of column dimensions and particle sizes. Additionally, advanced optical design in the diode array detector allows a wide dynamic range and high sensitivity, both of which are critical in the monitoring of small impurities in fine chemicals.

Introduction

The combined benefits are demonstrated by a separation of impurities found in a sample of octyl-dimethyl-4-aminobenzoate (Figure 1). The high pressure capability of the system allows the use of methanol, as well as acetonitrile, to explore the selectivity of the two solvents. At 1.5 mL/min, using a simple 2-min gradient and a 3.0 mm × 50 mm 1.8 µm column, the analysis time is only 3 min. The separation of the main components is shown in Figure 2.

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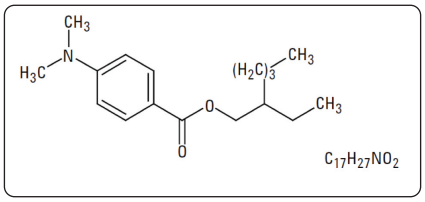

Figure 1
Structure of the cited compound.

The speed, resolution and flexibility of the system are further demonstrated by a separation of the sample using methanol or acetonitrile with low solvent consumption 2.1 mm id, 1.8 µm columns. The flow rate and gradient conditions are optimized for each solvent, to produce a gradient separation with maximum pressure of approximately 850 bar, a conservative setting for the 1200-bar capability of the Agilent 1290 Infinity LC. The separation of the main components, with the two organic solvents, is shown in Figure 3a (acetonitrile, top panel) and 3b (methanol, lower panel), where the chromatograms are zoomed to the region of peaks shown from approximately 1.2-2.5 min in Figure 2.

Configuration

- G4220A 1290 Infinity Binary Pump with Integrated Vacuum Degasser
- G4226A 1290 Infinity Autosampler
- G1316C 1290 Infinity Thermostatted Column Compartment
- G4212A 1290 Infinity Diode Array Detector

Conclusion

The combined high flow and high pressure capability of the system allows one to use high efficiency columns, producing rapid separations with remarkable resolution while conserving solvent over the use of 4.6 mm id columns. Impurity detection, due to high detector sensitivity and stability, is estimated to be < 0.01%.

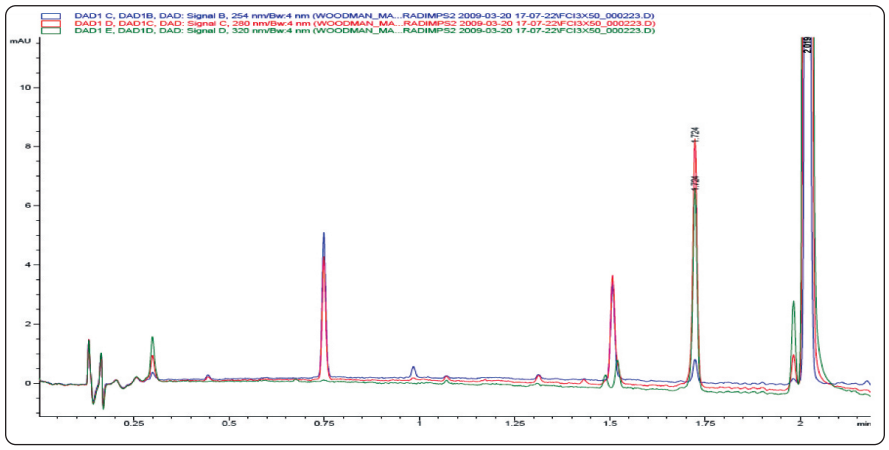

Figure 2
Initial separation conditions showing a need for greater resolution and selectivity. Sample: Octyl dimethyl para-aminobenzoate, 1 mg/mL. Gradient: 1.5 mL/min, 40% to 90% ACN/water over 2 minutes. Up to 460 bar on ZORBAX StableBond RRHT C18, 3 mm × 50 mm, 1.8 µm, 40 °C. 0.75 minute retention: 4-amino-benzoic acid; 2.1 minute retention: Octyl dimethyl para-aminobenzoate.

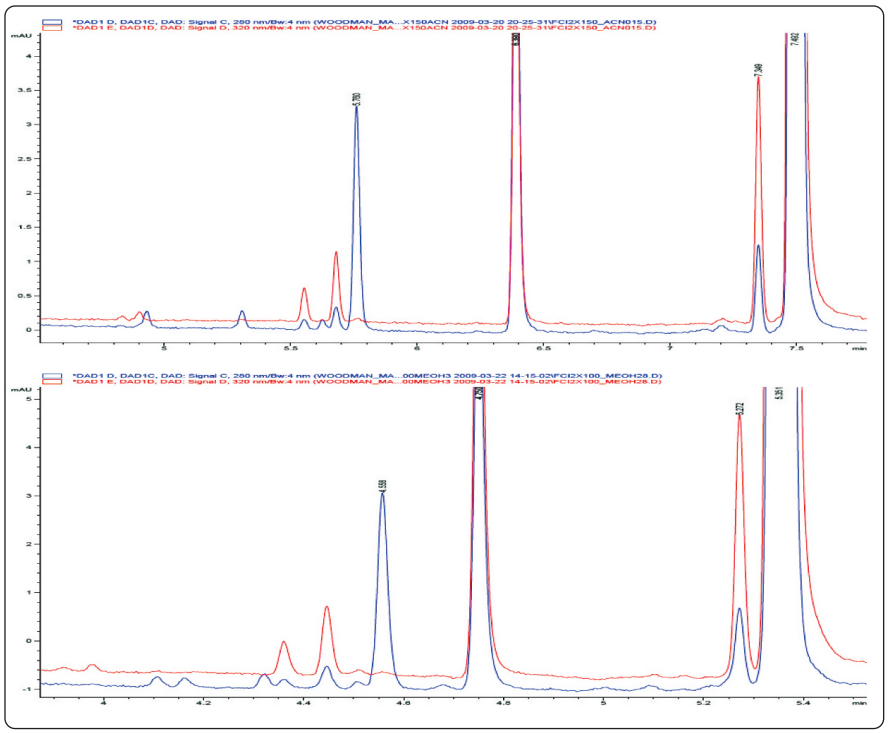

Figure 3
Results using ACN vs. MeOH with the same gradient slope on the 1290 Infinity LC. Sample: ODPABA working standard, 1 mg/mL. Conditions: ACN gradient 0.6 mL/min, 40% to 90% ACN/water over 7.4 minutes. Up to 850 bar on ZORBAX StableBond RRHD C18 2.1 mm × 150 mm, 1.8 µm, 40 °C. Methanol gradient 0.52 mL/min, 50% to 100% MeOH/water over 5.7 minutes. Up to 850 bar on ZORBAX StableBond RRHD C18 2.1 mm × 100 mm 1.8 µm, 40 °C. The increased selectivity of methanol allowed a shorter column to be used, decreasing run time and avoiding the use of more expensive acetonitrile mobile phase.

www.agilent.com/chem/1290

October 1, 2009
Publication Number 5990-4880EN

Agilent Technologies

Fast analysis of polyaromatic hydrocarbons using the Agilent 1290 Infinity LC and Eclipse PAH columns

Application Note

Environmental

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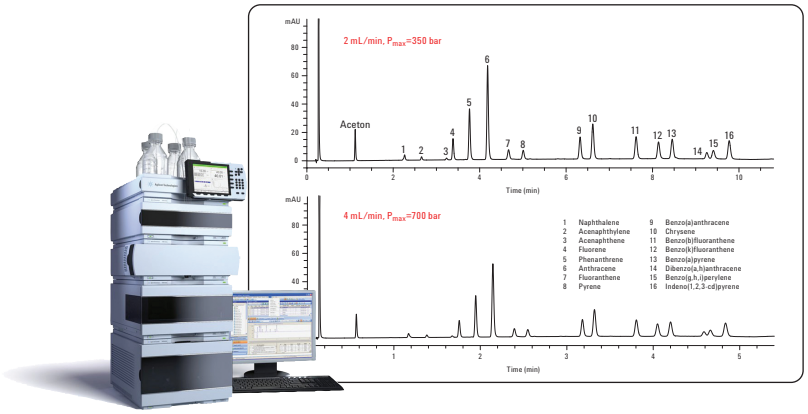

The Agilent 1290 Infinity LC has a broader power range (the combination of pressure and flow capabilities) than any other commercially available system. This is extremely useful for method transfer from one (U)HPLC to the Agilent 1290 Infinity LC system and allows the analyst to develop methods that are impossible to run on these other systems.

The flow and pressure capabilities are illustrated by a separation of 16 polyaromatic hydrocarbons (PAHs) at high pressure and flow rate. At 2 mL/min, the analysis time is approximately 11 min. Doubling the flow rate and gradient speed allows the sample to be analyzed in 5.5 min with a maximum pressure of 700 bar. The combination of high flow (4 mL/min) and pressure is useful in this case to increase the sample throughput. The separation of the PAHs is shown in Figure 1.

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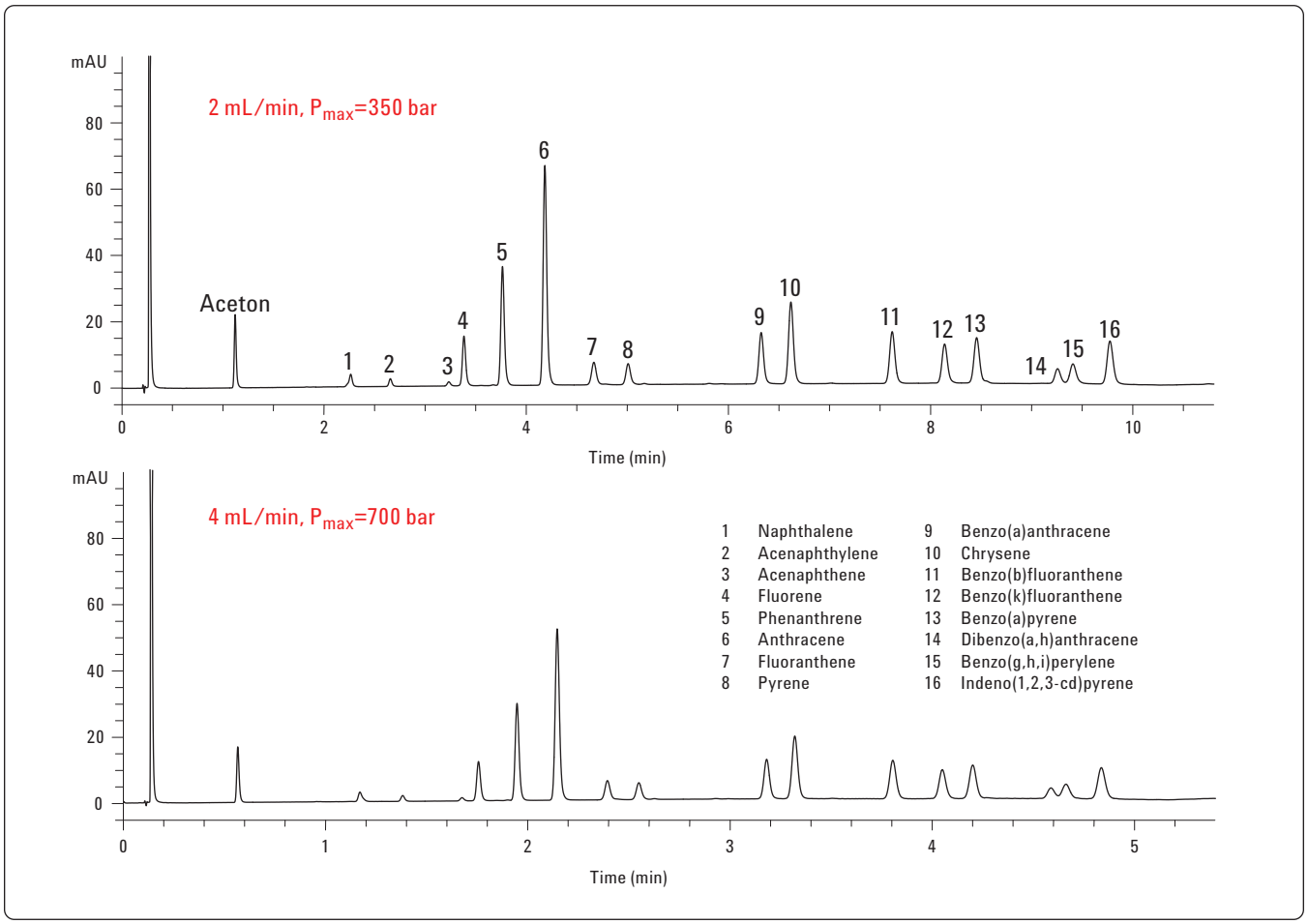

Figure 1
Analysis of 16 PAHs on the 1290 Infinity LC. Sample: standard solution of 16 PAHs, 50 µg/mL each.

Configuration:			
• G4220A	1290 Infinity Binary Pump with Integrated Vacuum Degasser		
• G4226A	1290 Infinity Autosampler		
• G1316C	1290 Infinity Thermostatted Column Compartment		
• G4212A	1290 Infinity Diode Array Detector		
Method:			
Column:	ZORBAX Eclipse PAH 4.6 mm × 50 mm, 1.8 µm		
Mobile phase:	A = water, B = acetonitrile		
Flow rate and gradient:	2 mL/min	0–0.33 min	40% B
		0.33–10 min	40–100% B
	4 mL/min	0–0.17 min	40% B
		0.17–5 min	40–100% B
Injection volume:	0.2 µL		
Detector:	Sig = 254/10 nm, Ref = off, 40 Hz		
Temperature:	25 °C		

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December 1, 2009
Publication Number 5990-4934EN

Agilent Technologies

High-throughput method development for aldehydes and ketones using an Agilent 1290 Infinity LC system and an Agilent ZORBAX StableBond HD column

Application Note

Environmental

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Abstract

This Application Note describes the development of a fast method for the determination of 13 aldehyde and ketone derivates with the Agilent 1290 Infinity LC system. The method, which used acetone as organic co-solvent separates the analytes within 3.5 minutes.

Agilent Technologies

Introduction

Aldehydes and ketones are important compounds in the chemical industry. One of the most essential aldehydes is formaldehyde because it is used for the production of glued wood and synthetic resin. In addition, formaldehyde is one of the most used disinfectants and preservative agents worldwide. Another relevant aldehyde in the chemical industry is acetaldehyde. This chemical is frequently used as an organic solvent and is an important intermediate product in many industries. For example, acetaldehyde is principally used for the production of acetic acid. In general, aldehydes and ketones with middle carbon chain lengths are used as intermediate products during the production of gum, synthetic resin and plastic products. Therefore, many analytical methods exist for the determination of aldehydes and ketones in different matrices. The majority of these methods use the derivatization with 2,4-dinitrophenylhydrazine yielding the corresponding 2,4-dinitrophenylhydrazone. After that, an HPLC separation with UV detection at 360 nm is then performed.

The introduction of the Agilent 1290 Infinity LC system has improved LC-UV methods in several ways. The pressure of the Agilent 1290 Infinity LC system remains stable as high as 1200 bar at flow rates up to 2 mL/min. This is a significant enhancement in comparison to conventional HPLC systems. The most important advantage of the Agilent 1290 Infinity LC system is the small dwell volume of 125 µL (the volume from the point of mixing solvents A and B up to the column inlet including the autosampler). Because of this very small dwell volume, narrow bore columns can be used to shorten analysis time and reduce organic solvent consumption.

This Application Note focuses on LC method development for the determination of several aldehydes and ketones, as well as the advantages of the Agilent 1290 Infinity LC system.

A commercially available method development software package was used to determine the optimal method parameters. Four basic chromatographic runs were performed to determine the optimal column temperature and solvent gradient. These measurements comprised two linear solvent gradients from 5% to 100% B in 10 and 30 minutes at 20 °C and the same gradients at 40 °C. The measurements were performed on an Agilent ZORBAX StableBond RRHD C18 column (50 mm × 2.1 mm, 1.8 µm) by using acetone as an organic modifier. A method was then developed and experimentally confirmed with high agreement between prediction and experiment.

Experimental

All calculations were performed with Agilent ChemStation software version B.04.02 [65].

LC system

For method development, an Agilent 1290 Infinity LC system was used. The system consists of:

- Agilent 1290 Infinity Binary Pump with integrated degasser (G4220A)
- Agilent 1290 Infinity High Performance Autosampler (G4226A)
- Agilent 1290 Infinity Thermostatted Column Compartment SL (G1316B)
- Agilent 1290 Infinity Diode Array Detector (G4212A)

Analyte mixture

The mixture of aldehyde-2,4-dinitrophenylhydrazones and ketone-2,4-dinitrophenylhydrazones is a certified reference material from Sigma-Aldrich (Catalog No. 47651-U) diluted in acetonitrile. In the mixture, each analyte has a concentration of 30 µg/mL of carbon.

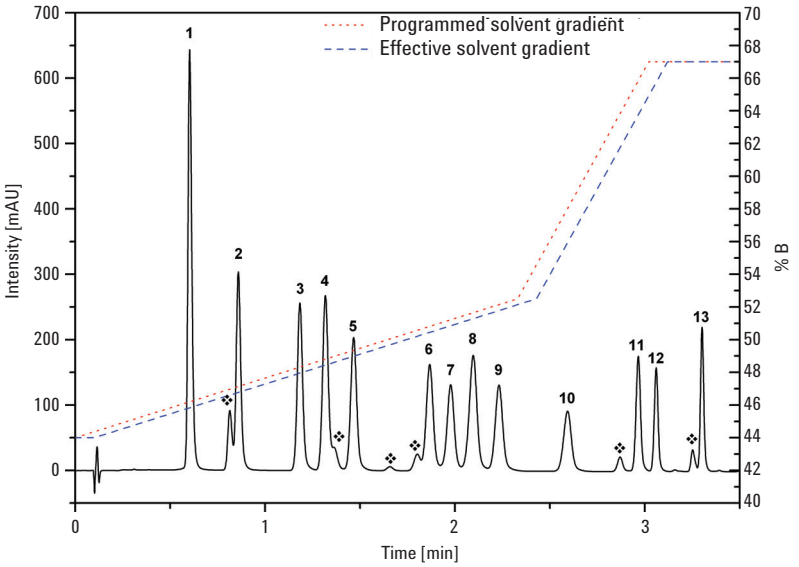
The elution order for all analytes depicted in all figures is:

1. Formaldehyde-2,4-dinitrophenylhydrazone
2. Acetaldehyde-2,4-dinitrophenylhydrazone
3. Acrolein-2,4-dinitrophenylhydrazone
4. Acetone-2,4-dinitrophenylhydrazone
5. Propionaldehyde-2,4-dinitrophenylhydrazone
6. Crotonaldehyde-2,4-dinitrophenylhydrazone
7. Methacrolein-2,4-dinitrophenylhydrazone
8. 2-Butanone-2,4-dinitrophenylhydrazone
9. Butyraldehyde-2,4-dinitrophenylhydrazone
10. Benzaldehyde-2,4-dinitrophenylhydrazone
11. Valeraldehyde-2,4-dinitrophenylhydrazone
12. m-Tolualdehyde 2,4-dinitrophenylhydrazone
13. Hexaldehyde-2,4-dinitrophenylhydrazone

Results and discussion

Figure 1 shows the computer-optimized separation of 13 aldehyde 2,4-dinitrophenylhydrazones and ketone-2,4-dinitrophenylhydrazones on an Agilent ZORBAX StableBond RRHD C18 column within 3.5 minutes. Acetone was used as an organic co-solvent. All peaks are baseline separated with a critical resolution of 1.6 between peak pair 6 and 7. The critical resolution was calculated by the tangent method. The impurities, which are present in the reference material and highlighted by stars were not included in the method development. Figure 1 also shows a comparison of the programmed and effective solvent gradient. Due to a very small dwell volume, there is only a minor difference between the programmed and effective solvent gradients compared to a conventional HPLC system, which exhibits a dwell volume of approximately 1000 µL. This means that at a flow rate of 1.2 mL/min, the programmed solvent gradient reaches the column inlet with a delay of 0.83 minutes, so that the elution of the early-eluting analytes occurs under isocratic conditions. In other words, the elution of the early-eluting analytes cannot be affected by the solvent gradient. Using the Agilent 1290 Infinity LC system with a dwell volume of 125 µL at a flow rate of 1.2 mL/min, the programmed solvent gradient reaches the column inlet after 6.25 seconds and enables fast separations within a few minutes.

The chromatogram shown in Figure 1 is a high pressure application. Due to the applied flow rate of 1.2 mL/min and the 1.8 µm particle packed column, a pressure drop of 1100 bar during the solvent gradient can be observed. Figure 2 shows an overlay of ten consecutive chromatograms, demonstrating the robustness and reproducibility of the develped method.



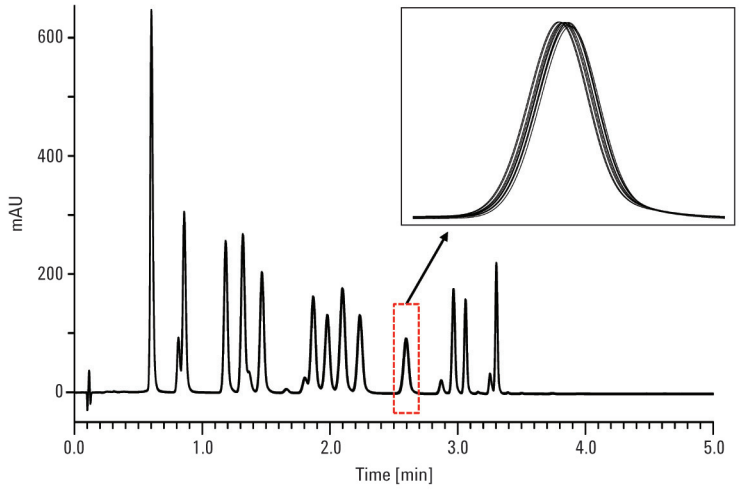
Chromatographic conditions:	
Stationary phase:	Agilent ZORBAX StableBond RRHD C18 (2.1 mm × 50 mm, 1.8 µm)
Mobile phase:	A: deionized water, B: acetone;
Solvent gradient:	0–2.33 min from 44% to 52.5% B, 2.33–3.02 min from 52.5% to 67% B, 3.02–3.50 min isocratic at 67% B;
Flow rate:	1.2 mL/min
Detection:	UV at 360 nm
Injection volume:	1 µL
Temperature:	33.4 °C
Pressure drop:	1100 bar.
Elution order of the analytes:	See experimental section; ❖impurities

Figure 1
Separation of 13 aldehyde-2,4-dinitrophenylhydrazones and ketone-2,4-dinitrophenylhydrazones.

Figure 2 shows that there are virtually no differences among the ten chromatograms. This conclusion is confirmed by the relative standard deviation (RSD) of retention times of the analytes, which ranges between 0.03% and 0.09%.

Conclusion

The Agilent 1290 Infinity LC system is suitable for developing fast HPLC methods. The separation of 13 aldehyde and ketone derivates was completed in around 3.5 minutes, using acetone as an organic modifier in the mobile phase. In addition, the method presented here illustrates that fast HPLC separations are only possible using HPLC systems with small dwell volumes. Finally, we have shown that the Agilent StableBond RRHD C18 column is suitable for separations where the pressure drop is greater than 1100 bar, without loss of separation efficiency.



See Figure 1 for Chromatographic conditions.

Figure 2
Overlay of 10 consecutive chromatograms of the separation of 13 aldehyde-2,4-dinitrophenylhydrazones and ketone-2,4-dinitrophenylhydrazones.

www.agilent.com/chem/1290

May 1, 2010
Publication Number 5990-5793EN

Agilent Technologies

Basic Performance of the Agilent 7700s ICP-MS for the Analysis of Semiconductor Samples

Application Note

Semiconductor

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Abstract

Agilent ICP-MS systems have become the benchmark for accurate low-level analysis of trace contaminants across a wide range of high-purity chemicals used in the semiconductor industry. As the first commercial ICP-MS to offer reliable and routine cool plasma operation, the Agilent 4500 Series ICP-MS set the standard for low level analysis of the previously problematic elements Na, K, Ca and Fe. Building on the success of the 4500 Series, the Agilent 7500s ICP-MS provided additional sensitivity, stability and matrix tolerance, while the Agilent 7500cs ICP-MS introduced new levels of performance through the provision of an Octopole Reaction System (ORS) operating in both collision and reaction modes.

The newly-developed Agilent 7700s ICP-MS further extends the performance of the technique for routine semiconductor applications by combining unmatched sensitivity, matrix tolerance, interference removal and stability in a single reliable and easy to use system.

Agilent Technologies

Introduction

The Agilent 7700 Series ICP-MS product line includes the 7700s model, which is configured specifically for semiconductor applications, with a PFA nebulizer, Pt interface and high-transmission ion lens. Development of the 7700 Series was focused on ensuring "ease of use", while improving on the sensitivity and interference-removal capability of the 7500cs. To meet these goals, many new features have been introduced in the 7700s, including much smaller size, easier maintenance, and lower cleanroom service requirements (20% lower exhaust flow and 3.5x lower pressure drop than the 7500cs). The 7700s also introduces a new, robust, frequency matching RF generator for improved performance in volatile organic solvents, and a 3rd generation ORS^3 collision reaction cell.

Basic Performance of the 7700s

The 7700s incorporates a new interface, ion lens and the newly-developed ORS^3, to deliver better ion transmission and lower backgrounds than the 7500cs. In general, the sensitivity (cps/ppt) of the 7700s is approximately 40% higher than that of 7500cs, and the new off-axis lens configuration reduces random background noise by half. The result is that the 7700s has much lower Detection Limits (DLs) and Background Equivalent Concentrations (BECs) than its predecessor. Table 1 shows the normal plasma DLs and BECs obtained on the 7700s in a matrix of 1% HNO_3. Elements with a * may also be measured in cool plasma (BEC and DL in brackets). Figure 1 shows the preferred-mode DL of the 7700s compared to the 7500cs. In all cases, the 7700s DL was lower, notably for elements with plasma-based interferences (for example ^{56}Fe, ^{78}Se).

Improved Interference Removal

The newly developed ORS^3 has longer rods and a smaller internal diameter, and operates at higher frequency and higher cell gas pressure than the cell fitted to the 7500cs. As a result, higher bias voltages may be used, which improves the removal of polyatomic interferences in helium (He) mode with Kinetic Energy Discrimination (KED), and also promotes Collision Induced Dissociation (CID) for relatively weakly-bound polyatomic ions such as ArO^+ (dissociation energy 0.6 eV) and $ArAr^+$ (dissociation energy 1.3 eV). More effective removal of these background (plasma-based) polyatomic ions provides much lower DL and BEC for many elements, although reaction mode (H_2 cell gas) provides the lowest DL and BEC for several elements including Fe and Se in the highest purity semiconductor samples.

Table 1. 7700s ICP-MS BECs and DLs in 1% HNO_3.

Mass	Element	BEC ppt	DL ppt	Mode
7	Li*	0.2 (0.01)	0.06 (0.01)	No gas
9	Be	0.04	0.05	No gas
11	B	6	1	No gas
23	Na*	200 (0.5)	4 (0.3)	No gas
24	Mg	0.2	0.07	No gas
27	Al*	20 (0.2)	0.6 (0.3)	No gas
39	K*	250 (0.5)	10 (0.3)	H_2
40	Ca*	5 (2)	2 (1)	H_2
48	Ti	0.2	0.2	He
51	V	0.1	0.1	He
52	Cr*	10 (0.03)	0.7 (0.09)	He
55	Mn*	0.6 (0.05)	0.6 (0.1)	He
56	Fe*	7 (0.5)	0.6 (0.2)	H_2
59	Co*	0.08 (0.01)	0.09 (0.06)	He
60	Ni*	1 (0.03)	0.6 (0.1)	He
63	Cu*	6 (0.6)	0.8 (0.4)	He
64	Zn	0.6	0.5	He
71	Ga*	0.07 (0.01)	0.05 (0.01)	He
74	Ge	0.01	0.05	He
75	As	0.07	0.4	He
78	Se	0.5	0.9	H_2
85	Rb*	0.2 (0.01)	0.2 (0.03)	He
88	Sr	0.01	0.04	He
90	Zr	0.06	0.05	He
93	Nb	0.01	0.02	He
98	Mo	0.04	0.07	He
107	Ag	0.04	0.03	He
114	Cd	0.02	0.06	He
118	Sn	0.5	0.2	He
121	Sb	0.03	0.04	He
138	Ba	0.01	0.02	He
178	Hf	0.01	0.01	He
181	Ta	0.01	0.01	He
182	W	0.01	0.03	He
197	Au	0.05	0.05	He
205	Tl	0.01	0.03	He
208	Pb	0.1	0.09	He
209	Bi	0.02	0.04	He
232	Th	0.01	0.01	He
238	U	0.01	0.01	He

*Elements may also be measured in cool plasma (BEC and DL in brackets).

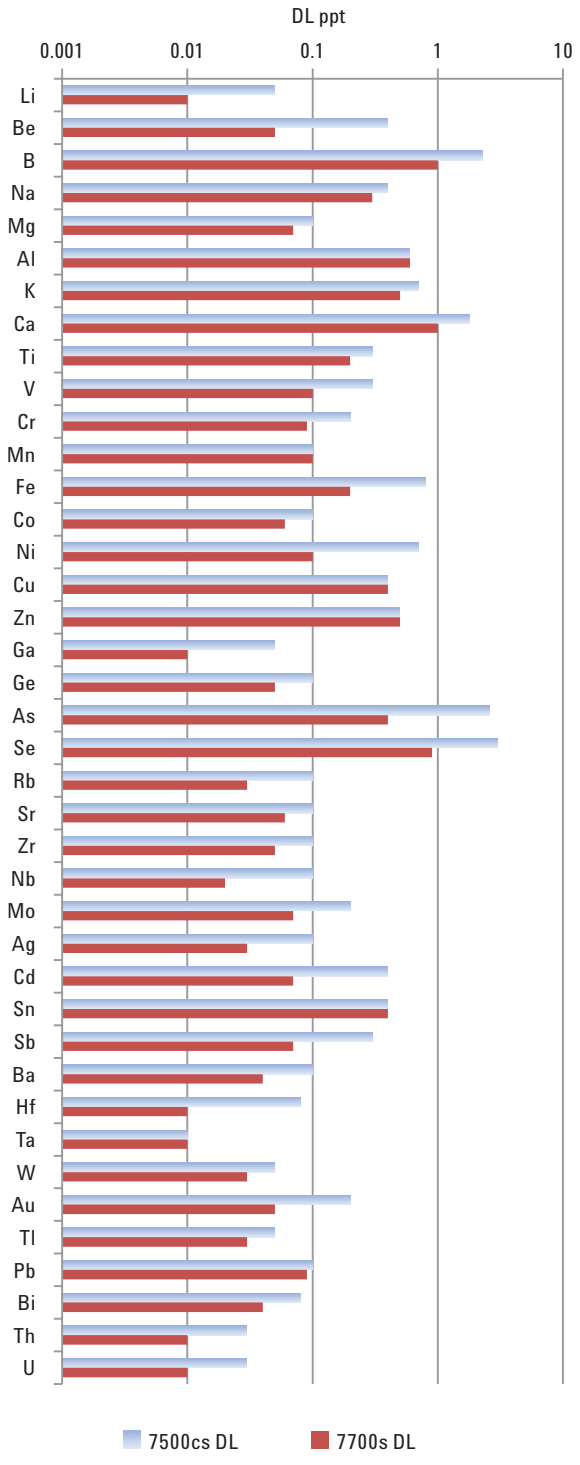

Figure 1. DL comparison for Agilent 7700s and 7500cs ICP-MS.

The enhanced He mode operation of the ORS[3] on the 7700s delivers dramatically improved detection of phosphorus, with DLs and BECs improved by a factor of 10 to 50 compared with conventional collision/reaction cell ICP-MS. The 7700s He mode calibration curve for phosphorus in 1% HNO_3 is shown in Figure 2.

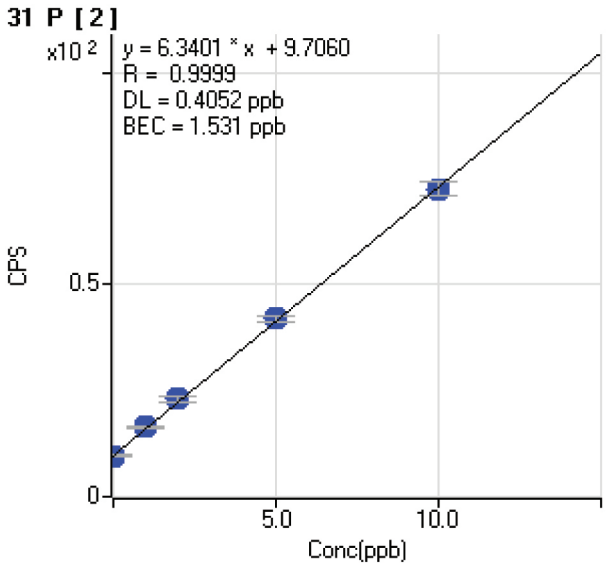

Figure 2. 7700s ICP-MS He mode calibration for ^{31}P in 1% HNO_3.

The new ORS[3] also delivers better performance in reaction mode with H_2 cell gas, due to the higher cell gas density and the higher energy of the collisions between the interfering ions and the cell gas. This gives lower DLs for elements such as Si (measured directly at mass 28), and reduces the need for highly reactive cell gases such as NH_3, which has a strong tendency to form multiple cluster ions in the cell and is therefore not generally suitable for variable or complex process chemical matrices, or for multi-element analysis. The performance of the 7700s in H_2 reaction mode is illustrated in Figure 3, which shows the calibration curve, DL and BEC for ^{28}Si in a matrix of 1% HNO_3. It should be noted that the pure water used in the examples in Figures 2 and 3 was normal Milli-Q water and not further purified. Silicon is expected to be present at high concentration in such water and blank contamination would therefore have contributed to the BEC.

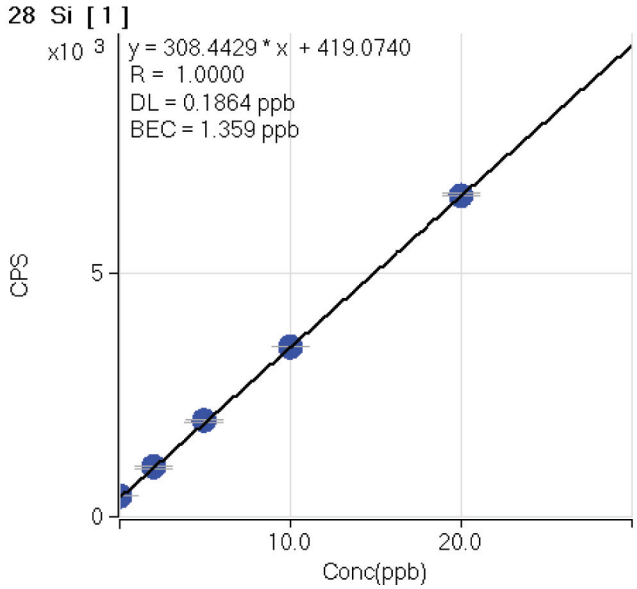

Figure 3. 7700s ICP-MS H_2 mode calibration for ^{28}Si in 1% HNO_3.

Long Term Stability

Long term (9 hours) stability was measured for several elements spiked at 100 ppt in 1% HNO_3. Sampling and data acquisition was done every 35 minutes. Raw count rate signal drift was within ± 5% over the 9 hour sequence, and the %RSD was < 3% for all analytes. The excellent long term stability of the 7700s operating in He mode is shown in Figure 4.

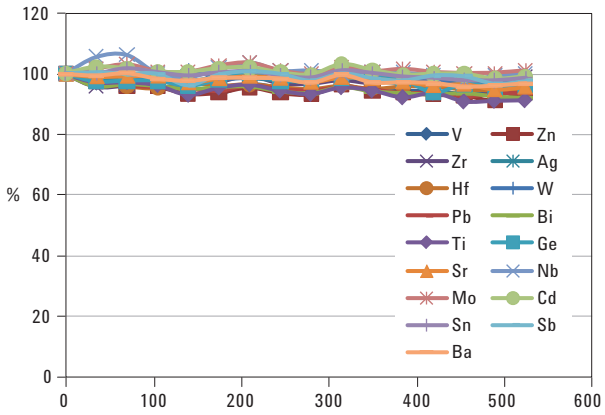

Figure 4. Nine hours stability of 7700s ICP-MS operating in He mode (100 ppt spike in 1% HNO_3).

Other Reaction Gases

The 7700s has both collision (He) and reaction (H_2) cell gas lines fitted as standard, but can also have an optional 3rd cell gas line fitted for specialized applications. In the semiconductor industry, this includes analyses where optimum interference removal requires a highly reactive cell gas such as NH_3. While the requirements for such highly reactive cell gases are very small, there are some specific cases where such gases offer the lowest DLs.

One example of the use of highly reactive cell gases is illustrated in Figure 5, which shows the calibration for V in concentrated HCl, using NH_3 mode. The intense ClO interference which affects the only useful isotope of V at mass 51 is not very reactive with H_2, so H_2 cell mode does not give sufficiently good interference removal for the lowest DL to be achieved in the highest purity HCl, such as (TAMAPURE-AA100 (20%)). As can be seen in Figure 5, the 7700s ICP-MS operating in NH_3 mode gives effective removal of the ClO interference in undiluted (20%) HCl, providing a BEC and DL of only 2.3 ppt for V.

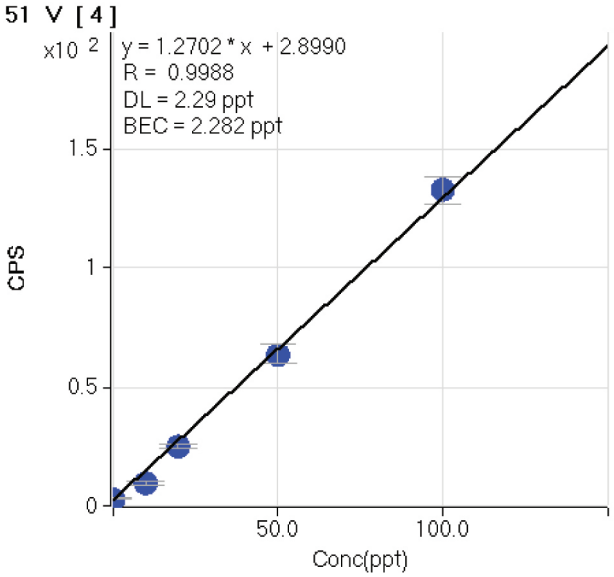

Figure 5. Vanadium calibration in undiluted HCl, using NH_3 mode.

Improved Analysis of Organic Solvents

The 7700s incorporates a newly developed frequency matching RF generator, which responds more quickly to any change of impedance in the plasma, compared to a conventional fixed frequency matching system. A wide range of organic solvents can therefore be introduced without causing disturbance of the plasma.

The most important organic solvent in the semiconductor industry is isopropyl alcohol (IPA). IPA is frequently used to clean silicon wafers and must be analyzed periodically to check for contamination by trace metallic elements. For most organic solvents (inducing IPA), an organics torch with a 1.5 mm internal diameter (id) injector is used, but a torch with a 1.0 mm id injector is also available for the most volatile solvents.

Figure 6 illustrates the single-ppt and sub-ppt DLs and BECs achieved on the 7700s ICP-MS for the analysis of undiluted IPA. The elements Ti, V, Co, Zn, As, Sr, Zr, Nb, Mo, Ru, Rh, Pd, Ag, Cd, Sn, Ba, W, Ir, Au, Tl, Bi, Th and U were measured in normal hot plasma conditions (in He, H_2 or no gas mode) and the remaining elements were measured in cool plasma.

Figure 7 shows the excellent raw count rate stability for a 100 ppt spike of several elements in undiluted IPA, measured continuously over 4 hours. Total signal variation was < 5 %RSD.

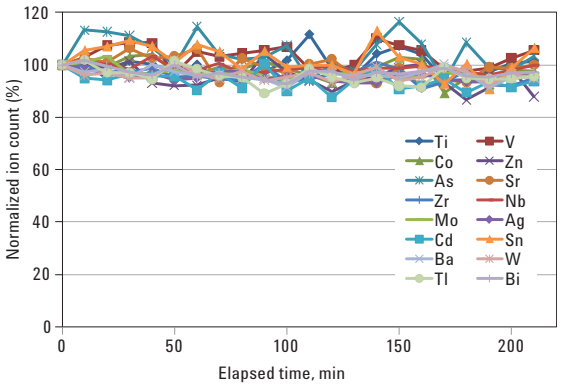

Figure 7. Four hours stability of He mode (100 ppt spiked into IPA).

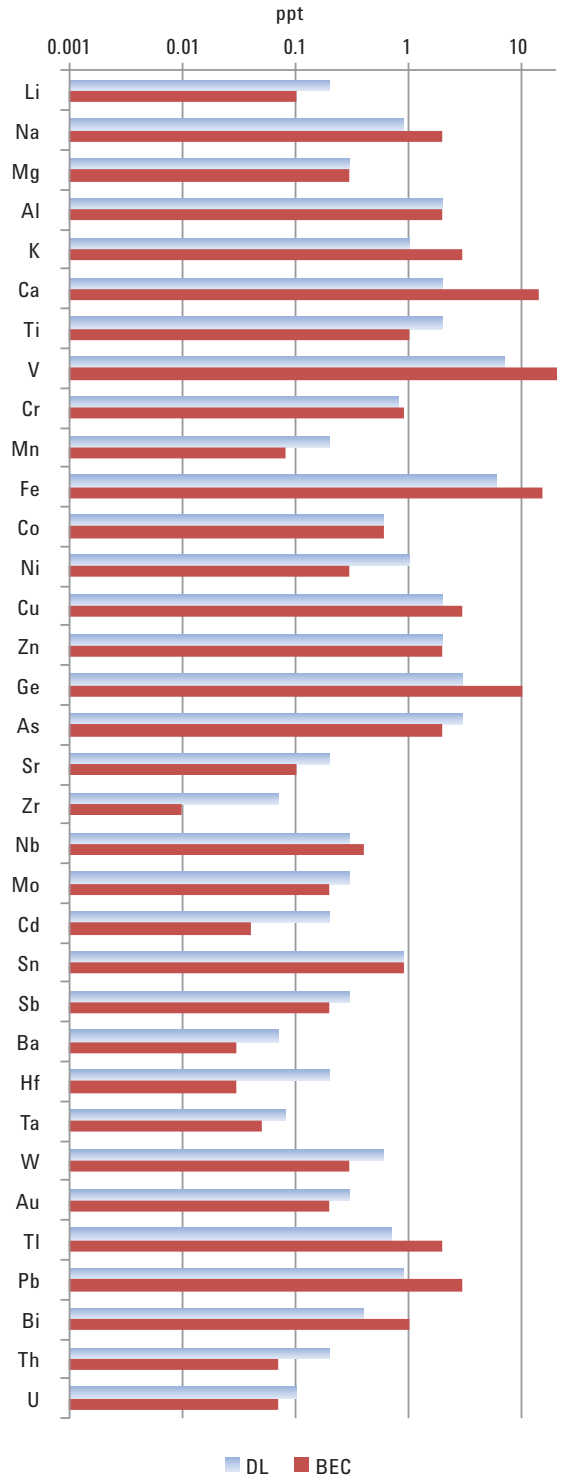

Figure 6. 7700s ICP-MS DLs and BECs in undiluted IPA.

Cool Plasma Operation

As the manufacturer responsible for the development of the first ICP-MS capable of routine operation under cool plasma conditions (4500 Series), Agilent was largely responsible for the initial acceptance and widespread use of ICP-MS in the semiconductor industry. In each subsequent generation of Agilent ICP-MS instrument, cool plasma has been further refined, allowing analysts to make use of the widest range of analytical techniques for interference removal. The 7700s provides a further enhanced cool plasma mode of operation, with improved robustness and stability due to the use of the new frequency matching RF generator.

The exceptional cool plasma performance of the 7700s is demonstrated in Figure 8 which shows the long term stability of several elements in 1% HNO_3.

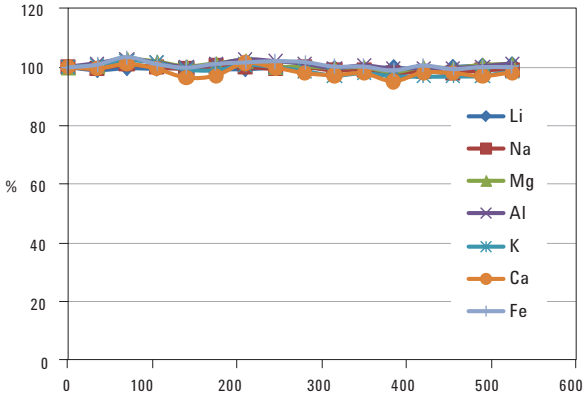

Figure 8. Nine hours stability of cool plasma mode (100 ppt spike in 1% HNO_3).

Cool plasma on the 7700s is also suitable for the measurement of organic solvents. Due to the high volatility of IPA (boiling point 82.4 °C), it may be difficult to keep the ICP stable, particularly when switching between cool and hot plasma modes within an analysis. This issue is resolved with the new frequency matching generator of the 7700s, as demonstrated in the data shown in Figure 9. The elements in Figure 9 were measured in cool plasma, while the corresponding data shown in Figure 7 were measured in normal hot plasma conditions. The plasma conditions were switched automatically between cool plasma and hot plasma for each sample in the analytical sequence, illustrating the robustness and matrix tolerance of the new 7700s RF generator.

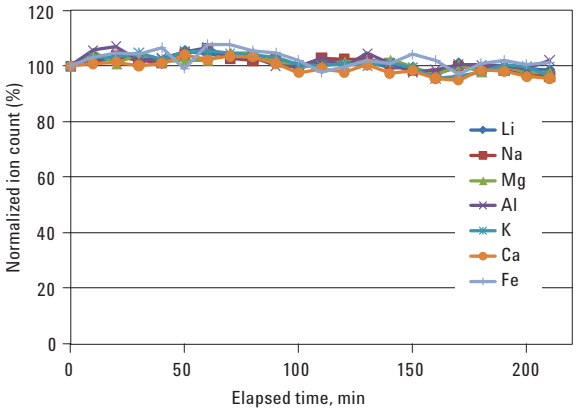

Figure 9. Four hours stability of cool plasma mode (100 ppt spiked into IPA).

Conclusions

In addition to greatly reduced cleanroom setup and operating costs due to its small size and low service requirements, the Agilent 7700s ICP-MS provides lower DLs and BECs, and higher sensitivity than the Agilent 7500cs ICP-MS.

Moreover, the newly developed, 3rd generation ORS^3 can be fitted with up to 3 cell gas lines (2 are included as standard), allowing total flexibility. The new ORS^3 cell improves performance for several critical elements by increasing the efficiency of both collision and reaction mode, and providing enhanced CID. These developments now allow several elements like phosphorus to be determined at lower concentrations than previously possible.

The robust RF generator of the 7700s also improves the analysis of volatile organic solvents, simplifying the analysis of a variety of process chemicals. Improved matrix tolerance and stability is provided, both for conventional analysis and cool plasma operation.

Details regarding the operating conditions and performance achieved in the analysis of specific semiconductor chemicals are provided in the tuning guide that ships with every Agilent 7700s ICP-MS instrument.

For More Information

For more information on our products and services, visit our Web site at www.agilent.com/chem/icpms.

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Printed in the USA
August 12, 2010
5990-6195EN

Agilent Technologies

Evaporation from 2-mL Vials on the Agilent 7696A Sample Prep WorkBench: Septa Unpierced, Septa Pierced with a Syringe Needle, Septa with an Open Hole

Application Note

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Introduction

In the course of sample analysis by gas chromatography, the vial septum may be pierced multiple times before each injection, often with multiple injections. Once the septum is pierced, solvent evaporation from the vial occurs. This usually does not create a reproducibility problem for GC analysis, even with multiple injections, unless the time between runs is an hour or longer. With the Agilent 7696A Sample Prep WorkBench, the number of times a septum is pierced may be greater, and the time before the final sample is analyzed may be much longer than is typical in GC.

Another problem that arises with the Agilent 7696A Sample Prep WorkBench is the need to withdraw large volumes from 2 mL vials. For example, transferring 0.5 mL solvent or sample from one vial to another can create a partial vacuum in the source vial. This results in poor reproducibility because the degree of vacuum varies from vial to vial and the amount of liquid actually transferred also varies. One way to eliminate this problem is to prepierce the septum with a small off-center hole so that no vacuum is created and the syringe needle is still wiped by the septum when withdrawn from the vial.

The evaporation rates of hexane (bp = 70 °C) and isooctane (bp = 100 °C) were measured at ambient temperature for three different septum scenarios to determine the magnitude of the problem. The three scenarios are as follows: a new unpierced septum, a septum prepierced approximately nine times, and a septum cored to prevent vacuum formation. Evaporation from the new, unpierced screw cap vial septa was considered negligible. Evaporation was greater with the septa pierced with a syringe needle and much greater with the cored septa.

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Experimental

Hardware

Vials: 2 mL glass screw cap (5182-0714)

Septum caps: With PTFE/red silicone rubber (5185-5820)

Septum types:

A = new, unpierced
B = pierced approximately 9 times with syringe needle
C = new, cored off-center with a 0.5 mm hole

The type B septa were prepierced with GC injections. The type C septa were cored with a miniature "cork borer" made from a brass tube (1/16" od × 0.035" id). One end was filed to create a sharp inner edge. The holes created were about 0.5 mm id.

Fifteen empty vials plus caps were weighed. Five contained type A septa, five contained type B and five contained type C. Vials were filled with about 1 mL of solvent each, reweighed, and placed in a Agilent 7696 sample tray. Vials were weighed again after 24 and 96 hr at room temperature (23 °C).

Results

The %loss/hr for the different septum types for hexane is:

A = 0
B = 0.3
C = 0.9

The %loss/hr for the different septum types for isooctane is:

A = 0
B = 0.1
C = 0.3

Table 1 lists average evaporation rates from vials with the different septa.

Conclusions

This data provides a rough idea of the effect solvent evaporation has on our preparation results. It is up to the user to determine what level of evaporation can be tolerated based on the specific method and length of time between initial and final samples in the preparation. When a method requires vacuum relief holes in the septa, the transfers should be performed early in the method if possible, and even perhaps as a separate method so that vials can be recapped before significant evaporation occurs.

Table 1. Average Evaporation Rates from Vials with the Different Septa

Solvent: hexane, bp = 70 °C

	Septum:	A		B		C	
	After:	**%loss**	**%loss/hr**	**%loss**	**%loss/hr**	**%loss**	**%loss/hr**
	24hr	0.00	0.00	7.27	0.30	21.06	0.88
	96hr	0.03	0.00	29.21	0.30	84.55	0.88

Solvent: isooctane, bp = 100 °C

	Septum:	A		B		C	
	After:	**%loss**	**%loss/hr**	**%loss**	**%loss/hr**	**%loss**	**%loss/hr**
	24hr	0.12	0.01	2.74	0.11	6.84	0.29
	96hr	0.65	0.01	11.38	0.12	28.26	0.29

A New, unpierced septa
B Septa prepierced about nine times
C Septa cored to prevent vacuum formation

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Printed in the USA
November 10, 2010
5990-6846EN

Agilent Technologies

Permanent Gases on a COX Module Using a Agilent 490 Micro GC

Application Note

Micro Gas Chromatography

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Abstract

This application note demonstrates the capabilities of the COX column with the Agilent 490 Micro GC, including separation of permanent gases and backflush possibilities to ensure extended column lifetimes.

Introduction

Separation of permanent gases is usually performed on a Molsieve column. This column offers the best separation for all permanent gases but also has some severe drawbacks. Water and carbon dioxide do not elute from a Molsieve column under regular GC conditions. A bake out at high temperatures (250 – 300 °C) is needed to fully regenerate the column. Regeneration is very time consuming in a Micro GC usually taking overnight or longer because the maximum temperature is 180 °C. In addition, it is likely that regeneration from moisture does not occur at this temperature.

If there is no need to separate oxygen and nitrogen, the COX column is a better alternative. It delivers good separation of permanent gases, and carbon dioxide elutes from the column. COX is an ideal alternative for a Molsieve column, offering prolonged lifetime and instrument uptime.

Agilent Technologies

Experimental

Instrumentation

An Agilent 490 Micro GC system with a COX column module was used for these experiments. The COX column module was equipped with a heated injector and an optinal precolumn with backflush.

Conditions

Column temperature	100 °C
Carrier gas	Argon, 100 kPa
Backflush to vent time	13 s
Injection time	80 ms
Injection temperature	110 °C
Sample line temperature	100 °C
Sampling time	30 s
Stabilization time	5 s
Run time	200 s

Sample Information

Standard gas samples were used. Concentrations were in % levels.

Table 1. Repeatability Figures Per Component on Peak Area

Run	He	H_2	N_2	CO	CH_4	CO_2
1	943213	16024030	20593423	1439534	1535598	1064007
2	947355	16092042	20685887	1444814	1538714	1062243
3	949818	16142635	20749728	1446996	1544418	1070193
4	949808	16167426	20781405	1449939	1542239	1066091
5	952725	16194789	20815739	1453498	1539162	1066940
6	952107	16206479	20826967	1456289	1543749	1063772
7	954648	16228802	20856620	1455219	1548126	1074325
8	954635	16249294	20879589	1456795	1547760	1079645
9	955454	16251565	20883920	1456611	1552320	1064839
10	955872	16250493	20901246	1473831	1547242	1065483
Average	951563.5	16180756	20797452	1453353	1543933	1067754
St. Dev	4053	75870	97930	9249	5122	5456
RSD%	0.43	0.47	0.47	0.64	0.33	0.51

Results and Discussion

The above settings produce the chromatogram shown in Figure 1, with repeatability data in Table 1.

The chromatogram shows a baseline separation of helium and hydrogen. Oxygen and nitrogen eluted as a single peak but separate from carbon monoxide and methane. Carbon dioxide eluted perfectly.

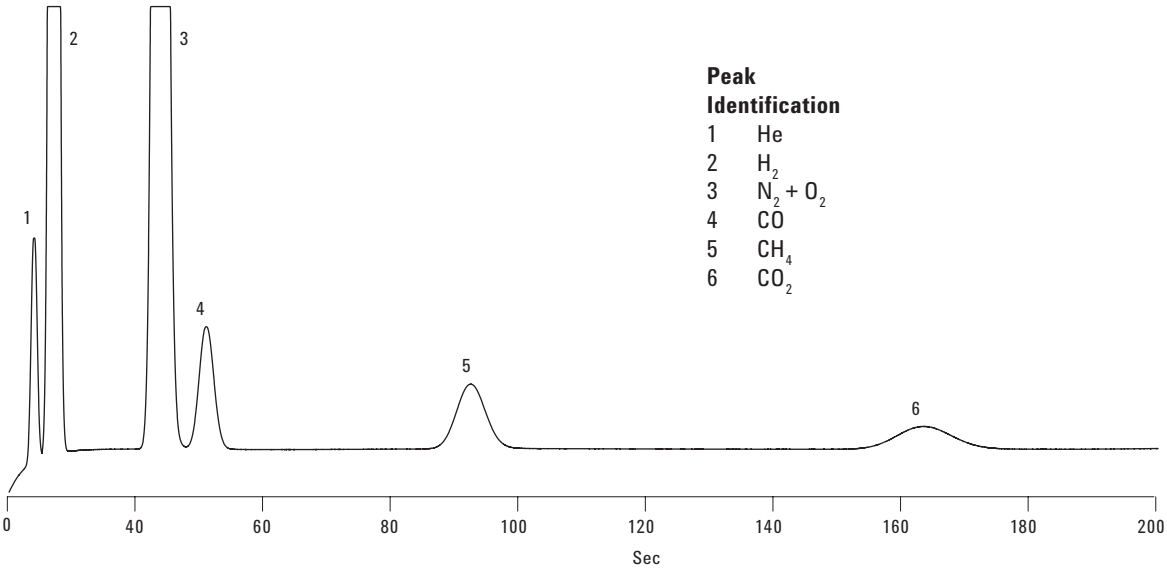

Figure 1. Excellent baseline separation of a gas sample on a COX column.

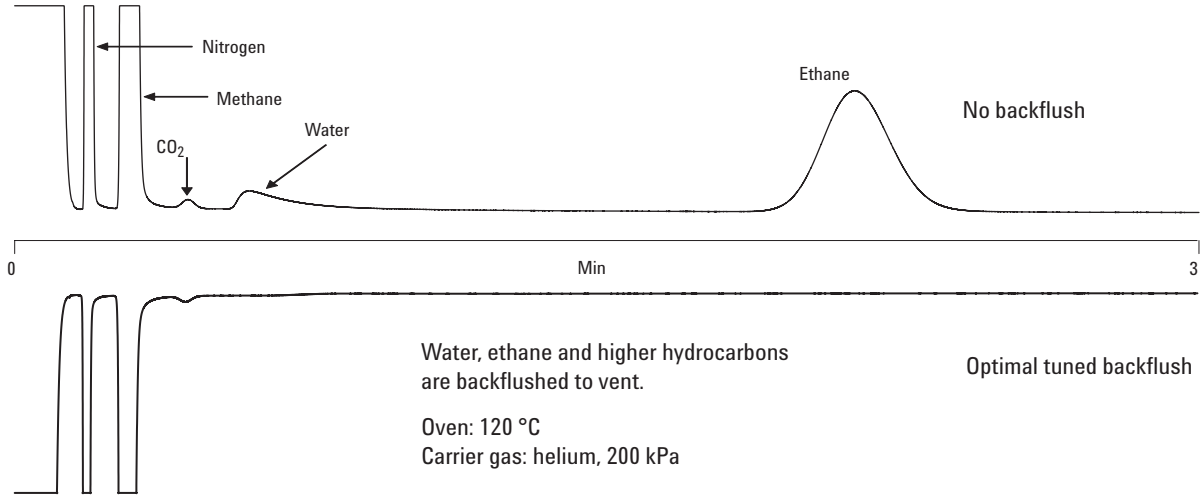

Figure 2. Backflush of water and ethane.

Other components such as water and higher hydrocarbons were backflushed to vent.

If the backflush time is set at a high value then virtually all the sample components enter the analytical column and eventually elute. However, if higher hydrocarbons are present the COX column is polluted because these components elute late and can influence the succeeding analysis.

Figure 2 shows the elution of water and ethane if no backflush is applied. If the backflush time is optimally tuned, water, ethane and higher hydrocarbons are backflushed to vent and does not enter the analytical column.

Conclusion

For the analysis of permanent gases the COX column is a good alternative to the commonly used Molsieve column.

Although the COX column does not separate oxygen and nitrogen, it does separate hydrogen and helium. In addition, carbon dioxide is analyzed and water elutes from the COX column. Repeatability figures are good, ensuring reliable analysis results.

The COX module can be equipped with a precolumn. This allows backflush of higher components and prolongs column lifetime.

The Agilent 490 Micro GC is a rugged, compact and portable "lab-quality" gas analysis platform. When the composition of gas mixtures is critical, this fifth generation Micro Gas Chromatograph generates more data in less time for faster and better performance.

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Printed in the USA
December 28, 2010
5990-7054EN

Agilent Technologies

Direct analysis of trace metallic impurities in high purity hydrochloric acid by Agilent 7700s ICP-MS

Application Note

Semiconductor

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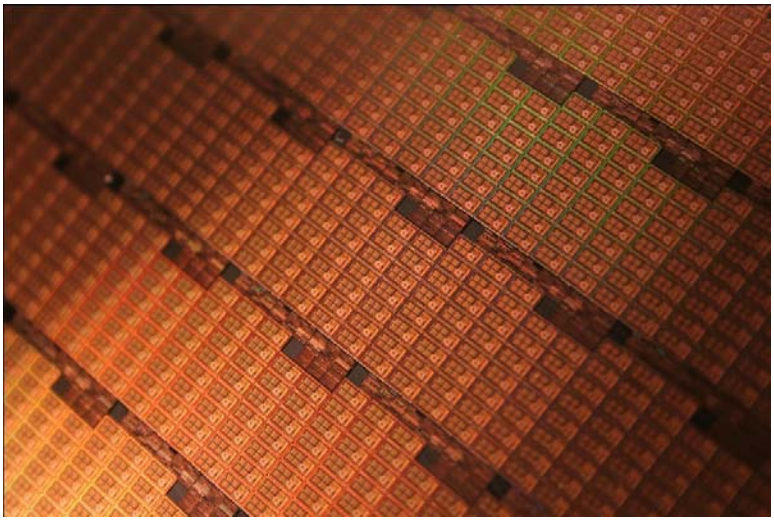

Abstract

This application note illustrates the advanced analytical performance and robustness of the Agilent 7700s ICP-MS for the direct determination of metallic impurities in high purity concentrated hydrochloric acid (HCl). The 7700s incorporates a third generation Octopole Reaction System, the ORS^3, which effectively removes polyatomic interferences, allowing ultimate detection limits to be achieved for elements that suffer from severe chloride-based interferences. For example, the polyatomic ion $^{40}Ar^{35}Cl^+$ can be eliminated by the ORS^3 allowing the direct measurement of As at mass 75, and permitting accurate analysis of As at trace levels in undiluted concentrated HCl. Enabling direct analysis of concentrated acids eliminates the dilution step from the sample preparation procedure, and so significantly reduces the potential for sample contamination.

Agilent Technologies

Introduction

Hydrochloric acid is frequently used to remove metallic impurities on the surface of silicon wafers. Together with hydrogen peroxide, this cleaning method is well known as RCA Standard Clean 2 (SC-2). The manufacturing process of semiconductor devices requires routine monitoring of contaminants in HCl, and ICP-MS is the accepted tool for this purpose. Although HCl is diluted prior to use for SC-2, the concentration of industrial grade HCl is usually 20% or 35%, depending on the method of production. Because HCl is highly corrosive; direct introduction of concentrated HCl into an ICP-MS is normally avoided. Moreover, introduction of HCl at high concentration leads to the formation of a large number of polyatomic ions in the ICP, which cause significant spectral interferences with some key elements of interest, for example, $H_2{}^{37}Cl^+$ on $^{39}K^+$, $^{35}Cl^{16}O^+$ on $^{51}V^+$, $^{35}Cl^{16}OH^+$ on $^{52}Cr^+$, $^{35}Cl^{37}Cl^+$ on $^{72}Ge^+$, $^{37}Cl_2{}^+$ on $^{74}Ge^+$, and $^{40}Ar^{35}Cl^+$ on $^{75}As^+$. Consequently, some methodology for the analysis of high purity HCl by ICP-MS has recommended sample pre-treatment steps to remove the chloride matrix, which can lead to analyte loss and sample contamination. However, the Agilent 7700s ICP-MS is manufactured using robust and anti-corrosive materials, which means that undiluted HCl can be analyzed directly, while the ORS^3 drastically improves the efficiency of removing polyatomic ions, allowing many elements to be determined at lower detection limits than were previously possible.

Experimental

Instrumentation

An Agilent 7700s ICP-MS fitted with an optional third cell gas mass flow controller (in addition to the standard collision (helium) and reaction (hydrogen) gas lines) was used throughout. The optional cell gas line is required for specialized applications, including analyses where optimum interference removal requires a highly reactive cell gas such as ammonia. The standard 7700s sample introduction system was used, consisting of the following parts (part numbers for replacements are shown in brackets):

- Pt sampling cone (G3280-67036)
- Pt skimmer cone with Cu base (G3280-67064)
- PFA nebulizer with uptake rate of 200 µL/min (G3139-65100)
- Quartz torch with 2.5 mm internal diameter injector (G3280-80001)
- Quartz spray chamber (G3280-80008).

Materials and reagents

High purity hydrochloric acid, TAMAPURE-AA100 (20%), was purchased from TAMA Chemicals, Japan. Undiluted HCl was introduced directly into the ICP-MS, to eliminate any sample preparation steps and thereby significantly reduce the potential for sample contamination.

Calibration standard solutions were prepared by spiking a mixed multielement standard (SPEX Certiprep) into an acid blank at 10, 20, 50 and 100 ppt.

Results and discussion

Detection limits and background equivalent concentrations

Forty two elements were measured using the 7700s operating in multiple tune modes. Data was acquired in an automated sequence of cool plasma, no gas and gas modes, during a single visit to the sample vial. Sample-to-sample run time was approximately 6 minutes. Data for each of the modes was combined automatically into a single report for each sample. Detection Limits (DL) and Background Equivalent Concentrations (BEC) are show in Table 1. DLs were calculated from 3σ of 10 measurements of the acid blank.

Table 1. 7700s ICP-MS DLs and BECs in 20% high purity HCl

Element	m/z	Mode	DL ppt	BEC ppt
Li	7	cool	0.016	0.004
Be	9	no gas	0.13	0.11
B	11	no gas	4.5	9.7
Na	23	cool	0.44	1.3
Mg	24	cool	0.11	0.22
Al	27	cool	0.79	1.1
K	39	cool/NH_3	0.40	0.50
Ca	40	cool/NH_3	1.1	2
Ti	48	He	0.71	0.68
V	51	NH_3	2.1	2.0
Cr	52	cool/He	4.5	12
Mn	55	He	1.57	2.84
Fe	56	cool	2.4	4.2
Co	59	He	0.20	0.13
Ni	60	He	3.03	4.43
Cu	63	cool	0.49	0.59
Zn	64	He	2.1	2.9
Ga	71	He	0.47	0.31
Ge	74	He	2.1	13
As	75	He	4.0	16
Se	78	He	5	5.5
Sr	88	He	0.21	0.061
Zr	90	He	0.11	0.03
Nb	93	He	0.34	0.43
Mo	98	He	0.52	0.67
Ru	101	He	0.05	0.01
Pd	105	He	0.57	0.51
Ag	107	He	0.056	0.033
Cd	114	He	0.41	0.52
Sb	121	He	2	2.8
Te	125	He	5.4	1.1
Ba	138	He	0.076	0.067
Hf	178	He	0.06	0.015
W	182	He	0.094	0.13
Re	185	He	0.49	0.54
Ir	193	He	0.1	0.07
Au	197	He	0.15	0.4
Tl	205	He	0.054	0.024
Pb	208	He	0.37	0.56
Bi	209	He	0.44	0.33
Th	232	He	0.01	0.003
U	238	He	0.032	0.013

Cr and K determination

Cool plasma is a proven technique used to remove plasma-based interferences. Although it has been largely superseded by Collision Reaction Cell (CRC) methodology, cool plasma remains the most effective analytical mode for some elements in certain matrices. Furthermore, the 7700s provides an enhanced cool plasma mode of operation, delivering improved robustness and stability due to the use of the new frequency matching RF generator. Used together with the ORS[3], cool plasma has recently been demonstrated to provide a new, powerful mode to remove interferences[1].

Because the major isotope of chromium ($^{52}Cr^+$) suffers an interference from $^{35}Cl^{16}O^1H^+$, chromium was determined using cool plasma and He mode. With cool plasma (low plasma RF power), production of $ClOH^+$ ions is suppressed because of its high ionization potential (I.P.) of 11 eV[2]. For further analytical improvement, He mode was used in combination with the cool plasma conditions to completely eliminate any remaining $^{35}Cl^{16}OH^+$ ions. The resultant calibration curve for ^{52}Cr is shown in Figure 1.

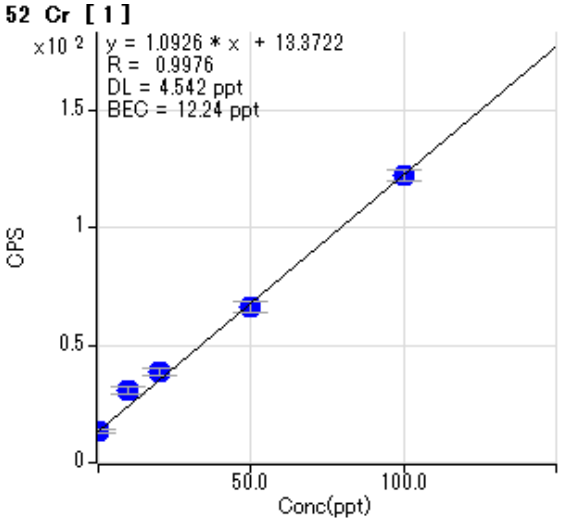

Figure 1. ^{52}Cr calibration curve obtained using He mode and cool plasma

The approach of using the ORS³ with cool plasma is also effective for other elements such as potassium. In order to suppress the interference of $H_2{}^{37}Cl^+$ on $^{39}K^+$, ammonia was selected as the cell gas with cool plasma. While there are very few cases where such highly reactive cell gases are required, there are some specific cases where such gases offer the lowest DLs. The intense $H_2{}^{37}Cl^+$ interference that affects K at mass 39 is not very reactive with H_2, so H_2 cell mode does not give sufficiently good interference removal for the lowest DL to be achieved in the highest purity HCl. The calibration curve for K (shown in Figure 2) illustrates the effective removal of the $H_2{}^{37}Cl^+$ interference using this novel mode of acquisition, providing a K BEC of 0.5 ppt and DL of 0.4 ppt in undiluted (20%) HCl.

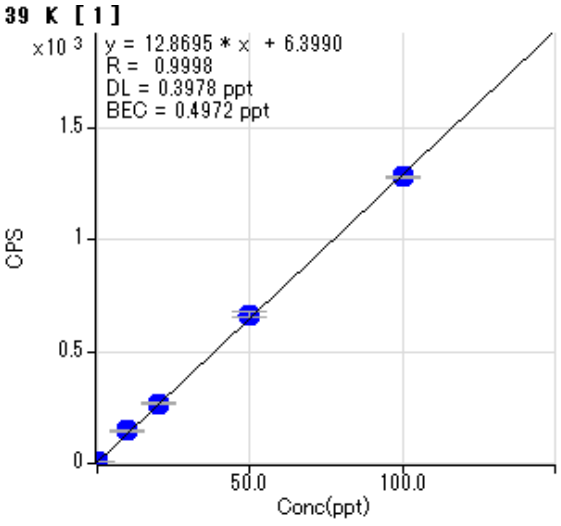

Figure 2. ^{39}K calibration curve obtained using NH_3 mode and cool plasma

Ge and As determination

The newly developed ORS³ of the 7700s improves the removal of polyatomic interferences using He mode with Kinetic Energy Discrimination (KED), and also promotes Collision Induced Dissociation (CID) for relatively weakly bound polyatomic ions.

Germanium has 3 major isotopes at 70, 72 and 74 amu that suffer from Cl-based polyatomic interferences, namely $^{35}Cl^{35}Cl^+$, $^{35}Cl^{37}Cl^+$ and $^{37}Cl^{37}Cl^+$. As the dissociation energy of Cl_2^+ is approximately 4 eV (3.95 eV for $^{35}Cl^{35}Cl^+$)³, CID of Cl_2^+ would be unlikely to happen with the previous generation ORS, which provided a collision energy of only 0.9 eV in He mode. In contrast, in the ORS³ of the 7700s, the collision energy is increased to 5 eV, facilitating CID of several polyatomic ions including Cl_2^+. The performance of the 7700s with ORS³ operating in high energy He mode is illustrated in Figure 3, which shows the calibration curve, DL and BEC for ^{74}Ge in a matrix of 20% HCl.

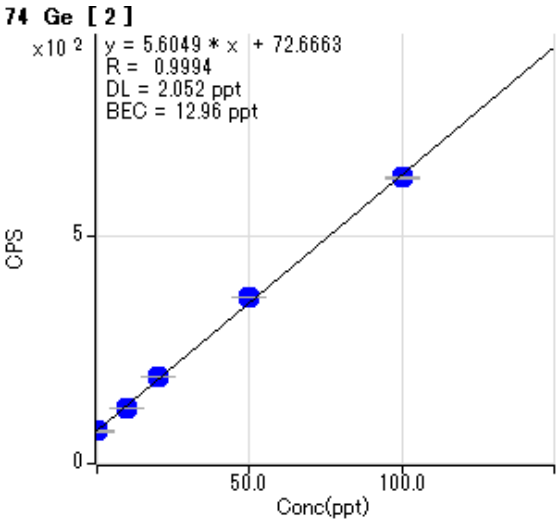

Figure 3. ^{74}Ge calibration curve obtained using high energy He mode

Arsenic has a single isotope at *m/z* 75, that can suffer an interference from the polyatomic ion $^{40}Ar^{35}Cl^+$ that readily forms in a chloride matrix, making it extremely difficult to determine ^{75}As at low levels directly at mass 75. The ArCl interference on As can be avoided by indirectly measuring As at 91 amu as the AsO^+ ion, which is formed either by applying cool plasma conditions or via the use of O_2 cell gas in the CRC. The latter approach utilizes hot plasma conditions but the measurement of As at mass 91 can still be affected by a $CaClO^+$ interference that forms from $CaCl^+$ when O_2 cell gas is used. Furthermore, AsO^+ at mass 91 suffers an isobaric interference from $^{91}Zr^+$, an overlap that does not occur under cool plasma conditions, since Zr is not ionized in a cool plasma. However, as with Cl_2^+, the higher collision energy of He mode in the ORS³ of the 7700s means that the $ArCl^+$ ion can also be dissociated by CID. This allows As to be determined at low levels

directly at 75 amu in 20% HCl, thus avoiding the use of both cool plasma and O_2 cell gas. A typical calibration curve for As in 20% HCl is shown in Figure 4.

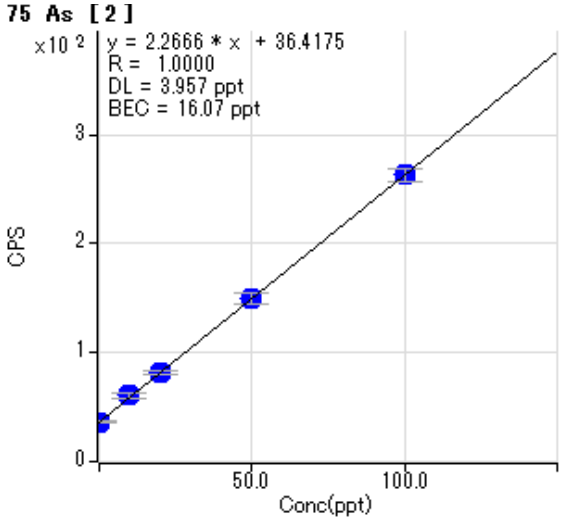

Figure 4. ^{75}As calibration curve obtained using high energy He mode

V determination

The $^{35}Cl^{16}O^+$ interference on $^{51}V^+$ can also be eliminated using NH_3 as the cell gas, but under normal hot plasma conditions (1600 W). The increased collision energy due of the ORS^3 improves the reaction efficiency and gives a significant improvement in the DL and BEC, as shown in Figure 5.

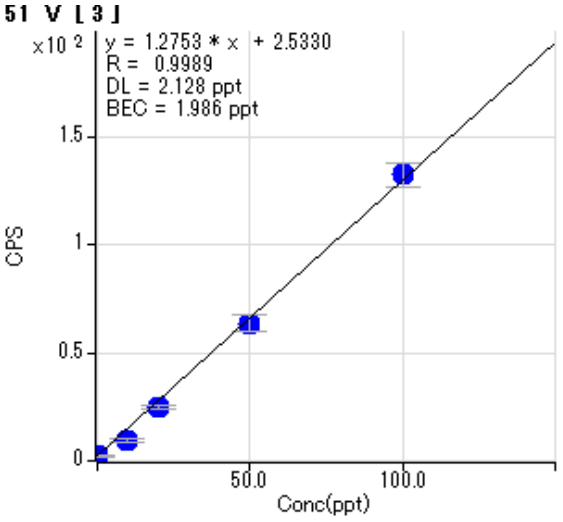

Figure 5. ^{51}V calibration curve obtained using NH_3 and normal plasma mode

Conclusions

The high performance of Agilent ICP-MS systems for the analysis of trace metallic impurities in concentrated HCl has been described previously[4]. Now, the Agilent 7700s ICP-MS with unparalleled cool plasma performance and ORS^3 collision/reaction cell further improves the detection limits for the analysis of high purity acids. The newly developed ORS^3 can be fitted with up to 3 cell gas lines (2 are included as standard), allowing total flexibility in both collision and reaction modes. The ORS^3 cell improves performance for several critical elements by increasing the efficiency of both collision and reaction mode, and providing enhanced dissociation of certain polyatomic ions by CID. These developments now allow several elements like Cr, K, Ge, As and V to be determined at lower concentrations than previously possible in a chloride matrix.

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Published May 4, 2011
Publication Number 5990-7354EN

Agilent Technologies

Onsite additive depletion monitoring in turbine oils by FTIR spectroscopy

Fast, easy antioxidant measurement

Application Note

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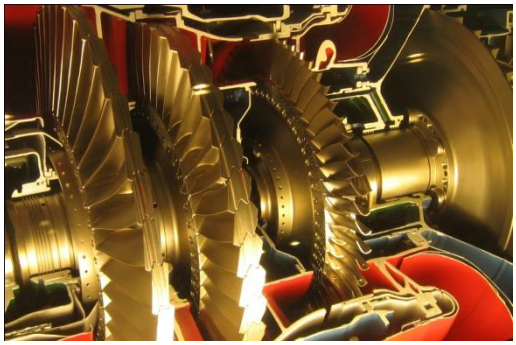

Abstract

Agilent 5500t FTIR spectrometers can independently measure phenolic and aminic antioxidants in turbine oil and provide the time sensitive results necessary to assist in preventing a non-scheduled shutdown by ensuring reliable operation of the turbine equipment. The 5500t FTIR system alerts, at pre-set warning levels, when the phenolic and aminic antioxidants are at or approaching minimal concentration milestones, and thus helps prevent turbine oils from reaching the critical point in the oxidation cycle of oil. Measurement is quick, easy and can be performed at-site. It requires no sample preparation, calibration, or electrode maintenance involved with voltammetric systems.

Agilent Technologies

Introduction

The Agilent 5500t FTIR (Fourier transform infrared) spectrometer, a compact, easy-to-use and affordable system, provides the ability to perform real-time, onsite analysis of high value assets such as turbines. With 5500t FTIR spectrometers, the lubrication specialist has the ability to simultaneously monitor key parameters such as oxidation, additive concentrations and levels of water in lubricants. This application note will demonstrate the ability to monitor the depletion of key additives using the 5500t FTIR spectrometer.

Antioxidants in turbine oil

The phenolic and aminic antioxidants in turbine oils function as preservatives, which prevent the oil from oxidizing and forming harmful varnish deposits. Oxidation causes turbine oils to quickly lose viscosity and wetting characteristics, which protect metal contact surfaces and prevent wear. Oxidation arises from a combination of sources including elevated temperatures, extreme pressures, high shear conditions, the presence of water and metal particles, and is accelerated by electrostatic sparking, particularly in certain gas turbine systems. Antioxidants inhibit the formation of these decomposition products, however once the antioxidants are consumed, the process accelerates exponentially and at a certain critical point, corrective action has negligible benefit. The 5500t FTIR system measures both the antioxidant levels and the amount of oxidation present, to ensure that corrective action is taken before this critical point is reached.

Measuring antioxidants in turbine oil with the Agilent 5500t FTIR

The primary and most abundant antioxidant is the phenolic antioxidant, which works synergistically with the aminic antioxidant. It is postulated that the phenolic antioxidant protects the workhorse aminic antioxidant, which has the ability to recharge itself over and over during the cycles of oxidation. This is consistent with data we have obtained, as will be demonstrated later in this application note.

The phenolic and aminic antioxidants in turbine oil have prominent absorbance bands in select regions of the infrared spectrum, thus enabling FTIR spectroscopy to be an ASTM preferred means of measurement. Figure 1 shows one of the major infrared bands of the phenolic antioxidant in turbine oil and the change in the band, as a function of time, as the antioxidant is depleted. Similarly, Figure 2 illustrates the incremental diminishment of the aminic antioxidant as the turbine oil ages. These bands are so characteristic of these two species that they are often called 'fingerprint bands' and they are the functional groups that are automatically tracked by the 5500t FTIR spectrometer software.

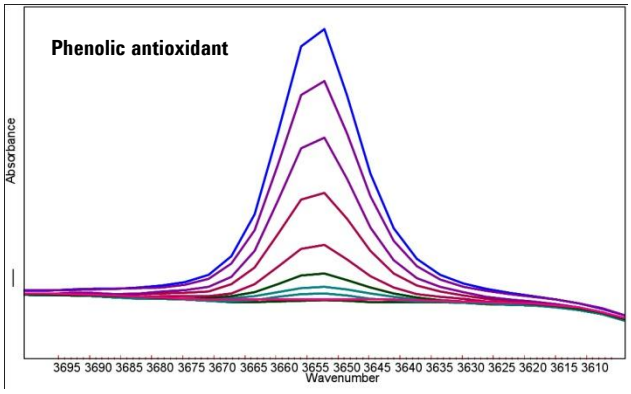

Figure 1. FTIR spectral overlay of the phenolic antioxidant functional group bands depleting as a function of time. The strongest band (light blue) is that of new ISO 32 turbine oil and the weakest absorbance (light green) is from turbine oil that has started to show some oxidation.

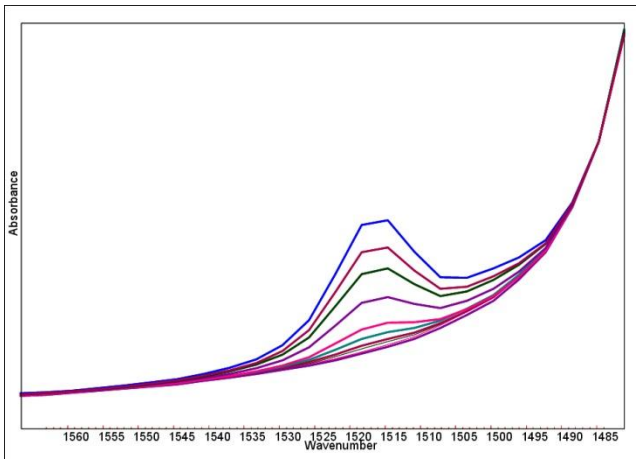

Figure 2. FTIR spectral overlay of the aminic antioxidant functional group depleting as a function of time. The strongest absorbance (red) is aminic antioxidant in new ISO 32 turbine oil and the weakest bands (blue and green) are from turbine oil with spent antioxidant.

The 5500t FTIR software (Figure 3) stores the FTIR spectrum of the initial new or reference oil. When in service used oil is measured, its spectrum is overlaid and compared to the reference oil. The user is provided a weight % for each phenolic and aminic antioxidant as well as a visual overlay of the spectral regions associated with each additive. The turbine oil methods also provide oxidation and nitration as a percentage of an upper limit, which is set from oxidation tests. The 5500t FTIR software is also programmed to inform the user via a yellow 'Monitor Frequently' warning when each additive is nearing the critical depletion points. Likewise, a red 'Change Immediately' warning is displayed on any additive, or other component such as water or oxidation, which has reached a critical threshold. Therefore, if both the phenolic and aminic antioxidants are in the red zone the critical saturation point for oxidation is imminent. The oxidation and ppm water are also provided with visual comparisons to the reference oil.

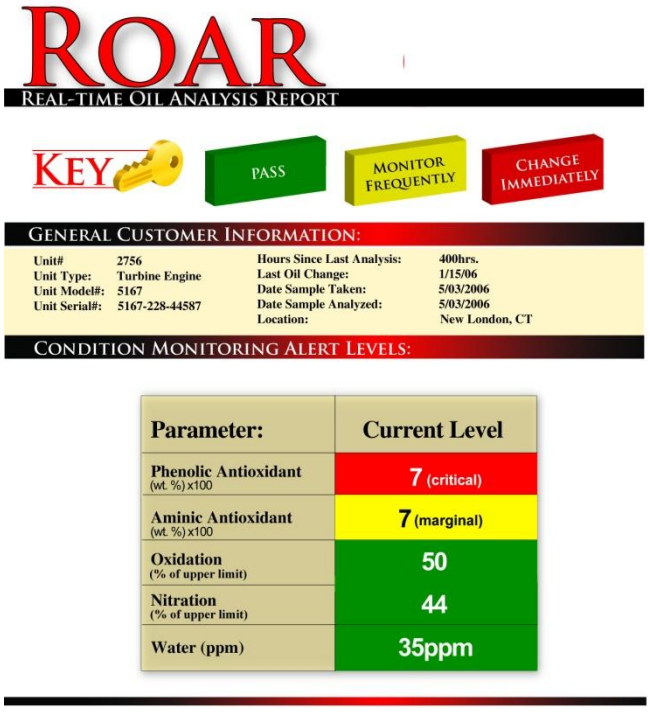

Figure 3. Agilent 5500t FTIR software presents the user with the specific concentration of phenolic and aminic antioxidants as well as crucial information about oxidation by-products and level of water contamination

The relationship between antioxidant depletion and oxidation

We will demonstrate the relationship of antioxidants and oxidation formation as well as the ability of the 5500t FTIR system to both predict and detect oxidation formation before the critical point is reached. Metallic iron and copper, known oxidation catalysts were added to used Chevron ISO 32 turbine oil that was in service 4 months in a steam turbine system. The iron and copper catalysts accelerate the inherent thermal oxidation mechanism, and are used in most oxidation potential tests such as RPVOT (D2272), Universal Oxidation Test (D6514 and D5846), and TOST (D943).

This mixture was heated at 135 °C for 26 days at atmospheric pressure in air, and small samples of the oil were removed every 2 to 3 days. The samples were analyzed using a 5500t FTIR spectrometer and the peak area measurements for phenolic antioxidant, aminic antioxidant, and oxidation products were recorded and plotted as a function of time as shown in Figure 4. As shown, the phenolic antioxidant diminishes to about 40% of the original amount in a relatively short time, however, the aminic antioxidant is observed to stay above 80% for almost the whole life span of the oil. Some of the initial drop in the phenolic antioxidant is due to evaporation which is a known problem with certain more simple phenolic antioxidants. The aminic antioxidant is observed to have three stages:

- Stage 1: The aminic antioxidant level is fairly constant and remains at this level approximately halfway thru the useful life of the oil. The initial slight increase in aminic may be due to volatiles in the oil, which can evaporate from the new oil during high temperature operation, thus slightly increasing the concentration of the aminic antioxidant.
- Stage 2: The aminic antioxidant depletes rapidly by about 25% at the mid-way point in the useful life of the oil.
- Stage 3: After the phenolic drops below 30% of the original concentration (70% depletion) the aminic begins a rapid descent from 80 to 40%. At this

critical point, the oxidation process accelerates exponentially. Corrective action would need to be taken prior to this stage in order to extend the useful lifespan of the oil.

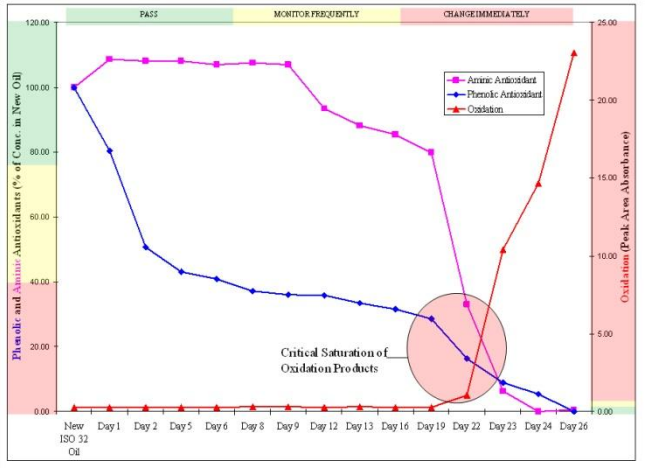

Figure 4. The additive depletion (% relative to new oil concentrations, left scale) and oxidation formation (right scale) trend analysis in thermally stressed ISO 32 turbine oil generated using the Agilent 5500t FTIR spectrometers

Lube 'useful life' measurements – Agilent 5500t FTIR versus voltammetric methods

As we have demonstrated in this application note, the 5500t FTIR system measures each antioxidant species individually, as well as providing a direct measurement of the degree of oxidation in the oil.

Cyclic voltammetric methods rely on mixing an exact amount of an oil sample with exact amounts of an electrolyte solution, the solution is shaken, at which point the antioxidants are extracted into the electrolyte solution. The results require a sample of the new oil for comparison and the used oil results are given in % depletion instead of exact concentrations such as weight %. This also causes inaccurate results if the used oil has been mixed with slightly different brands of oils. Another potential drawback to this technique is the antioxidant extraction from oil is never 100% efficient (typical extraction efficiencies are 75 to 95%), so not all of the active antioxidants are being measured. The pipetting required for voltammetric methods is not as accurate for higher viscosity oils, especially with gear oils or greases. Separate electrolyte solutions are needed for measuring oxidation and additional different solutions are needed to analyze crankcase or polyol ester based oils. The voltammetric method doesn't measure water or nitration, and contaminants in the oil such as EHC hydraulic fluid may cause inaccurate results. However, the 5500t FTIR spectrometer can detect the presence of contaminants such as EHC hydraulic fluid in turbine oils or gear oil in turbine oil.

The 5500t FTIR system requires only a drop of neat oil for its measurements and no sample preparation, whereas, voltammetric systems require careful pipetting techniques and an extraction step using an electrolyte solution. The FTIR system comes fully calibrated for weight % antioxidant functional groups in turbine, gear, hydraulic, and crankcase oils. Metal particles, water, or organic salts (that is, ionized carboxyls such as copper carboxylates) will not interfere with the antioxidant measurements using the 5500t FTIR system. The 5500t FTIR system has virtually no learning curve, requires no maintenance nor special chemicals or reagents for antioxidant measurement. Since the antioxidants can be monitored independently using the 5500t FTIR, re-additization can be carefully controlled and monitored. The effectiveness of top-offs, bleed and feed, filtration, and dehydration can be monitored as well. Mixing oil brands is not recommended, but the weight % phenolic and aminic antioxidants are still accurate measurements no matter what mineral oil basestocks are mixed together.

Conclusions

Agilent 5500t FTIR spectrometers are capable of independently measuring phenolic and aminic antioxidants in turbine oil and provide the time sensitive results necessary to assist personnel in preventing a non-scheduled shutdown by ensuring reliable operation of the turbine equipment. The 5500t FTIR system is designed to alert, at pre-set warning levels, when the phenolic and aminic antioxidants are at or approaching minimal concentration milestones, and thus help prevent turbine oils from reaching the critical point in the oxidation cycle of oil.

The capability of measuring additives in turbine oil by FTIR spectroscopy eliminates the issues associated with other measurements, including the need for sample preparation, calibrating, and maintaining electrodes based on voltammetric systems. The measurements are more rapid than electrode based antioxidant monitoring equipment, and minimize the dependency on the skill of the operator and the operating condition of the equipment. As importantly, the ability to measure antioxidant levels at-site via FTIR means that the results will be more convenient, more frequent, and obtained far more rapidly than samples that are sent for offsite analysis to a traditional oil analysis lab.

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Published May 1, 2011
Publication Number 5990-7801EN

Agilent Technologies

Onsite quantitative FTIR analysis of water in turbine oil

Application Note

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Introduction

The availability of the Agilent 5500t FTIR spectrometers, which are compact, easy-to-use and affordable systems, provides new capabilities for real-time, on-site analysis of high value assets such as turbines. With the 5500t FTIR spectrometers, the lubrication specialist now has the ability to monitor key parameters such as oxidation, additive depletion and levels of water in lubricants. In this application brief, we will demonstrate that the Agilent 5500t FTIR spectrometer has the sensitivity, accuracy and reproducibility to determine the level of water in turbine oils without the difficulties associated with the conventional Karl Fischer technique.

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Water in turbine oil

An important parameter to measure

The amount of water in turbine oil is critical to the performance and longevity of the equipment. Excessive amounts of entrained water in the turbine oil can cause premature failure of the turbine unit, typically due to changes in the physical properties induced by the presence of water. Physical properties of oil affected by the presence of water include viscosity (measure of the oil's resistance to flow), specific gravity (density of the oil relative to that of water), and the surface tension (a measure of the stickiness between surface molecules of a liquid). All of these properties are important for the ability of the oil to coat, lubricate, and protect the critical mechanical clearances. In addition, water in turbine oil can accelerate additive depletion and contribute to chemical degradation mechanisms such as oxidation, nitration, and varnish formation.

On-site analysis is highly desirable

The ability to measure water on-site, as soon as possible after drawing the sample, is a substantial benefit in obtaining accurate water level results. Off-site analysis for trace water in oil may be compromised due to variability of water concentration introduced by storage, transportation, or shipment of a sample. Furthermore, turbine oils contain demulsifying additives that cause microscopic water droplets to separate from the oil and concentrate in layers at the bottom and sides of containers. This demulsifying action takes time to occur, and can cause large variations in analytical measurements. Also, oil samples can sometimes pick up or lose water simply depending on the type of sample container used.

Measuring water in turbine oil

Karl Fischer (KF) coulometric titration is typically used to determine the amount of water in turbine oils. Karl Fischer has some practical draw backs for on-site analysis including complicated sample preparation, the use of hazardous and expensive chemical reagents, and length of time required to perform the analysis. However, KF analysis is considered the "gold standard" method for analyzing water in oil because it provides accurate and precise answers.

FTIR spectroscopic analysis eliminates many of the concerns associated with measuring water via Karl Fischer titration. The spectroscopic method, can be performed in far less time than KF measurement, does not require reagents and when a rugged and easy-to-use FTIR system such as the 5500t instrument is used, FTIR is ideal for on-site analysis. Karl Fischer titrations require about 10-15 minutes to perform, with the instrument properly conditioned and equilibrated overnight. For KF analysis the oil must be carefully weighed on a high precision balance before and after injecting into the titration vessel. Following each analysis the KF instrument takes another 5-10 minutes to re-equilibrate. The FTIR analysis takes about 2 minutes to perform and is immediately ready for the next sample analysis after a simple cleaning with a tissue.

This application brief will demonstrate that FTIR spectroscopic analysis using the 5500t FTIR is as accurate and precise as the Karl Fischer method within the analytical range necessary for measuring water in turbine oil. Using the 5500t, we have developed two FTIR methods for water in turbine oil and have calibrated and evaluated them against the Gold Standard Karl Fischer procedure.

Water in turbine oil - the FTIR method

Used turbine oil (C&C Oil Co.) was homogenized with water and aged overnight at 70 °C to make a very high water standard. This standard was then diluted with various amounts of a used turbine oil mix, which contains oil in-service four months and another more degraded oil with a dark amber color. These dilutions had various amounts of water based on how much "as is" oil was added. The samples were mixed well and allowed to equilibrate for about an hour before they were analyzed by coulometric Karl Fischer titration (Metrohm 756 KF Coulometer) to determine the concentration of water. The samples were run in

duplicate by KF before the infrared spectra were acquired using the 5500t FTIR spectrometer. The water concentrations for the prepared standards ranged from 22-3720 ppm (parts per million). The water IR absorbance measurement for each standard sample was plotted versus the corresponding KF water data to obtain a residual least squares linear regression. The IR spectra were also analyzed using a partial least squares method to develop a regression model for the quantitative predictions of water in oil.

Calibration results

The IR analysis and calibration models indicate a very good correlation between the 5500t FTIR measurements and the Karl Fischer water data. Two different methods were developed for the quantitative measurement of water in oil using the 5500t spectrometer. The first is a relatively simple conventional IR absorbance model following Beer's Law that uses the region of the IR spectrum in which water strongly absorbs, known as the O-H stretch region. The second method uses multiple regions of the IR spectrum with partial least squares (PLS) chemometric modeling to reduce the effects of noise, baseline variance, and other interfering factors.

Beer's law model

In the first method, a peak area absorbance measurement provides a detection limit of about 30 ppm water in oil (Figure 1). The IR spectra of 15 samples with KF water values ranging from 7-270 ppm were used to build a linear calibration curve that follows Beer's Law (Figure 2). The weakest water absorbance in Figure 1 is new turbine oil with 30 ppm of water (Red) and the strongest water absorbance is shown in blue with a KF water value of 1460 ppm. The calibration plot is shown in Figure 2 with a correlation coefficient of R2=0.977 and a standard error of validation (SEV) of ~40 ppm (20-270 ppm range). The addition of higher water concentration standards to the calibration improves the correlation coefficient to R2=0.996.

Therefore, this calibration is optimized for the low levels of water (<500 ppm), but is still quite accurate for predications of higher water levels above 500 ppm if necessary.

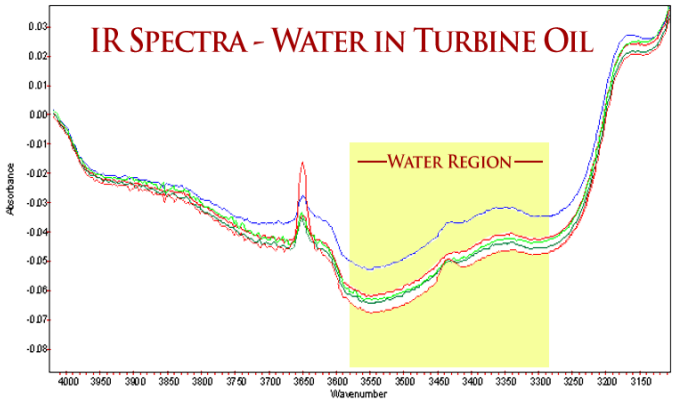

Figure 1. The overlaid IR spectra of turbine oil with the water absorbance region expanded, water values from bottom to top are 30 ppm (red), 80 ppm (dark green), 217 ppm (light green), 533 ppm (red), and 1460 ppm (blue)

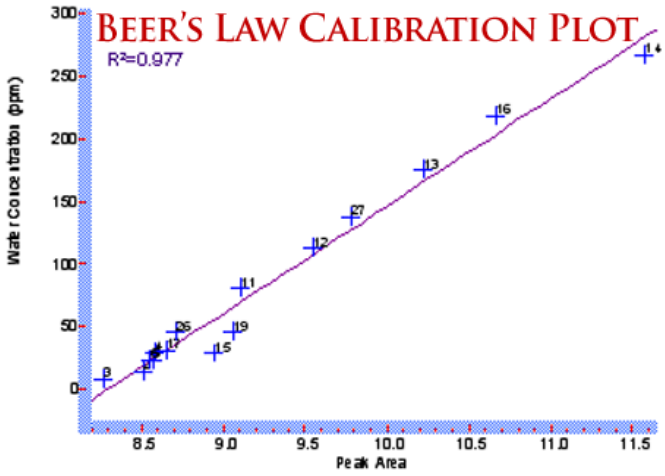

Figure 2. The calibration plot of KF water values (ppm) versus peak absorbance area for water in turbine oil using a Beer's Law peak area method

Pls model

The PLS chemometric model uses more sophisticated mathmatics to develop models that are typically more robust and accurate than the conventional Beer's Law IR absorbance method demonstrated above. Whereas both the PLS and the Beer's law quantitative methods for water in oil are sufficient for classification into 100 ppm ranges (i.e. <100 ppm, 100-200 ppm, 200-300 ppm, etc.), the PLS method provides the most accurate

KF water prediction values over the whole range of 30-1500 ppm.

In order to develop the PLS method for water in oil, we used 23 standards covering a range from 7-1460 ppm water. We then recorded the IR spectrum and measured the water level by the KF method. The two sets of results were correlated with partial least squares and the predicted versus actual KF values are plotted in Figure 3 and indicate a correlation coefficient of R^2=0.990.

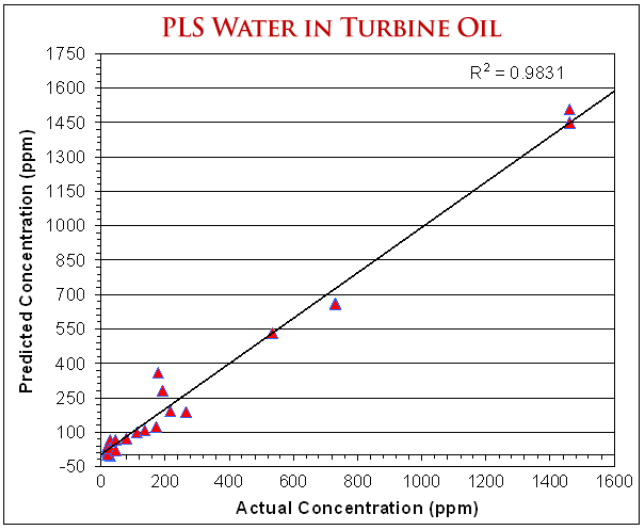

Figure 3. The PLS predicted versus actual plot of KF values using Agilent 4500 Series FTIR spectrometer

Predictions

To validate each FTIR method, 15 unknown mixtures were made by mixing used turbine oils with hydrated turbine oils, and running them by KF (in duplicate) and by FTIR (in triplicate). The coulometric KF performance was verified using 100 ppm and 1000 ppm NIST reference standards. It was found that thorough mixing was important to obtain quality data, due to the heterogeneous nature of water in turbine oil. Environmental and experimental factors caused the KF duplicate measurements to typically vary by 30-60 ppm, measured consecutively in the 100-1000 ppm range. The FTIR water predictions indicated similar variations in replicate measurements of the same sample. The averages of the replicate measurements by KF and FTIR are compared in Table 1. Good agreement with the KF measurements is observed for both FTIR methods, however, the PLS predictions are statistically better in the 100-1500 ppm range. The standard deviation between the averaged PLS predictions and the averaged KF data (0-700 ppm range) are all below 30 ppm, except for one sample (#11). The Beer's Law method predictions are better in the 0-100 ppm range, and are sufficient to classify the water concentrations into ranges as follows: <100 ppm, 100-200 ppm, 200-500 ppm, and 500+ ppm.

Validation Sample	Beer's Law (ppm water)	PLS (ppm water*)	KF (ppm water)
Turbine Oil 1	26.5	-	27.5
Turbine Oil 2	160	194.6	199.7
Turbine Oil 3	125.2	139	145.1
Turbine Oil 4	15.1	-	12.4
Turbine Oil 5	21	-	19.8
Turbine Oil 6	63	64.5	40.8
Turbine Oil 7	251.8	219.3	215.3
Turbine Oil 8	117.9	70.3	111.1
Turbine Oil 9	539.3	685.4	663.3
Turbine Oil 10	350	300	246
Turbine Oil 11	340.7	367.3	285.7
Turbine Oil 12	251.8	244.4	206.5
Turbine Oil 13	2979.3	3780.5	367.4
Turbine Oil 14	1100.3	1375	1027.5
Turbine Oil 15	1219.2	1541.9	1362.4

Conclusions

We have shown that the Agilent 5500t FTIR Spectrometer is capable of measuring water in oil at the levels that are critical to the reliable operation of the turbine equipment. The capability of measuring water in turbine oil by FTIR spectroscopy eliminates the issues associated with Karl Fischer measurements including the need for expensive and hazardous consumables, the time required for the KF measurement as well as the dependency on the skill of the operator and the operating condition of the KF equipment.

As importantly, the ability to measure water levels at-site via FTIR means that the results will be more accurate, more reproducible and obtained far more rapidly than samples that are sent for off-site analysis to a traditional oil analysis lab. We have observed that low ppm levels of water are observed to change on an hourly basis if left open to air - a sample that initially was 200 ppm can have less than 100 ppm if left in an open sample container overnight. This is also true if the sample container is not filled to the top, and water can evaporate into the head space (air) of the jar. One can only imagine the level of error that is introduced when half filled jars are sent to off site labs.

The Agilent 5500t FTIR spectrometer can detect water at the necessary warning levels. The system can alert when water reaches 100 ppm and then issue a critical warning if the water reaches 200 ppm. In addition to the analysis of water, Agilent's Mobility spectrometers can measure the depletion of additives and determine the levels of oxidation and nitration by-products in turbine oils.

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Published May 1, 2011
Publication Number 5990-7806EN

Agilent Technologies

Simple, automated measurements of the photocatalytic properties of colorimetric species using the Agilent Cary 60 UV-Vis spectrophotometer with fiber optics

Application Note

Chemicals

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Summary

The Agilent Cary 60 UV-Vis spectrophotometer is the new, improved successor to the award-winning Cary 50 UV-Vis. In this short review, this instrument platform was evaluated for its potential to measure small (40 µL) samples of methylene blue *in situ* during exposure to high-intensity UV irradiation. Methylene blue is combined with other compounds used in a variety of applications, including use in cosmetics and sun screen products and environmental remediation in contaminated air and polluted water.

Introduction

By virtue of its unique optical design, we have previously demonstrated that the Cary 60 UV-Vis has no measureable effect on photobleaching of the polyaromatic hydrocarbon probe methylene blue[1], which has been shown to be particularly susceptible to photobleaching during continuous readings in UV-Vis instruments from other manufacturers[2]. In the present study, we extend our observations to applications in which photobleaching is desirable, induced by an external, high-intensity UV lamp. This is of significant benefit when analyzing the photochemical properties of electron-quenching compounds, such as methylene blue, that can have protective properties against a broad range of ailments associated with UV exposure, including cancer[3].

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Using the fiber optics microprobe accessory with the Cary 60, the purpose of this study was to develop an automated method of analysis to study the induced photobleaching of samples *in situ*, as opposed to conventional approaches that require sampling be made manually using a cuvette resulting in less accurate results and significant increase in time per analysis (Wang et al[4]).

In the present study, we use fiber optics in aqueous samples at 20 °C under conditions of normal laboratory fluorescent ambient lighting. This approach allows users to take the instrument to the sample, rather than the conventional approach in spectroscopy in which the sample is presented to the instrument. The unique optical configuration of the Cary 60 makes this possible mainly by virtue of the high-intensity xenon flash lamp combined with the latest electronics, enabling the system to effectively monitor small changes in absorbance without any effect of ambient light. Key benefits of this approach are discussed further in this document.

Apparatus and materials

Part Number	Description
G6860AA	Cary 60 UV-Vis with WinUV software and PC
7910035600	Fiber optic microprobe
G6866A	Fiber optic probe coupler

Methods and results

The Cary 60 instrument platform was fitted with the fiber optics coupler and microprobe as shown in Figure 1. Baseline readings were taken using purified water.

A solution of methylene blue (12.5 ppm, 60 mL) was prepared by diluting stock solution (400 ppm) in purified water. Incrementally, small aliquots of a solution of titanium (0.50 g/L, 200 µL) were added in order to assess and evaluate the effects of the photodegradation rate of methylene blue against time in the presence of titanium. Samples were placed in a Class 2 safety cabinet where they were irradiated with high-intensity UV light to induce photochemical reaction, being stirred continuously during the analysis, and the absorbance measured at 20 °C using a fiber optic microprobe.

Figure 1. The Cary 60 UV-Vis fitted with the fiber optics microprobe accessory provides a simple mechanism to measure a sample *in situ* and remote to the instrument

Using the Scanning Kinetics application module in the Cary WinUV software package, scans were taken at 2-min intervals from 400–800 nm over a period of 20 min. The effect of the high-intensity UV lamp on the photokinetic properties of the methylene blue solution was assessed in terms of peak height and blue-shifting of the spectra (Figure 2).

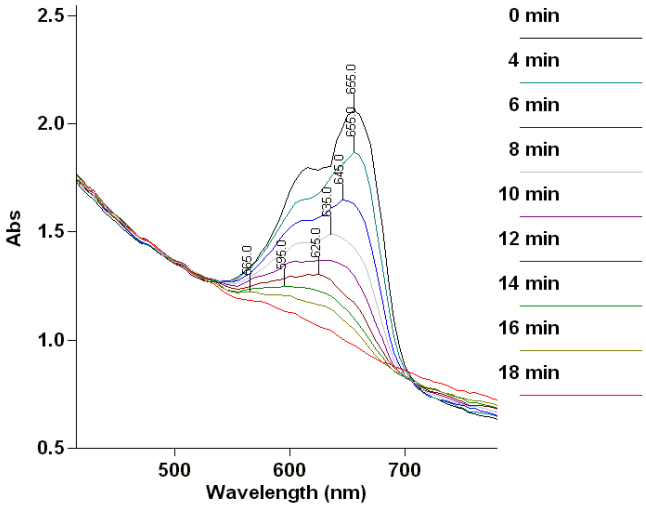

Figure 2. Scanning kinetics using fiber optics *in situ* of methylene blue under the exposure of a high-intensity UV lamp (Oriell 500 W Hg(Xe) lamp) over a period of 20 minutes within the range 400 to 800 nm. Labels refect maximum absorbance wavelengths

Discussion

Results in Figure 2 show significant photobleaching of the methylene blue solution and a blue-shift of the maximum absorbance peak at 655 nm over the 20-min period.

These results are comparable with those taken in a cuvette at room temperature, which conventionally demands transfer of aliquots of the sample to an instrument remote from the reaction chamber. This approach results in inaccurate data, particularly so when dealing with photosensitive samples such as methylene blue[2].

Conclusion

Results discussed above demonstrate that the Agilent Cary 60 instrument fitted with the fiber optics microprobe accessory provides a simple, cost-effective, rapid and versatile system for the automated measurement of photocatalytic reactions *in situ*. As far as the authors are aware, this is the first time this approach (i.e., with fiber optics) has been successfully accomplished.

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Published May 1, 2011
Publication Number 5990-7864EN

Agilent Technologies

Direct measurement of metallic impurities in 20% ammonium hydroxide by Agilent 7700s ICP-MS

Application Note

Semiconductor

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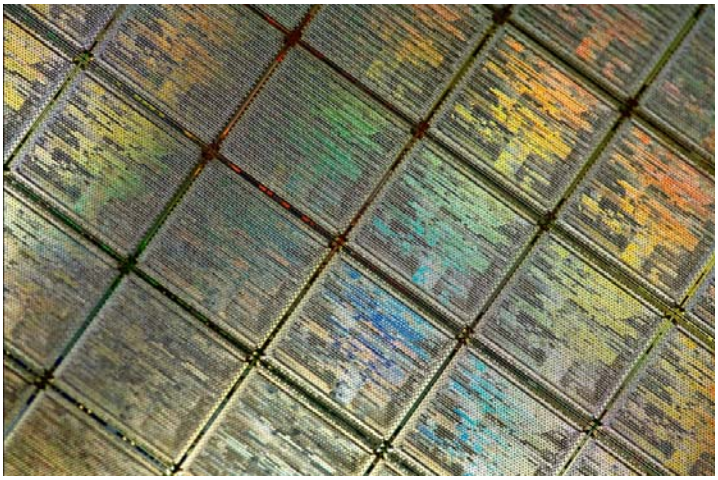

Abstract

Ammonium hydroxide (NH_4OH) is a chemical used in the manufacture of semiconductor devices, and must therefore be analyzed for trace metal impurities. The direct analysis of undiluted (20%) NH_4OH using ICP-MS is challenging, because its high vapor pressure causes plasma instability. However, dilution of the samples would degrade detection limits, so the ability to directly analyze 20% NH_4OH is important. The Agilent 7700s ICP-MS employs a unique, high-speed frequency-matching ICP RF generator that can maintain a stable plasma even when 20% NH_4OH is aspirated. The 7700s also features the most effective technologies for removing spectral interferences in high-purity chemicals, making it ideally suited to semiconductor chemical analysis. An Agilent 7700s, using an inert sample introduction system, was used to measure trace elements in high purity 20% NH_4OH. Detection limits in the single digit ppt or sub-ppt range were obtained for 48 elements, and long term (~7 hours) stability of approximately 5% RSD was achieved for a spike level of 100 ppt in 20% NH_4OH, demonstrating the ability of the 7700s to routinely measure trace contaminants in high purity 20% NH_4OH.

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Introduction

Many chemicals are used throughout the manufacturing process of semiconductor devices. Metallic impurities present in the chemicals and chemical mixes used can cause contamination and give rise to defects in the final product, so their levels must be strictly controlled. ICP-MS is the standard technique used for the measurement of metallic impurities in semiconductor chemicals. Among all the process chemicals, those that are used as part of the silicon wafer cleaning process are particularly important, as they are in direct contact with the wafer and can therefore impart impurities to the wafer surface. There are over 100 cleaning steps throughout the manufacture of a typical semiconductor device, and one of the typical solutions used for wafer cleaning is a chemical mix developed by RCA, commonly referred to as Standard Clean 1 (SC-1). SC-1 is a mixture of NH_4OH, hydrogen peroxide (H_2O_2) and ultra-pure water (UPW) in the ratio 1:1:5, and is used to remove surface particles by lightly etching the wafer. There is a clear requirement for a highly sensitive and reliable analytical methodology to measure metallic impurities in the high purity chemicals from which SC-1 is produced.

While UPW and H_2O_2 are easy matrices to analyze, the analysis of high purity NH_4OH by ICP-MS is very difficult for two reasons. Firstly, NH_4OH is a strong alkali, which causes some metals to readily precipitate as insoluble hydroxides. This presents difficulties for determination using the method of standard additions (MSA), because MSA is performed by sequentially spiking an acid-based multi-element standard into the sample. At higher concentrations, some metals will precipitate when spiked into the undiluted NH_4OH, making accurate determination by MSA impossible. However, the NH_4OH used in SC-1 is high purity grade with a maximum metallic impurity level of 100 ppt, so higher level spikes can be avoided if the ICP-MS detection limits are sufficiently low. If MSA spikes are at the low ppt level (<100 ppt), minimal precipitation should occur, and MSA can in fact be used to analyze undiluted NH_4OH. To be able to produce good MSA calibrations below 100 ppt of course requires the interference removal technology employed in the ICP-MS to be extremely effective for all interferences.

The Agilent 7700s ICP-MS, with the third generation Octopole Reaction System (ORS^3), has the widest range of interference removal technologies of any ICP-MS instrument. In addition to conventional no gas mode, the ORS^3 operates in both collision (He) mode and reaction (e.g. H_2) mode, and cool plasma mode is also available. The interference removal approach selected depends on the analytical requirement: for this application, calibration down to the 10 ppt level is required for all analytes and therefore the most efficient interference removal mode is required for every analyte. Switching between modes is fully automated and all analytes are measured with a single visit to the sample vial. The small size of the ORS cell allows very fast switching between cell gas modes so the additional time required for multi-mode operation is minimized.

The second challenge when analyzing undiluted NH_4OH is that the plasma becomes unstable due to the high vapor pressure of undiluted NH_4OH, so routine, direct analysis of undiluted NH_4OH has not previously been possible. The accepted method of analyzing NH_4OH is to remove the matrix by heating to near dryness and then re-dissolving the residue in 1% HNO_3 prior to measurement by ICP-MS [1]. Although this method is widely used, laboratories favor elimination of the sample preparation step, to shorten analysis time and reduce the risk of sample contamination and loss of volatile analytes. The Agilent 7700 Series features a unique RF plasma generator design with high-speed frequency-matching that can instantaneously adjust to changes in plasma load – for example when switching from aqueous to a high vapor pressure solvent. This produces a very stable plasma capable of tolerating the direct aspiration of undiluted NH_4OH.

The combination of high sensitivity, effective removal of interferences, and high-speed frequency-matching ICP RF generator enables the Agilent 7700s to measure low ppt level metallic impurities directly in undiluted high purity NH_4OH.

Experimental

An Agilent 7700s was fitted with an inert sample introduction system (Agilent part # G4912-68002) comprising a PFA double pass spray chamber and demountable torch fitted with a 1.5 mm ID sapphire injector. Standard Pt interface cones and PFA concentric nebulizer were used. A solution of Li, Zn, Sn and Pb prepared in 2% NH_4OH was used to tune the instrument. These elements were chosen since they form amphoteric oxides or hydroxides (which can display both acidic and basic properties), and are therefore stable in NH_4OH. The normal tuning solution containing Li, Y, Ce and Tl in 2% nitric acid may also be used, but a thorough rinsing with UPW prior to analysis is necessary to prevent residual acid mixing with the NH_4OH standard and samples. Once the tuning conditions have been established, the system should be rinsed with UPW and then 20% NH_4OH for an hour to ensure that the sample introduction system is free from acid. Operating parameters are shown in Table 1. The acquisition used a multi tune mode method, performed with a single visit to the sample vial, and data for each of the modes (cool plasma, no gas, He mode and H_2 mode) was combined automatically into a single report for each sample. Total run time per sample, including uptake and rinse, was 8m 20s.

High purity grade NH_4OH (20% as NH_3) was used (TAMAPURE-AA100, TAMA Chemicals, Kawasaki, Japan), and the MSA calibration standard solutions were prepared by spiking a mixed multi-element standard (SPEX CertiPrep, Metuchen, NJ, USA). Calibration levels, added sequentially into a blank of undiluted NH_4OH, were at 10, 20, 50 and 100 ppt.

Results

DLs and BECs

3 σ detection limits (DLs) and background equivalent concentrations (BECs) obtained for 48 elements in high purity NH_4OH are shown in Table 2. For Se and Te, the lowest detection limits were obtained using H_2 mode, while for Na, Mg, Al, K , Ca, Cr, Mn, Fe, Co, Ni and Cu, cool plasma mode was used. For the remainder, He mode was used except for Be and B which were run using no gas mode. The DLs were calculated from 10 measurements of the blank. All DLs were single figure ppt or lower, as were the BECs, aside from Ca at 11 ppt. This demonstrates the ability of the 7700s to remove spectral interferences, and also the high quality of this NH_4OH product. Since no individual analysis mode gives the ultimate DLs for every element, the 7700s offers the ultimate performance for semiconductor chemical analysis by allowing the user to automate analysis methods with no gas, He and H_2 modes, plus cool plasma. This flexibility, coupled with pre-defined methods and simple, automated optimization routines makes the 7700s the most powerful ICP-MS for semiconductor analysis.

Table 1: 7700s Operating Parameters

	Normal Plasma	Cool Plasma
Forward power (W)	1600	600
Carrier gas (L/min)	0.8	
Make up gas (L/min)	0.1	0.5
Sampling depth (mm)	7	18
Cell gas flow (mL/min)		
He mode	5	-
H_2 mode	6	-
Cell KED (V)	3	-
Uptake time (s)	60	
Acquisition time (s)	350	
Rinse time (s)	90	

Table 2: 7700s ICP-MS DLs and BECs in 20% NH_4OH

Element	m/z	Mode	DL/ppt	BEC/ppt
Li	7	cool	0.014	0.003
Be	9	no gas	0.33	0.1
B	11	no gas	2.6	16
Na	23	cool	0.43	0.38
Mg	24	cool	0.17	0.32
Al	27	cool	0.26	0.67
K	39	cool	0.25	0.38
Ca	40	cool	1.9	11
Ti	48	He	2.4	1.4
V	51	He	0.67	0.31
Cr	52	cool	0.3	0.4
Mn	55	cool	0.078	0.026
Fe	56	cool	1.5	2.1
Co	59	cool	0.23	0.052
Ni	60	cool	0.88	0.42
Cu	63	cool	3	1.8
Zn	66	He	1.7	0.8
Ga	71	He	1.7	0.68
Ge	72	He	4.3	1.6
As	75	He	6.5	3.8
Se	78	H_2	8.4	4.6
Rb	85	He	0.022	0.028
Sr	88	He	0.86	0.29
Zr	90	He	0.35	0.2
Nb	93	He	0.057	0.076
Mo	98	He	0.24	0.16
Ru	101	He	0.26	0.1
Rh	103	He	0.41	1.4
Pd	105	He	0.18	0.092
Ag	107	He	0.11	0.12
Cd	111	He	0.66	0.35
Sn	118	He	2.3	1.5
Sb	121	He	2.3	0.92
Te	125	H_2	1.3	1.3
Cs	133	He	0.41	0.21
Ba	138	He	0.27	0.13
Hf	178	He	0.24	0.086
Ta	181	He	0.047	0.036
W	182	He	0.16	0.071
Re	185	He	0.14	0.061
Ir	193	He	0.15	0.16
Pt	195	He	0.39	0.48
Au	197	He	0.4	0.17
Tl	203	He	0.21	0.27
Pb	208	He	0.75	1.1
Bi	209	He	0.16	0.15
Th	232	He	0.085	0.025
U	238	He	0.064	0.013

Calibration linearity

Figures 1a and 1b show examples of the calibration plots achieved for elements in two different modes: V (He mode) and Fe (cool plasma mode). Excellent linearity at the ppt level was achieved in each case, demonstrating the interference removal power of the 7700s in both modes.

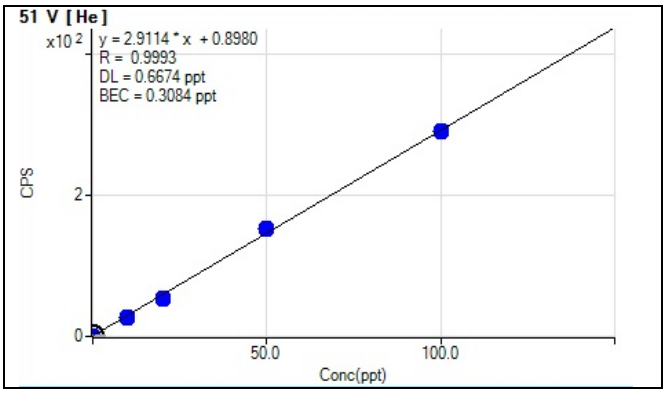

Figure 1a: Calibration plot showing spikes at 0, 10, 20, 50 and 100 ppt for V in 20% NH_4OH

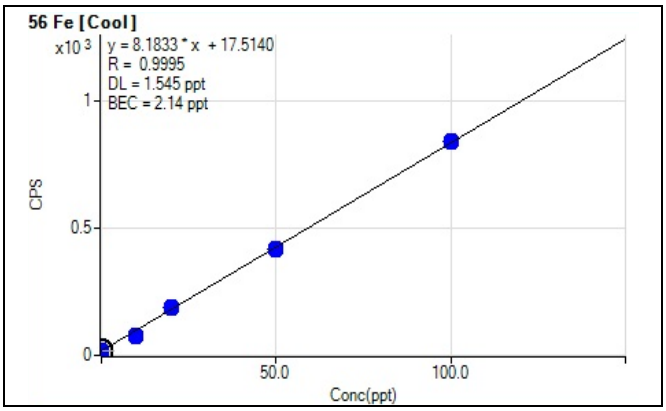

Figure 1b: Calibration plot showing spikes at 0, 10, 20, 50 and 100 ppt for Fe in 20% NH_4OH

Long term stability

If present at a high enough concentration in an alkaline solution, many metals form insoluble hydroxides which easily form precipitates or can be adsorbed on to the surface of the vessel wall, and the likelihood of precipitation or adsorption increases with time. Keeping metal concentrations low (at a maximum of 100 ppt) reduces the risk of precipitation, and monitoring signal stability over an extended period is an effective way to confirm the chemical stability of the analytes. A long term (just under 7 hours) stability test was performed for 22 elements spiked at 100 ppt in 20% NH_4OH. Signal intensity was measured every 45 min while blank samples of 20% NH_4OH were introduced between each spike measurement. Figure 2 shows the excellent stability (approx. 5% RSD) of the spikes over the test period, confirming the applicability of direct analysis using MSA at spike concentrations up to the 100 ppt level in 20% NH_4OH.

Conclusions

The direct analysis of 20% NH_4OH was performed successfully using an Agilent 7700s ICP-MS. The high speed frequency-matching RF generator of the 7700 produces a stable plasma when 20% NH_4OH is aspirated, while the availability of multiple interference removal technologies assures low DLs and BECs for all 48 elements measured. The routine applicability of direct measurement using MSA has been demonstrated by limiting spike concentrations to 100 ppt, which avoids precipitation in the alkaline matrix. As a result, labs are no longer forced to carry out matrix removal in order to analyze high purity 20% NH_4OH by ICP-MS, and direct analysis is routinely possible.

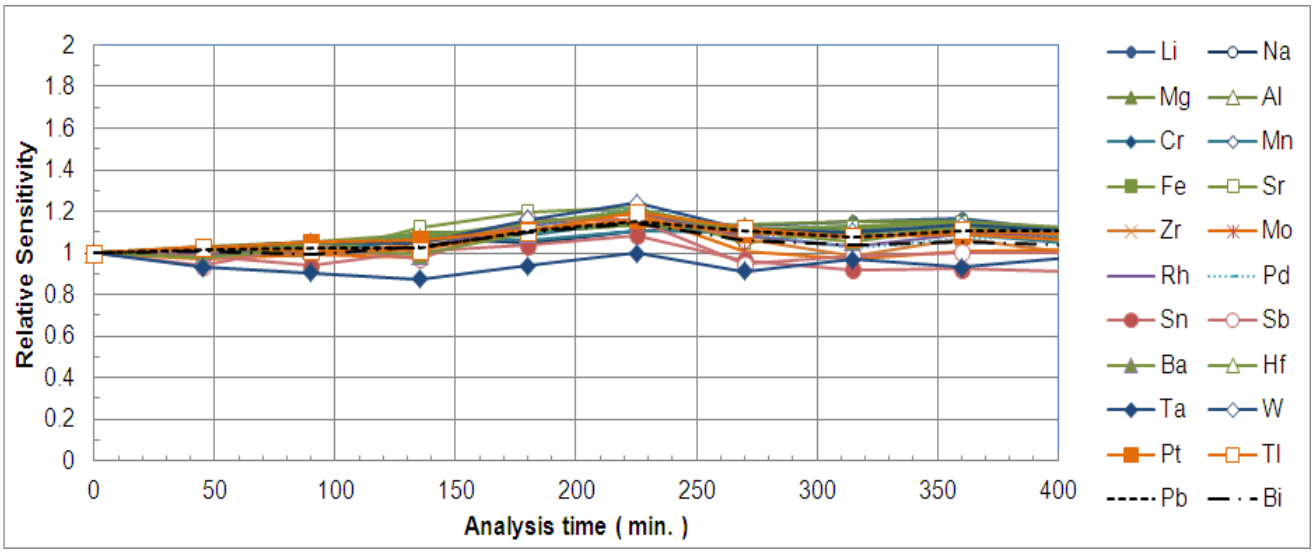

Figure 2: Signal stability of a representative selection of 22 elements spiked at 100 ppt in 20% NH_4OH

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www.agilent.com/chem

Published May 4, 2011
Publication Number 5990-7914EN

Agilent Technologies

Returning to Fixed Pathlength Infrared Spectroscopy: Gaining Detail and Removing the Obstacles

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Introduction

This article discusses the benefits of making infrared (IR) transmission measurements from liquids with a fixed pathlength. The pros and cons, mainly cons, of traditional fixed pathlength cells are reviewed first, with the main "cons" being difficulties with filling and cleaning, and the need to protect the IR windows from moisture. ATR has become a practical alternative method for a liquid, however, the technique, by nature, is a surface-based measurement and there are significant limitations in regard to physical pathlength, which is very short.

A new system that provides a fixed pathlength IR transmission measurement for liquid sample handling and analysis is reviewed. The system features and integrated FTIR and provides three user-selectable pathlengths that are factory fixed at the time of purchase; nominally set to 30, 50 and 100/150 microns that can be used without the customary drawbacks of a fixed pathlength cell. A special sampling point, called a DialPath head (Figure 1A/B), is used to locate the sample between a pair of specially designed zinc selenide (ZnSe) windows. These are constructed not to generate any optical interference pattern in the recorded spectrum. The sampling point is easily accessible and sample preparation is reduced to applying a drop of liquid on the lower "window" and after the measurement the window is cleaned by a wipe with a tissue, Q-tip or paper towel.

Fixed pathlength measurements have the ability to provide fine detail in the measured spectrum. This is an important fact for quality-based measurements where subtleties or small variations differentiate "good" from "bad" materials. Some example applications are reviewed that illustrate the benefits of fixed path measurements. Comparisons are made with a standard laboratory-based FTIR equipped with fixed pathlength transmission cells to confirm equivalency. The featured applications include measurements of dilute solutions, alternative fuels and food products (dairy products and edible oils).

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Background and the use of fixed pathlength cells

Originally, infrared spectroscopy was developed as a quantitative technique for liquid petroleum products (fuels and lubes) and polymers. It was later that it became the universal tool for material identification, as we know of today. The combination of material identification and quantitative response has made infrared spectroscopy unquestionably the most versatile instrumental method for chemical and physical analysis, covering a wide range of applications. As with any measurement, maintaining quantitative integrity by reproducible and accurate sampling is essential. In the infrared, maintaining a measureable pathlength, which is not trivial, is required for the accurate analysis of liquids. There are at least five critical factors to be considered and addressed:

- The need for a pathlength compatible with the absorption characteristics of the liquid in the mid-infrared (5000 cm^{-1} to 400 cm^{-1}/2.0 µm to 25 µm)
- Mechanical design issues of an accurate and reproducible short pathlength
- The filling, emptying and cleaning of the cells and the influence of the sample
- Window material selection based on the properties of the sample, and the optical characteristics of the window
- Alternative methods of sampling that reduce or overcome the difficulties associated with the sample...are they good substitutes?

It is obvious that there are important issues related to making infrared spectral measurements that become practical challenges. The first is the high infrared absorption cross section of most materials. Unlike other spectral regions, where cells or cuvettes are used with pathlengths measured in millimeters or centimeters, infrared measurements require pathlengths measured in microns. Generating a reproducible film of a sample this thin is a challenge. For years practical infrared spectral analysis has been performed with different methods of handling of liquid samples whereby the pathlength is controlled to the accuracy required for the analysis.

The standard, for 40 years, is the fixed pathlength cell, where the optical pathlength is generated by the use of thin spacers sandwiched between a pair of infrared transmitting windows. Two versions of these cells are used; demountable cells and sealed cells. Demountable cells are dismantled to simplify “filling”, “emptying” and cleaning. The windows are separated, and the sample is dropped into the void in the spacer, and then the top window is carefully replaced to form a sandwich with the liquid; taking care not to trap air. The problem with this approach is that assembly can be difficult and there is uncertainty in the pathlength formed. At best, it is a semi-quantitative approach to sample handling.

Sealed cells are required for accurate sampling. In a sealed cell the sample holder, the windows and the spacers have to be permanently fixed together. Such a cell is filled via special sample ports where the liquid is injected from a syringe into the cell. While this sounds simple, in practice it has significant practical drawbacks. Filling, where the liquid is “squeezed” into the confined space, which is at most 100 microns thick, is the first challenge. This can require the application of pressure from a syringe. This step requires extreme caution because the hydraulic pressure generated can damage the cell and can cause leaks. Originally, cells were sealed with special lead spacers treated with mercury to form an amalgam seal. Today, the use of these materials are not permitted, and non-toxic alternatives such as tin, steel or aluminum foils are used, sometimes in combination with an adhesive. Teflon sheet spacers are used in demountable cells and occasionally in sealed cells. However, the sealing integrity of Teflon-based spacers is questionable.

The next practical issue is emptying and cleaning the cell. As indicated above, a sealed, fixed pathlength cell is filled via filling ports. These are implemented by the use of a special drilled window, which is sealed against the metal front plate of the cell. This front plate has input tubes with female Luer fittings that couple to the

male Luer tip of a syringe. The entire assembly, mounting plates, seals, windows and the selected spacer form the sealed, fixed pathlength infrared cell. This is a fragile, complex component that requires skilled assembly, and careful use, maintenance and storage.

These cells have been the mainstay of liquid sample handling of liquids for nearly fifty years. They are not ideal, they are expensive, and they are difficult to fill, empty and clean. If handled correctly, they are usually filled and emptied by a pair of syringes connected to the filling ports of the cell. This action takes skill and dexterity, and if not carried out carefully it will lead to the formation of bubbles; a serious interference in the measurement. Incorrect use can lead to cell damage, with resultant leakage of fluid. Also, short pathlengths (less than 50 µm thick) are especially difficult to use with samples of medium to high viscosity. Emptying and cleaning are equally difficult, and again a syringe is used to draw out the sample, and then to flush solvent through the cell until the cell is clean. Careful selection of the solvent is important to ensure dissolution of the sample, ease of removal and to ensure inertness towards the windows.

The best windows for good infrared transparency are sodium chloride and potassium bromide. While these are good optically speaking, they are water soluble and are readily attacked (etched) by moisture in the sample or by humidity in ambient air. Calcium fluoride and barium fluoride are water insoluble and moisture resistant they have a restricted range of infrared transparency (optical cut-offs at 1100 cm^{-1} for CaF_2 and 870 cm^{-1} for BaF_2). A practical alternative is to use windows made from zinc selenide (ZnSe). This material provides transparency similar to NaCl, and can be used to 650 cm^{-1}. The material is very durable and is not attacked by water. Unfortunately, it is not in common use as a cell window because ZnSe has a high index of refraction (Index = 2.4) and it introduces an interference pattern (sine wave) into the spectrum of most liquids. This interference is above an acceptable level and in most cases is impossible to remove from a final spectrum.

In summary, practical issues interfere with the ability to obtain fixed pathlength infrared measurements of liquids in traditional cells:

- The pathlength is required to be between a few micrometers (µm) and a few hundred micrometers (<200 µm, <0.2 mm)
- The pathlength must be accurately defined and reproducible
- Fixed pathlength cells are difficult to fill, empty and clean
- Window materials need to be carefully selected; materials such as ZnSe, which appear to be ideal, are unsuitable because of optical interference caused by a high index of refraction

Practical alternatives for fixed pathlength infrared measurements

In the 1980s the application of ATR was extended to include liquids. Commercial accessories based on cylindrical internal reflectance elements (IREs) or horizontally mounted IREs provided a practical solution. Zinc selenide turns out to be a good match for this application because of its optical range, hardness, high index and water insolubility. Consequently, ATR has become a de facto standard for the handling of liquids. ATR is a surface phenomenon and the physical optical pathlength is only a few microns deep. The effective pathlength can be extended by multiple internal reflections, where the liquid sample has multiple interactions with the internal reflections. Optical geometries with nine or ten reflections produce an "effective pathlength" in the range of 10 µm to 25 µm, dependent on the analytical wavelength.

There are downsides to the ATR measurement linked to the mechanism of the internal reflection. First, the physical pathlength, per reflection is short and is wavelength and index dependent. Consequently, the actual, physical pathlength is not absolute and is effectively unknown and variable.

Also, zinc selenide, a popular IRE substrate, is ionic and its surface is chemically reactive. Practical alternatives to zinc selenide exist, with diamond being a candidate. Commercial accessories exist based on diamond with configurations that provide from single to nine reflections for liquid handling. Diamond is an ideal substrate; it is very hard and is chemically inert. Optically it is limited in size and optical transmission with a loss in throughput performance for configurations with multiple reflections (3x and 9x).

The success of horizontal ATR accessories and diamond tipped ATR sampling systems must not be underestimated. Most laboratories have implemented these systems for liquid sample measurements. However, the approach is a compromise for many measurements. Non-reproducibility is an issue, but this can be improved by integration of the ATR into a dedicated instrument with rigid, permanent mounting. Although some non-reproducibility (linked to the index of refraction) may still exist, the permanent mounting of the IRE provides a fixed sampling point and is a popular method for routine sample handling.

The benefits offered by an integrated ATR measurement can be improved by the combination of the ATR with an optimized FTIR spectral engine. In such systems the sample can be applied to the sampling point from a dropping pipette, and the analysis completed in a few seconds. Cleaning is reduced to simply wiping material off the ATR sampling surface with a soft tissue, possibly followed by the use of a small amount of solvent. Moving forward, a similar easy-to-use interface would provide the ideal scenario for a fixed pathlength measurement. Such a system would offer the benefits of real extended pathlength, with the simplicity of a "drop-it-on"/"wipe it off" sampling point, and a measurement that is not compromised by the sample.

An integrated measurement system from Agilent Technologies, the 5500 Series FTIR and sample handling system, has been developed and introduced, fulfills this "idealized" concept for fixed pathlength sample handling. The implementation covered in this article uses a three-position version of the company's 5500 DialPath FTIR rotary head, providing pathlengths of 30, 50 and 100 µm for the fixed path transmission measurements. This head, shown in Figure 1, is equipped with a slightly curved (bowed) zinc selenide window, which rotates to form a rigidly defined pathlength with the sample. Figure 1B shows the head located at position 1, which provides a nominal 30 µm optical path; the other two locations provide nominal 50 µm and 100 µm paths, respectively.

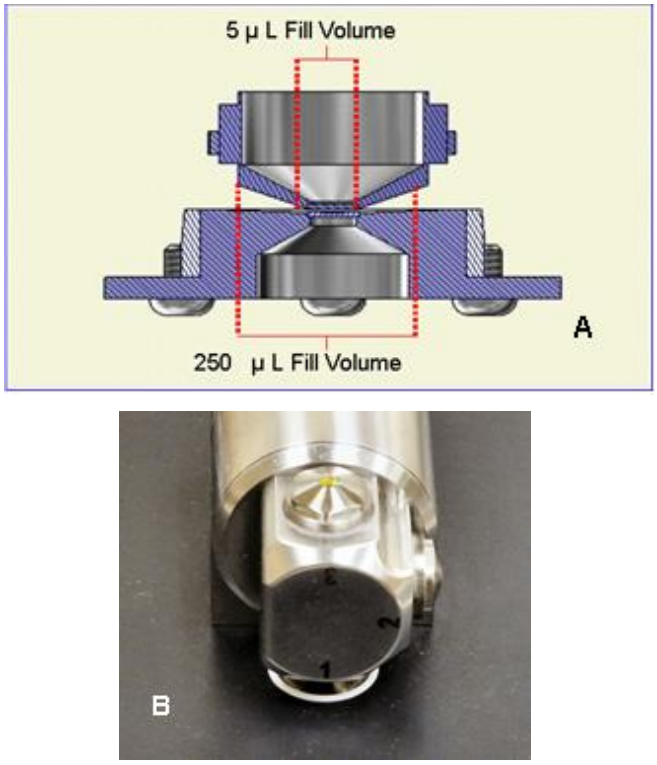


Figure 1. The 5500 DialPath FTIR sampling point concept (A); provides a user selectable pathlength, with one of three fixed/calibrated optical paths, designated 1, 2 and 3 (B)

This configuration provides the simplicity of the ATR sampling concept where the sample is dropped on to the small circular window, the sampling head is rotated in place, and the measurement made, in a few seconds. The liquid forms a uniform capillary film between the lower window and the window in the rotary head. The sweeping action of the rotary head produces a uniform film without any bubble interference. The slight curvature of the optical surface eliminates the opportunity to form an optical interference situation

between the two zinc selenide windows. The optical, mechanical and water insolubility benefits of the zinc selenide windows are realized without the negative impact of optical interference. The lack of optical interference can be appreciated by Figure 2, where the three baselines (100% lines) for the empty window cavities are presented. These spectra, recorded in approximately 13 seconds have a nominal 8000:1 SNR across the analytical range of 2100 cm^{-1} to 1100 cm^{-1}.

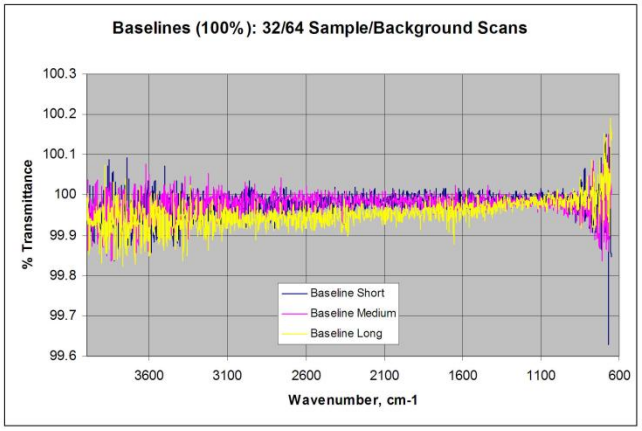


Figure 2. 100% Baseline performance; spectra from long, medium and short pathlengths presented with an average SNR of 8000:1 (2100 cm^{-1} to 1100 cm^{-1})

The SNR represented in Figure 2 is a significant result because it shows a flat 100% line without any artifacts caused by optical interference. The spectrum from a fixed pathlength cell constructed from zinc selenide windows would be dominated by a large sinusoidal pattern. This occurs with or without the sample in place. The lack of any interference pattern is further substantiated by the adherence to the square root law, where the SNR of the system is proportional to the square root of the number of scans (Figure 3). An excellent linear correlation is observed for the practical measurement timeframes; the presence of interference would result in significant deviation and curvature to this line.

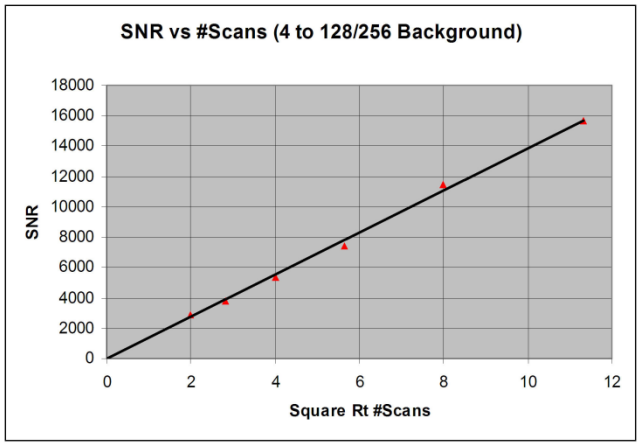


Figure 3. The adherence of the measurement system to the square root law of measured SNR

It is appropriate to compare the spectral data from a standard diamond ATR system with the fixed pathlength (5500 DialPath) measurement (Figure 4).

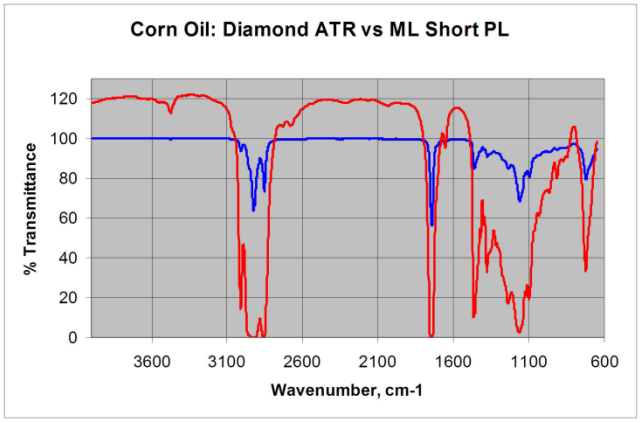
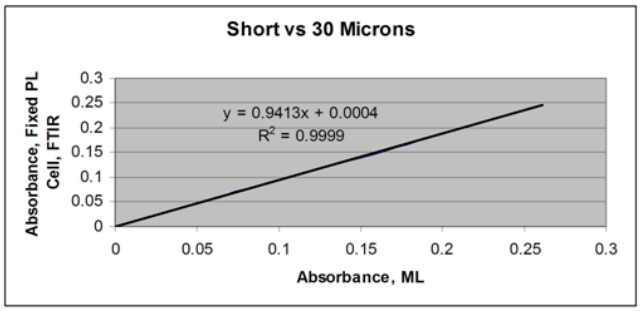


Figure 4. Comparison of the effective pathlength of a diamond ATR integrated system with the short fixed pathlength (~30 µm) transmission spectrum for corn oil

Both systems provide good quality spectral data, however, if one is looking for characteristic details in the spectrum for property measurements, such as the degree and type of unsaturation of an edible oil, then a long, fixed path measurement is required. One minor optical issue is that the high index of the ZnSe windows can be detected by the shift in the baseline of the corn oil above 100%. This result is the difference between the low index of the air (used for background), versus the higher index of the corn oil.

Analytically this is not a problem because the shift can be compensated from the absorbance form of the spectrum.

The reproducibility of the pathlength and the ability to dial in a longer pathlength are important attributes. The pathlength is defined by the height of the head from the measurement surface; a mechanical adjustment fixed at manufacture. The actual pathlength can be calibrated from the spectral response of fixed calibrated pathlengths in a standard lab instrument. The unit used for the data here was not pre-calibrated to exact values. The data shown in Figure 5 is taken from a series of standard xylene solutions prepared in carbon tetrachloride and recorded on the 5500a FTIR system. A parallel set of spectra were obtained on a commercial FTIR (PerkinElmer Spectrum 100) with a set of calibrated fixed pathlength, KBr cells (30µm, 50µm and 100µm). The results (Figure 5) indicate a high level of correlation between the two different sets of fixed pathlength spectra, providing calibration equations for the three 5500a system pathlengths; short = 31.9 µm, medium = 52.6 µm, and long = 114.7 µm.



	ML Pathlengths	PL Equation	Correlation
Short	31.9	y = 0.9413x + 0.0004	R2 = 0.9999
Medium	52.6	y = 0.9497x - 0.0013	R2 = 0.9998
Long	114.7	y = 0.8721x + 0.0018	R2 = 0.9992

Figure 5. Example calibration for the short pathlength (No 1) of the Agilent 5500 DialPath FTIR system based on comparisons with a calibrated fixed pathlength cell for a series of xylene solutions

These experiments have demonstrated that the fixed pathlengths of the 5500 DialPath system are highly reproducible, and once calibrated provide an accurate duplication of the fixed pathlength performance of the standard, calibrated fixed pathlength cells.

Practical applications of a fixed pathlength measurement system

The ability to measure with known fixed pathlengths is important for a wide range of applications. An obvious application is for the analysis of very dilute solutions where a pathlength of 100 µm or more is required. The application shown in Figure 6, are spectra of dilute solutions (<1% solute) of methanol are measured in a non-polar solvent (carbon tetrachloride).

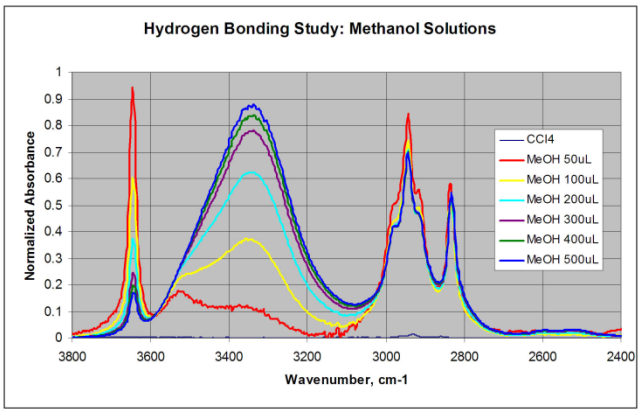


Figure 6: Dilute solutions of methanol in carbon tetrachloride; a study of the effects of hydrogen bonding in non-polar solvents

This is a classical measurement where changes in intermolecular hydrogen bonding are demonstrated. The normal condensed phase spectrum of methanol exhibits a broad absorption centered at 3450 cm^{-1} assigned to polymeric hydrogen bonding. Upon dilution with the non-polar solvent, this hydrogen bond profile changes as indicated in the red and yellow band profiles of Figure 6. These spectra correspond to the transition, through oligomeric forms to the non-bonded form with the narrow absorption at 3630 cm^{-1}. This experiment is only practical with a long path measurement (100+ µm in this case). The ATR method is impractical for this type of application.

The largest benefit of the open architecture of the 5500 DialPath system is the ability to handle medium to high viscosity liquids. Typical applications that are constrained by viscosity are measurements on vegetable oils (including cooking and edible oils), dairy products (such as milk, cream and butter products) and automotive products, including fuels, lubricating oils

and greases. While an ATR liquid measurement system might be used for some of these applications, the increased spectral detail of a longer pathlength is preferred for product quality and performance-related measurements. Figure 7 is important for both edible and cooking oils and products derived from these materials, such as biodiesel fuels. Recent regulations on food quality and safety have focused on the need to eliminate trans unsaturated fats from food preparation. The total level of unsaturates and the type of unsaturates, including the trans configuration can be determined from the spectral region from 1000 cm^{-1} to 650 cm^{-1}. In the case of biodiesel, many quality parameters are linked to components formed in the esterification process. These components, such as free acid, free glycerol and glyceride fragments can be determined from the spectrum. These include the OH stretching region featured in Figure 7A where residual water (from esterification) and free glyceride components can be detected and measured. These measurements require the extended pathlengths used in the spectra shown in Figure 7A/B (100+ µm pathlength).

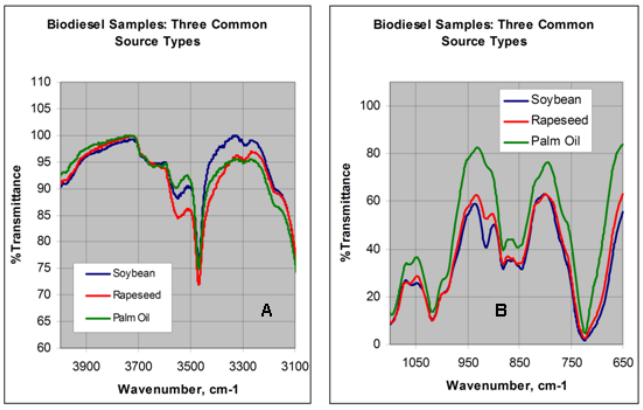


Figure 7. Detailed information from the base ester components used in the production of biodiesel methyl esters; hydroxyl (A) and unsaturation (B) functionalities

Another important issue for biodiesel is the level and type of unsaturation; a parameter linked to the chemical reactivity of unburned fuel residues in the engine oil. Three common types of biodiesel are illustrated in Figure 7B, ranging from the rapeseed derivatives (common in Europe), the soy based product (USA), and the palm oil based product often used in Latin America and the Caribbean. These differences correlate with unsaturation and chain length. These considerations equally apply to edible oils, where unsaturation, molecular weight and reactivity are relevant to use at high temperatures.

Another important application of fixed path infrared measurements to biodiesel fuel is in the qualification of biodiesel blends. While biodiesel may be used as 100% of the methyl ester fuel, it is seldom used or distributed in that form. 100% biodiesel has a negative impact on vehicle emissions and it can attack materials used in the fuel system of a vehicle (tubing, seals and gaskets) Many vehicle/engine manufacturers, do not recommend its use; its use may violate and even void the vehicle powertrain warranty. Typically the fuel is used diluted with hydrocarbon diesel fuel to give 5 % to 20 % in blends designated B5 to B20. Figure 8 illustrates the measurement of biodiesel blends covering the full range from B0 to B100. Good calibrations for this series are obtained as indicated in Figure 9.

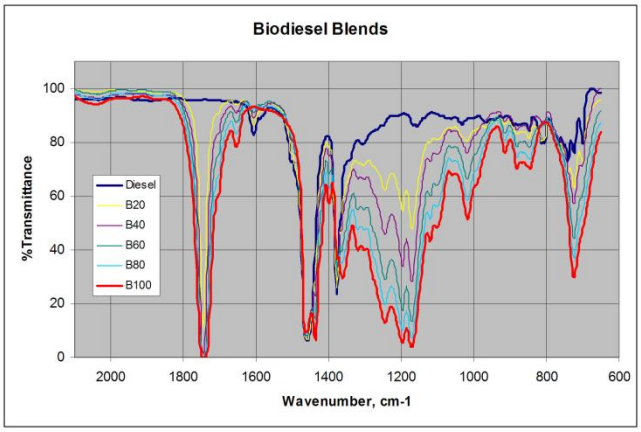


Figure 8. Measurement of biodiesel blends, experimental data from B0 (diesel fuel) to B100 (biodiesel) and intermediate biodiesel/diesel blends

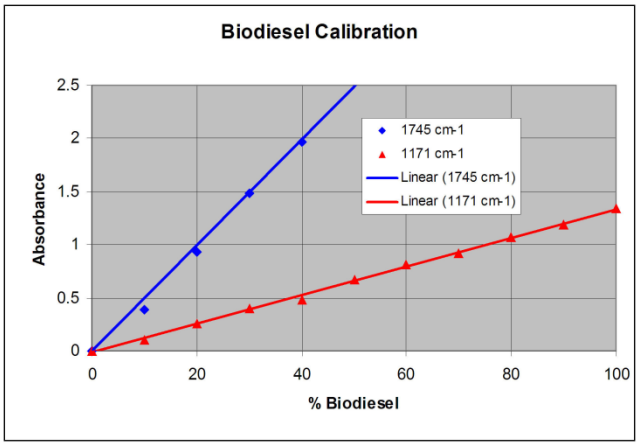


Figure 9. Quantitative measurement of biodiesel blends, B0, B10 to B90 and B10

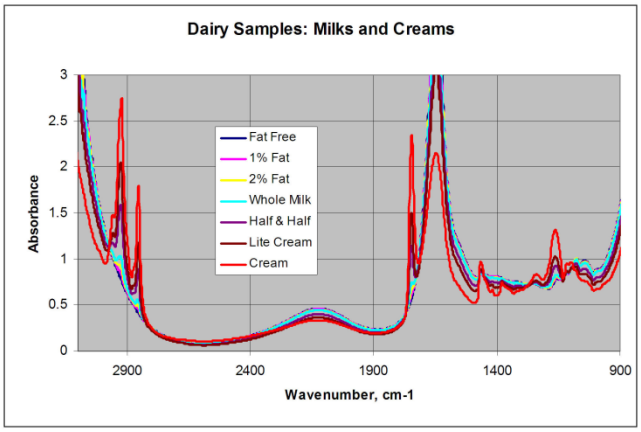


Figure 10. Dairy product spectra; short fixed pathlength (~30 mm), from fat free skim milk to standard heavy cream

The role of mid-infrared in the commercial analysis of milk and dairy products is well established. The measurement of raw milk in a fixed pathlength cell is used by regulatory agencies to control and standardize milk and dairy products. Standard methods exist for fat and protein content, which is used for the payment of the farmer. The performance and health of the dairy herd is also controlled, in pseudo real-time by monitoring fat/protein content. The results are used to control diet and medications. All of the relevant components in dairy products are derived from measurements of the infrared spectral data between 1800 cm^{-1} and 1000 cm^{-1}, a region that includes fat (ester), protein (amide bands) and sugars/lactose
(C-O-C, ether bands). Attempts to make these measurements in a standard sealed cell are fraught with difficulties. The accuracy of a fixed pathlength measurement is required, and the ease of handling high fat content materials, such as cream products, with the ease of cleaning, make the 5500a FTIR approach ideal for dairy product analysis.

Summary of the role and benefits of a “fixed” dial-a-pathlength system

This article has reintroduced the concept of making fixed pathlength mid-infrared transmission measurements without the complexity or the difficulties of the traditional sample handling method. A two-step approach summarized as “drop it on” and “wipe it off” is proposed, where the sample is put in place from a dropping pipette and is removed with the wipe of a paper towel. Anyone who has faced the challenges of working with the traditional fixed pathlength sealed cells can appreciate the ease of use and the simplicity of the system described. Traditional short path cells are impossible to fill with most liquids with average viscosity, and once filled, the cell can take five minutes or more to clean. The system described dramatically improves productivity and provides a platform for rapid, accurate quantitative analysis for all types of liquids.

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Published May 1, 2011
Publication Number 5990-7969EN

Agilent Technologies

Carboxylic Ester Analysis on Agilent PLgel MIXED-E with Gel Permeation Chromatography

Application Note

Materials Testing and Research, Polymers

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Introduction

Carboxylic acid esters are made by Fischer esterification, in which a carboxylic acid is treated with an alcohol in the presence of a dehydrating agent. Esters are widely found in fruit and vegetable odors, and in insect pheromones. Commercially they are used in fragrances and as flavorings in the food industry.

Agilent PLgel 3 µm MIXED-E columns simplify the analysis of carboxylic esters by gel permeation chromatography. These columns are ideal for low molecular weight samples that contain oligomeric fractions, as well as polymers, up to 30,000 MW.

Agilent Technologies

Analysis of a carboxylic acid ester

Figure 1 shows the rapid separation of carboxylic acid ester oligomers using two Agilent PLgel 3 µm MIXED-E columns to improve resolution.

Conditions

Column	2 × Agilent PLgel 3 µm MIXED-E, 300 × 7.5 mm (p/n PL1110-6300)
Eluent	THF
Flow rate	1.0 mL/min
Detector	RI
System	Agilent PL-GPC 50

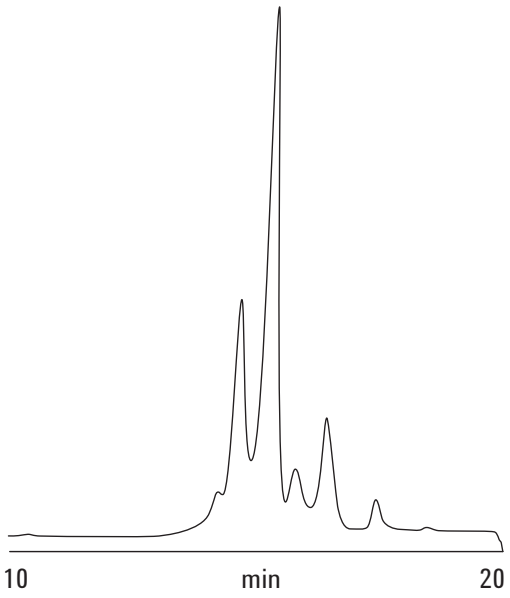

Figure 1. Separation of oligomers of a carboxylic acid ester on an Agilent PLgel 3 µm MIXED-E two-column set.

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Printed in the USA
April 21, 2011
5990-7982EN

Agilent Technologies

The ultratrace determination of iodine 129 in aqueous samples using the 7700x ICP-MS with oxygen reaction mode

Application note

Nuclear

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First presented at the 2011 Winter Plasma Conference, Zaragoza, Spain, Jan 30th–Feb 4th 2011.

Abstract

Determination of the radionuclide iodine 129 using ICP-MS has been improved by the use of an Octopole Reaction System (ORS) ICP-MS, operated in oxygen cell mode. Oxygen reaction gas removes the background signal at m/z 129, which is due to the presence of Xe as an impurity in the Ar plasma gas and to the polyatomic ion $^{127}IH_2^+$. NIST SRM standards with different $^{129}I/^{127}I$ ratios and at multiple concentrations were successfully measured, and a ^{129}I detection limit of 1.1 ppt was obtained. No sample preparation is required for aqueous samples and the method is capable of high sample throughput.

Agilent Technologies

Introduction

Iodine 129 (^{129}I) is a long-lived radionuclide (half-life of 15.7 million years), which has been released into the environment as a result of nuclear weapons testing and accidental releases from nuclear power plants and spent nuclear fuel reprocessing plants. Neutron activation analysis (NAA) and accelerator mass spectrometry are commonly used techniques for the measurement of ^{129}I at ambient (pre-nuclear age) levels, but these techniques are costly and time consuming (several weeks for NAA) involving radiochemical separation. Less sensitive but faster and more routine techniques used to monitor for ^{129}I release are liquid scintillation counting and gamma spectrometry, though measurements still take several hours and significant radiochemical sample preparation is required. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) can also be used to measure ^{129}I, however detection limits are compromised by a spectral overlap from ^{129}Xe, due to the Xe impurity present in the argon plasma gas.

Since ^{129}Xe is an elemental, rather than a polyatomic interference, magnetic sector ICP-MS does not have sufficient resolution to separate the 2 isobars, so a different approach is necessary. In this study, a quadrupole ICP-MS (Agilent 7700x) with an Octopole Reaction System (ORS) was used to remove the Xe interference on ^{129}I by reaction with oxygen gas in the ORS cell.

Instrumentation

The Agilent 7700x ICP-MS features a 3rd generation ORS cell (ORS^3), which features a longer, narrower octopole, and operates at higher frequency, higher cell gas flow rates, and higher collision energy than in previous ORS versions [1]. In combination, these enhancements give better interference removal efficiency in both collision and reaction modes. The ORS^3 cell operates effectively in both collision mode, using an inert collision gas (helium) with kinetic energy discrimination (KED), and reaction mode, using a reactive gas such as hydrogen. In this study, the ORS^3 was operated in reaction mode, using oxygen as a reaction gas. Oxygen reacts with Xe, converting Xe^+ ions to Xe atoms by charge transfer, and also removes the $^{127}IH_2$ overlap on ^{129}I by chemical reaction, thereby removing both the interferences on ^{129}I. Helium was also added to the cell to thermalize the ion beam. This minimizes the high mass tail of the ^{127}I peak, which might otherwise contribute to the signal measured at m/z 129 when ^{127}I is present at high concentration. The two cell gases (oxygen and helium) can be added as a pre-mixed blend or, for greater flexibility, added separately using independent mass flow controllers. The latter approach was used in this work, utilizing an optional low-flow 3rd cell gas controller. A standard Agilent glass concentric nebulizer and double-pass quartz spray chamber cooled to 2 °C were used for sample introduction. In order to ensure the chemical stability of iodine in aqueous solutions, samples are typically prepared in a basic diluent, such as tetramethyl ammonium hydroxide (TMAH). This ensures that iodine is not converted to volatile chemical forms that would rapidly be lost from the solution. Plasma parameters were automatically optimized to give 'Robust Plasma' conditions, using the pre-set plasma autotune feature in the Agilent ICP-MS MassHunter software. Robust plasma conditions are defined by a CeO^+/Ce^+ ratio of 1% or less and provide high matrix tolerance for routine use. Ion lens voltages were also autotuned for maximum sensitivity.

Optimization of oxygen flow rate

The optional low-flow 3rd cell gas mass flow controller of the 7700 Series ICP-MS can be used for several different gases and, when used for oxygen cell gas, the flow rate range is 0 to 1.12 mL/min (expressed as 0 to 100% flow, where 100% = 1.12 mL/min). Oxygen flow rate was optimized while aspirating a ^{127}I standard prepared in 0.5% TMAH (Tama Chemicals, Kawasaki, Japan) and monitoring m/z 127 and 129 (Figure 1). Assuming equal signal response for the 2 isotopes of I (127 and 129), the optimum cell gas flow rate was identified from the rate that gave the lowest background equivalent concentration (BEC) at m/z 129, using the measured ^{127}I sensitivity to calculate the BEC for ^{129}I. The lowest ^{129}I BEC achieved was 0.6 ng/L (ppt), obtained at an oxygen gas flow rate of 90% of maximum (1.01 mL/min), demonstrating the effective removal of the Xe and IH_2 interferences. Figure 1 shows the cell gas optimization plot.

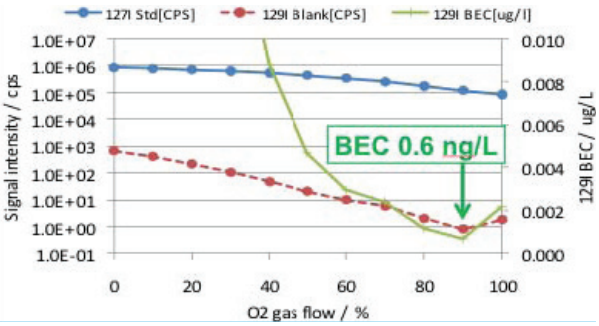

Figure 1. Profile of ^{127}I sensitivity, m/z 129 background and ^{129}I BEC versus oxygen flow rate

The Xe concentration in high purity argon varies depending on the Ar source (Xe is generally higher in bottled Ar gas than in liquid Ar tanks), but Xe peaks are always present at significant levels in ICP-MS spectra. The effectiveness of the ORS[3] in removing this Xe signal is demonstrated visually from the log scale spectra shown in Figure 2.

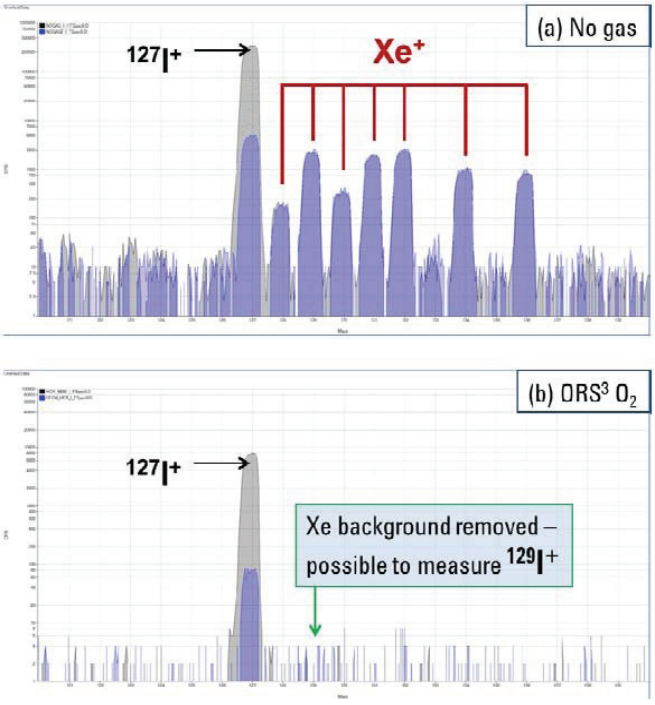

Figure 2. Mass spectra at mass range m/z 120–140 in (a) no gas mode and (b) oxygen reaction mode. In each case, the grey spectrum is 10 µg/L ^{127}I and the overlaid blue spectrum is the blank.

The upper spectrum (a) in no gas mode (no oxygen cell gas flow) clearly shows the Xe background arising from the Ar plasma gas and the background contribution from the polyatomic ion $^{127}IH_2^+$ that is produced in the plasma during the analysis of real samples. The grey spectrum is from a 10 µg/L ^{127}I solution, and the overlaid blue spectrum is from a blank. Switching on the cell gases (b) decreases sensitivity but the Xe^+ peaks disappear, as oxygen converts Xe^+ to Xe via a thermodynamically favorable charge transfer reaction ($Xe^+ + O_2 \rightarrow Xe + O_2^+$; $k = 1.1x10^{-10}$), freeing m/z 129 for the measurement of ^{129}I. Again, the grey spectrum is from the 10 µg/L ^{127}I solution, while the overlaid blue spectrum is from the blank, showing a small amount of memory from the iodine standard previously run. In oxygen reaction mode, m/z 131 also becomes available, allowing for the trace measurement of ^{131}I, though this is not particularly useful since its half-life is only 8 days.

Table 1 shows the ICP-MS operating parameters (at optimum oxygen flow rate). Total cell gas flow rate was 5.01 mL/min (4 mL/min He plus 1.01 mL/min oxygen).

Table 1. ICP-MS operating parameters

Parameter	Value
RF power (W)	1550
Sampling depth (mm)	8
Carrier gas (L/min)	1.05
Spray chamber temperature (°C)	2
He gas flow (mL/min)	4
Oxygen flow (mL/min)	1.01
KED (V)	10

Calibration standards

Calibration standards were prepared by diluting ^{129}I isotopic standards NIST SRM 3231 Level I and II (NIST, Gaithersburg MD, USA) with 0.5% TMAH in deionized water. The certified value for the $^{129}I/^{127}I$ ratio is $0.981x10^{-6} \pm 0.012x10^{-6}$ in Level I, and $0.982x10^{-8} \pm 0.012x10^{-8}$ in Level II. An intermediate standard with a $^{129}I/^{127}I$ ratio at the 10^{-7} level was prepared by spiking the Level I standard solution with ^{127}I at the appropriate concentration using a potassium iodide solution. The rinse solution was 1% TMAH.

Analytical data

Measurement of the NIST 3231 level I standard

In order to confirm the applicability of the method to measurement of ^{129}I at varying concentration levels, the $^{129}I/^{127}I$ ratio was measured in 4 different solutions containing NIST 3231 at different concentrations, as shown in the data in Table 2. In the case of the highest dilution (NIST 3231 diluted x100), ^{129}I was present at the single figure ppt level. For each solution, 5 replicate measurements of the $^{129}I/^{127}I$ ratio were acquired, with a total data acquisition time of 10 minutes for the 5 replicates. After subtracting the reagent blank, the measured $^{129}I/^{127}I$ ratio corresponded well with the certified value of $0.981x10^{-6}$ ($9.81x10^{-7}$) at all concentrations. RSD values were higher than is typically achieved for a more conventional isotope ratio measurement, due to the very low count rate measured for ^{129}I, although the RSD improved to 1.5% in the x10 diluted standard as the ^{129}I count rate increased to 70 cps.

Table 2. NIST 3231 Level I — measured iodine 129/127 ratio for each dilution. (Integration time was 0.3 s for m/z=127 and 15 s for m/z=129.)

Dilution factor	^{127}I (cps)	^{129}I (cps)	$^{129}I/^{127}I$	$^{129}I/^{127}I$ (average)	RSD (%)
100	6224116	6.1	$9.80x10^{-7}$		
	6092355	6.3	$1.03x10^{-6}$		
	6073353	6.4	$1.05x10^{-6}$	$9.81x10^{-7}$	8.6
	6125790	6.2	$1.01x10^{-6}$		
	6099791	5.1	$8.37x10^{-7}$		
50	14044748	12.3	$8.76x10^{-7}$		
	13933138	13.4	$9.64x10^{-7}$		
	13475103	13.6	$1.01x10^{-6}$	$9.93x10^{-7}$	7.8
	14128483	15.0	$1.06x10^{-6}$		
	14144548	15.0	$1.06x10^{-6}$		
20	36305910	33.8	$9.32x10^{-7}$		
	35573975	32.9	$9.24x10^{-7}$		
	36062147	36.4	$1.01x10^{-6}$	$9.62x10^{-7}$	3.9
	36295813	36.0	$9.93x10^{-7}$		
	36050890	34.3	$9.51x10^{-7}$		
10	75347525	72.6	$9.64x10^{-7}$		
	75216132	74.5	$9.90x10^{-7}$		
	73965391	71.2	$9.62x10^{-7}$	$9.68x10^{-7}$	1.5
	73792267	71.9	$9.74x10^{-7}$		
	74307176	70.7	$9.52x10^{-7}$		

Calibration plots for ^{127}I and ^{129}I

In order to confirm the calibration linearity for both iodine isotopes, the different dilutions of NIST 3231 Level I were processed as calibration standards, and the calibration plots generated are shown in Figure 3. Excellent linearity was achieved for both isotopes. The BECs for ^{127}I and ^{129}I were 0.65 µg/L and 1.9 ng/L, while the detection limits (3σ, n=10) for ^{127}I and ^{129}I were 0.14 µg/L and 1.1 ng/L, respectively. The BEC and DL for ^{127}I were higher due to carryover from previous measurements.

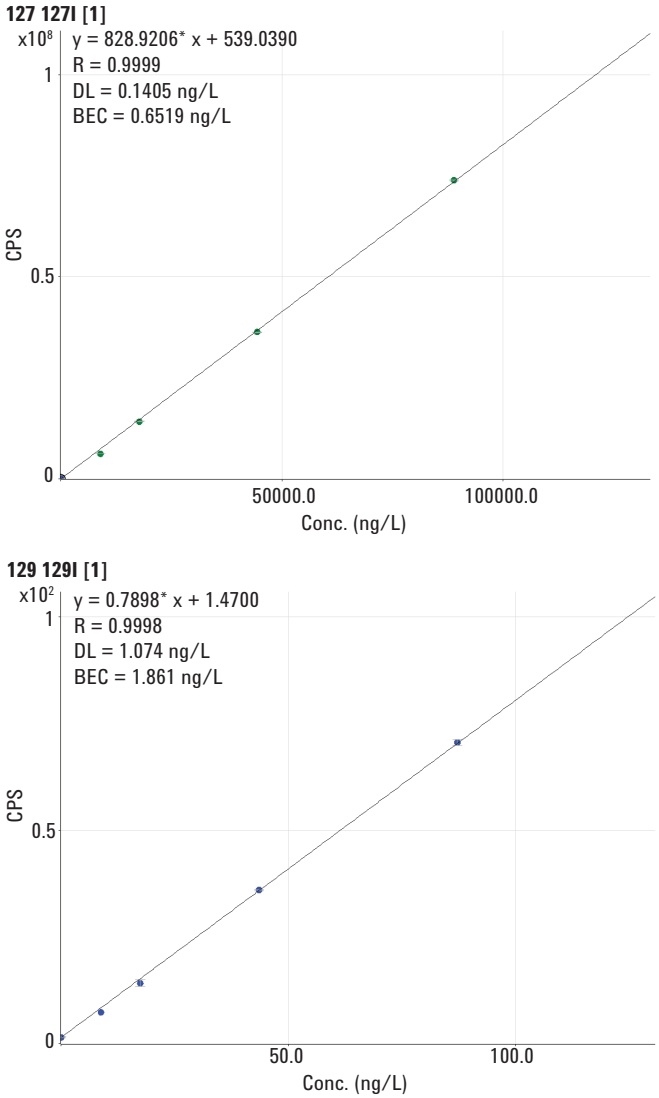

Figure 3. Calibration plots for ^{127}I (top) and ^{129}I (bottom) obtained from multiple dilutions of NIST 3231 Level I

Analysis of an 'intermediate' NIST 3231 standard and NIST 3231 level II

In order to validate the method, an 'intermediate' standard (nominal $^{129}I/^{127}I$ ratio 10^{-7}) was prepared by diluting NIST 3231 Level I 1:100 in a solution of potassium iodide (88.9 mg/L iodine). Measurement of the $^{129}I/^{127}I$ ratio in this intermediate standard gave good agreement with the target $^{129}I/^{127}I$ value of $1x10^{-7}$ (Table 3). NIST 3231 Level II ($^{129}I/^{127}I = 0.982 x10^{-8}$) was also measured and good agreement was again obtained (Table 4), though with higher RSD as the ^{129}I concentration approached the detection limit of the method.

Nevertheless, the fast, direct measurement at the 10^{-8} level for $^{129}I/^{127}I$ using standard liquid sample introduction and without sample preparation impressively demonstrates the capability of this method for rapid screening of samples for ^{129}I.

To confirm that overlap of ^{127}I on ^{129}I does not occur when ^{127}I is present at high concentration, spectra of NIST 3231 Level I, the prepared intermediate NIST 3231 standard, and a blank containing ^{127}I at the same concentration as in the standards (88.9 mg/L (ppm)) were acquired. The overlaid linear scale spectra are shown in Figure 4. Note there is no overlap of the ^{127}I high mass side peak tail on m/z 129. While some iodine hydride ($^{(127}I^{1}H$) can be seen at m/z 128, there is no IH_2 observed at m/z 129, as had been proposed in a previous study [2].

Table 3. Measurement of the 'intermediate' NIST 3231 standard

Dilution factor	^{127}I (cps)	^{129}I (cps)	$^{129}I/^{127}I$	$^{129}I/^{127}I$ (average)	RSD (%)
I 88.9 mg/L added 1/100 diluted NIST 3231 (expected 10^{-7})	76548859	7.3	$0.95x10^{-7}$	$0.99x10^{-7}$	7.9
	76618521	8.3	$1.08x10^{-7}$		
	76523125	6.8	$0.89x10^{-7}$		
	76849756	8.1	$1.05x10^{-7}$		
	76052388	7.6	$1.00x10^{-7}$		

Table 4. Measurement NIST 3231 Level II

Dilution factor	^{127}I (cps)	^{129}I (cps)	$^{129}I/^{127}I$	$^{129}I/^{127}I$ (average)	RSD (%)
NIST 3231 Level II $^{129}I/^{127}I = 0.982x10^{-8}$	536908532	4.0	$0.74x10^{-8}$	$1.07x10^{-8}$	22.4
	526648579	5.9	$1.13x10^{-8}$		
	518477906	7.3	$1.41x10^{-8}$		
	508547526	5.2	$1.03x10^{-8}$		
	503530493	5.3	$1.05x10^{-8}$		

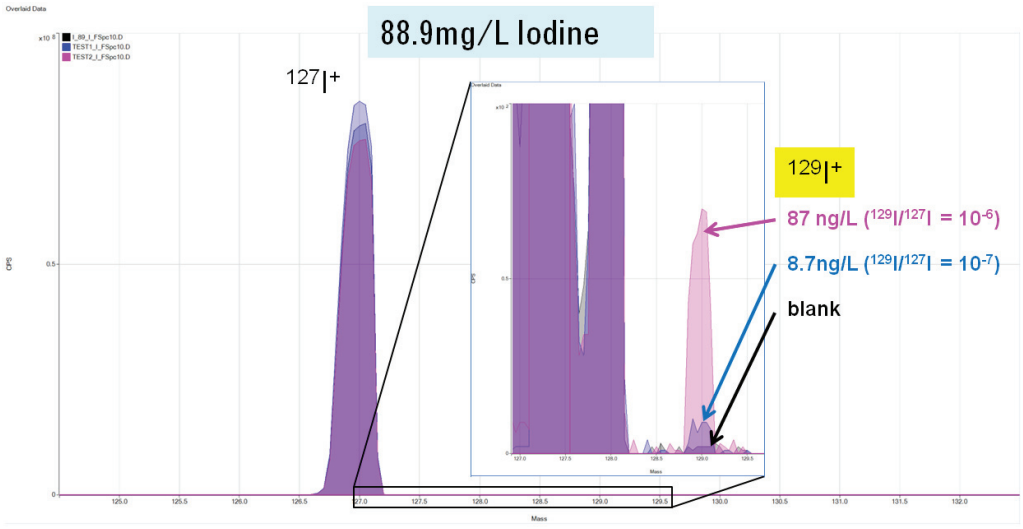

Figure 4. Overlaid spectra of 88.9 mg/L ^{127}I blank, NIST 3231 Level I (87 ng/L ^{129}I), and the intermediate standard (8.7 ng/L ^{129}I)

Conclusions

Improvements in the performance of ICP-MS for the direct measurement of ^{129}I in solution have been made possible by the use of an Agilent 7700x ICP-MS, featuring an ORS^3 cell operated in oxygen reaction mode. The new design features of the ORS^3 cell offer improved interference removal capability such that the Xe background due to the presence of Xe in the Ar plasma gas could be completely removed. A ^{129}I detection limit of 1.1 ppt was obtained and excellent abundance sensitivity prevented spectral overlap on ^{129}I from the presence of ppm levels of ^{127}I. NIST standards with different $^{129}I/^{127}I$ ratios and at multiple concentrations including the low level NIST 3231 Level II with a $^{129}I/^{127}I$ ratio of 10^{-8} were accurately measured without any sample preparation and with a standard liquid sample introduction system. The method is applicable to routine use and is capable of high sample throughput.

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www.agilent.com/chem

Published June 30, 2011
Publication number: 5990-8171EN

Agilent Technologies

Trace elemental analysis of trichlorosilane by Agilent 7700s ICP-MS

Application note

Semiconductor analysis

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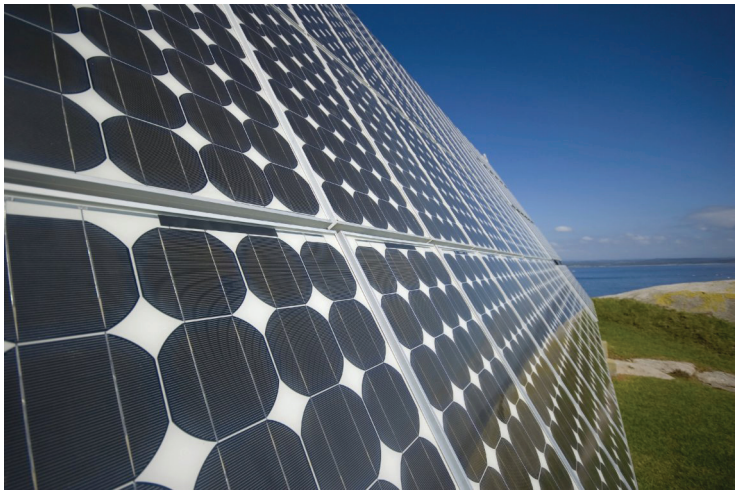

Abstract

Metallic impurities in trichlorosilane (TCS), an intermediate product used in the production of photovoltaic (PV) silicon, must be strictly controlled in order to produce the high purity PV silicon necessary for the manufacture of solar cells. A successful analytical method was developed, featuring measurement of impurities in TCS by an Agilent 7700s ICP-MS, following a sample preparation approach developed by Agilent. A spike recovery test demonstrated the validity of the method for 33 elements including boron and phosphorus, and two TCS samples were also analyzed. The ability to analyze TCS allows PV silicon manufacturers to check the TCS intermediate chemical for metallic impurities prior to the manufacture of PV silicon.

Agilent Technologies

Introduction

The search for alternative energy resources has intensified due to the exhaustion of natural fossil fuel resources and their contribution to global warming, together with associated geological, environmental, and political concerns. Among the many alternative methods of generating electricity, solar power or photovoltaic (PV) has experienced the highest annual growth since 2004. Photovoltaic panels, which are generally silicon wafer-based, convert energy from the sun into electricity, but the efficiency of this process is relatively low, so improving conversion efficiency is a key goal. The metallic impurity level in the polycrystalline silicon (polysilicon) used in the manufacture of the wafers that go into the PV panels must be strictly controlled, as impurities degrade the conversion efficiency. An effective method for the analysis of PV grade silicon by ICP-MS has already been developed by Agilent [1]. In an effort to further increase device efficiency, PV silicon manufacturers want to measure impurities in the chemicals used in the manufacture of polysilicon. This application note describes the application of the Agilent 7700s Octopole Reaction System (ORS) ICP-MS to the analysis of trichlorosilane, which is commonly used in the manufacture of ultrapure polysilicon.

Experimental

Instrumentation

A standard Agilent 7700s ICP-MS was used, with the addition of an Agilent Inert Sample Introduction kit consisting of a PFA concentric nebulizer, PFA double pass spray chamber and a demountable torch with 2.5 mm inner diameter platinum injector.

The 7700s has the widest range of available interference removal technologies of any ICP-MS instrument. In addition to conventional no gas mode, the 7700s can be operated in cool plasma mode and in ORS mode, which provides effective interference removal in both collision mode using an inert cell gas (He), and in reaction mode using a reactive cell gas (such as H_2). The 7700 Series ICP-MS features a third generation ORS cell (ORS^3) that features a longer, smaller internal diameter octopole, operating at higher frequency than in previous ORS versions. The ORS^3 can also be operated at higher cell gas flow rates and with higher bias voltages, which promotes greater collision energy. In combination, these features give improved interference removal efficiency in both collision and reaction modes.

The interference removal approach is selected depending on the analytical requirement. For this application, ultimate sensitivity is required for all analytes and therefore the most effective interference removal mode is required for every analyte/interference. A data acquisition protocol featuring four steps was used, and all instrument operating parameters are given in Table 1. During method development, some analytes were run in multiple modes, and the mode that gave the best detection limits in the sample matrix was determined for each analyte.

Table 1. ICP-MS operating parameters

	Method step			
	Step 1	**Step 2**	**Step 3**	**Step 4**
Plasma	Cool	Normal		
RF power (W)	600	1600		
Sample uptake rate (µL/min)	~160 (free aspiration)			
Sampling depth (mm)	16	8		
Carrier gas flow rate (L/min)	0.7			
Makeup gas (L/min)	0.8	0.5		
He cell gas flow rate (mL/min)	0		5	2
KED (V)	13	3		135

Analytes:	Step 1	^{7}Li, ^{23}Na ^{24}Mg, ^{27}Al, ^{39}K, ^{40}Ca, ^{55}Mn, ^{56}Fe, ^{63}Cu, ^{71}Ga
	Step 2	^{10}B, ^{138}Ba, ^{178}Hf, ^{181}Ta, ^{182}W
	Step 3	^{48}Ti, ^{51}V, ^{52}Cr, ^{59}Co, ^{60}Ni, ^{64}Zn, ^{75}As, ^{88}Sr, ^{90}Zr, ^{93}Nb, ^{98}Mo, ^{107}Ag, ^{114}Cd, ^{118}Sn, ^{121}Sb, ^{205}Tl, ^{208}Pb, ^{209}Bi, ^{232}Th, ^{238}U
	Step 4	^{31}P

The analytes measured in each step in the final method are also shown in Table 1. Step 1 employed cool plasma mode, and all remaining steps used normal or hot plasma. Step 2 used conventional no gas mode, Step 3 used He collision mode, and Step 4 was a modified He collision mode optimized for phosphorus determination. In the past, P has been measured indirectly as $^{31}P^{16}O$ at m/z 47, using cool plasma or O_2 cell gas. The improved performance of the ORS^3 enables a greater reduction of the interferences $^{15}N^{16}O$ and $^{14}N^{16}O^{1}H$ that overlap P at

mass 31, lowering the detection limit of P in He collision mode by a factor of 50, and making direct measurement of P possible at the required levels. Switching between modes is fully automated and all analytes were measured with a single visit to the sample vial, which helps to minimize sample contamination. The small size of the ORS cell allows for very fast switching between modes, so the additional time required for multi mode operation is minimized. The total analysis time was 8 minutes per sample.

Sample preparation

Trichlorosilane (TCS) is an intermediate compound used in the manufacture of high purity polysilicon. Since TCS is a volatile liquid that can easily be purified by distillation, it can be made from low-grade metallurgical grade silicon, purified, and then converted to high purity polysilicon. TCS is liquid at room temperature with high volatility (BP 31.8 °C). It easily decomposes to SiO_2 in air by hydrolysis, as shown below:

$$SiHCl_3 + 2H_2O \rightarrow SiO_2 + 3HCl + H_2$$

The direct sampling and on-line ICP-MS analysis of TCS in the manufacturing line is not feasible, because SiO_2 would deposit in the transfer tube and on the ICP-MS sample introduction and interface components. In addition, TCS must be chilled and handled in an inert environment to avoid evolution of HCl gas, and so an ICP-MS inside a clean mini-environment would need to be installed at each sampling point. The only practical approach is therefore to transfer liquid TCS to the lab for analysis. In this work, liquid TCS was transferred to a clean laboratory following appropriate safety precautions, and analyzed after careful sample preparation using the following procedure: Liquid TCS was converted to SiO_2 via gentle hydrolysis in an inert gas atmosphere, dissolved in HF solution and Si removed (as SiF_4 gas) by heating to dryness. The dry residue was then re-dissolved in 0.4% HCl solution before analysis by ICP-MS. Caution! The sample preparation step must be performed with great care and all appropriate safety precautions followed*.

Calibration standards

External calibration in a matrix of 0.4% HCl was used for quantification of the trace elements. Since the Si matrix is removed during the sample preparation, the sample residue re-dissolved in 0.4% HCl, is essentially free of any matrix. Four multielement calibration standard solutions were prepared at 0, 1, 2 and 5 ppb in 0.4% HCl. No internal standardization was used, to avoid the possibility of adding contamination.

Results and discussion

Detection limits

Detection limits (DLs) were calculated from 3 sigma of the calibration blank and are shown in Table 2. Low ppt DLs for V and As demonstrate the efficient removal of the ClO (on ^{51}V) and ArCl (on ^{75}As) polyatomic interferences in the HCl matrix. Also the phosphorus DL of 0.1 ppb shows the effectiveness of the optimized He collision mode parameters used in Step 4 for the removal of NO/NOH on P. DLs in the original sample were also calculated, by multiplying by a dilution factor of 7.5*, and all DLs were under 1 ppb in the original TCS sample.

Quantitative analysis

Table 2 also shows the results from the quantitative analysis of two TCS samples, after reagent blank subtraction. Sample A was a high purity TCS sample obtained from a semiconductor company and stored in a glass vial. Sample B was also obtained from a semiconductor company, but shipped in a stainless steel pressure vessel. As is clearly shown in Table 2, Sample B contained significantly higher levels of Fe, Ni and Cr than Sample A, indicating metallic contamination from the steel container. Sample A was found to be of high purity with only four elements above 1 ppb in the original sample.

*Agilent ICP-MS users requiring more detailed information on TCS sampling and analysis should contact their local Agilent ICP-MS applications team.

Table 2. Detection limits and quantitative analysis

m/z	Element	DL (final solution) (ppb)	DL (original sample) (ppb)	Analysis — Sample A (ppb)	Analysis — Sample B (ppb)
7	Li	0.0003	0.002	0.007	0.007
10	B	0.08	0.60	1.4	5.5
23	Na	0.002	0.01	0.53	15
24	Mg	0.001	0.010	2.5	1.4
27	Al	0.006	0.04	0.75	8.5
31	P	0.1	0.7	2.7	4.2
39	K	0.02	0.15	0.23	3.6
40	Ca	0.006	0.05	0.83	26
48	Ti	0.001	0.008	0.08	2.3
51	V	0.008	0.06	0.08	0.6
52	Cr	0.02	0.12	0.12	22
55	Mn	0.001	0.008	0.01	1.6
56	Fe	0.01	0.08	1.9	180
59	Co	0.0001	0.001	0.02	0.3
60	Ni	0.001	0.008	0.08	14
63	Cu	0.002	0.01	0.08	0.8
64	Zn	0.001	0.01	0.38	3.5
71	Ga	0.001	0.006	0.01	0.03
75	As	0.02	0.14	0.14	0.02
88	Sr	0.0001	0.0004	0.01	0.1
90	Zr	0.0002	0.001	0.08	1.0
93	Nb	0.0002	0.001	0.007	0.02
98	Mo	0.0003	0.002	0.08	1.3
107	Ag	0.004	0.03	0.03	0.02
111	Cd	0.0001	0.001	0.007	0.04
118	Sn	0.003	0.02	0.38	1.7
121	Sb	0.001	0.01	0.08	0.5
138	Ba	0.0002	0.002	0.007	1.4
181	Ta	0.0001	0.0004	0.007	0.2
182	W	0.0003	0.002	0.007	0.3
208	Pb	0.0002	0.002	0.007	0.8
232	Th	0.0001	0.001	0.007	0.007
238	U	0.0001	0.0002	0.007	0.007

Recovery test

In order to test the recovery efficiency of the method, and in particular to check for loss of volatile elements during the sample preparation procedure, a high purity sample of TCS was purchased (Tokyo Chemical Industry Co. Ltd., Tokyo, Japan). The TCS sample was divided into four aliquots, each containing 1.5 g of TCS, and sample preparation was performed as previously described. After hydrolysis and HF addition, but prior to the heating step to remove Si, one of the aliquots was spiked at the 5 ppb level with a multielement standard (Spex, Metuchen, NJ, USA). The samples were then evaporated to dryness and the dry residue was taken up in 0.4% HCl and analyzed. Figure 1 shows the recoveries achieved for the 5 ppb spiked sample. All elements gave good recoveries, including B, which can be problematic due to its volatility, demonstrating the validity of both the sample preparation and analytical methods.

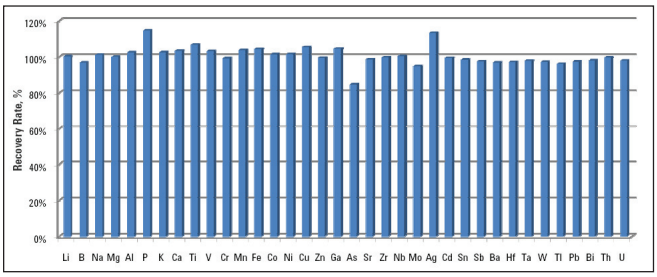

Figure 1. 5 ppb spike recovery test in TCS, confirming no loss of volatile elements during sample evaporation, and accurate recovery (between 80 and 120%) for all analytes.

Conclusions

Trichlorosilane has been successfully analyzed using the Agilent 7700s ICP-MS, following a sample preparation approach developed by Agilent. The ORS^3 cell improves He collision cell performance significantly, achieving a DL of 0.1 ppb for phosphorus from a direct measurement at m/z 31. A spike recovery test demonstrated the validity of the sample preparation and analytical methods for all elements including boron. The ability to analyze TCS allows PV silicon manufacturers to check the TCS intermediate product for metallic impurities prior to the manufacture of PV silicon.

References

1. Ultratrace Analysis of Solar (Photovoltaic) Grade Bulk Silicon by ICP-MS. Agilent Application Note, 5989-9859EN, Oct 2008.

www.agilent.com/chem

Published June 24, 2011
Publication number: 5990-8175EN

Agilent Technologies

Identification of Oxidation Products of L-Ascorbic Acid by HPLC

Application Note

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Introduction

Ascorbic acid is a water soluble sugar acid with antioxidant properties. The L-isomer of ascorbic acid is commonly known as vitamin C and is found naturally in fruits and vegetables. It is also added to fruit juices and other processed products as an antioxidant. Vitamin C is an essential nutrient in the human diet in the manufacture of collagen. In humans, absence of the vitamin leads to scurvy, a deficiency disease.

The acid has strong reducing power but when oxidized is converted to several compounds that do not have the same antiscorbutic or reducing properties. Given the importance of the vitamin in human health and its widespread use as an antioxidant in processed foods, study of its degradation products is merited. This note describes aspects of the rate of degradation of L-ascorbic acid and the nature of some of its degradation products using PLRP-S columns. PLRP-S is a rigid macroporous styrene/divinylbenzene HPLC phase with outstanding chemical and physical stability. The high surface area of the 100Å pore size enables retention of water soluble solutes.

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The aim of this study was to develop a rapid HPLC method for the quantitative and qualitative analysis of L-ascorbic acid.

Materials and Reagents

Reference samples: commercial L-ascorbic acid, oxalic acid, L-dehydroascorbic acid (DHAA) dimer.

Conditions

Columns: 2 x PLRP-S 100Å 5 µm, 150 x 4.6 mm (p/n PL1111-3500)
Eluent: 0.2 M NaH_2PO_4, pH 2.14
Flow rate: 0.5 mL/min
Inj Vol: 20 µL
Detector: UV, 268 and 220 nm

Results and Discussion

Reference materials

The retention of reference compounds is shown in Figure 1. L-ascorbic acid was well resolved from its possible degradation products.

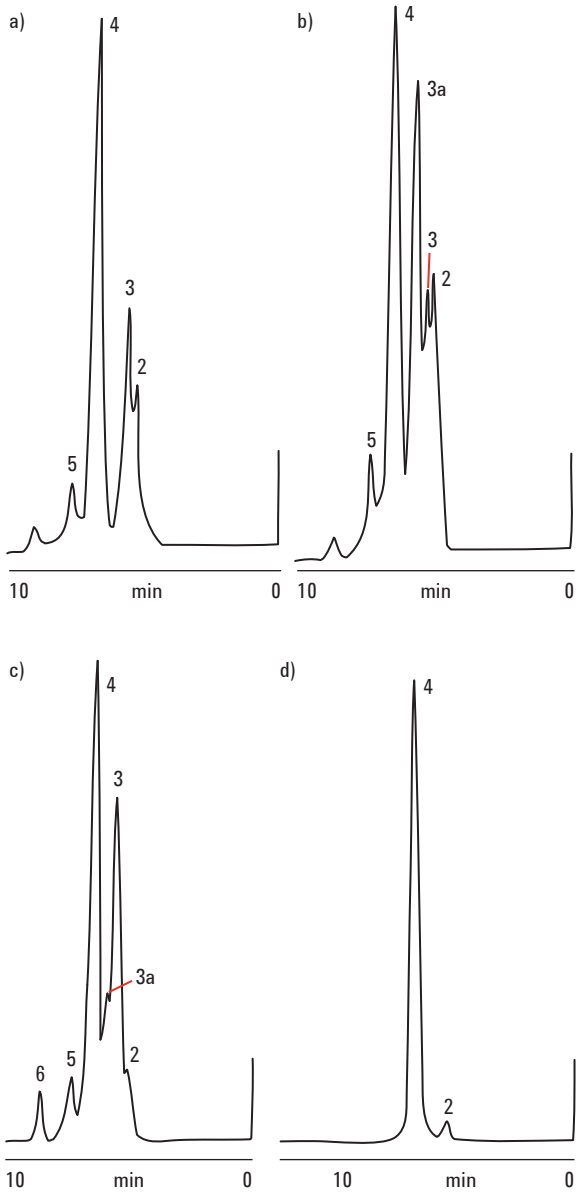
Bromine oxidation of L-ascorbic acid

HPLC of freshly prepared solutions of L-ascorbic acid treated with bromine produced the curves shown in Figure 2. Peak 1 is a high response at 220 nm, whereas peak 2 is a smaller response later identified as DHAA monomer with a free side chain. Peak height intensity was proportional to the amount of bromine added. Further addition of bromine was made to a cold solution of L-ascorbic acid (2 °C) to control degradation of DHAA. Homocysteine was then added and the reduction reaction went ahead at room temperature.

Effect of homocysteine on DHAA

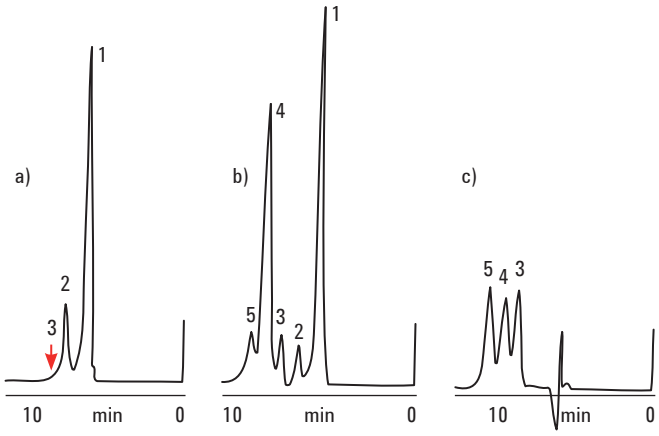
The reaction of homocysteine with DHAA was examined to distinguish the DHAA peak from surrounding peaks in the commercial samples (Figure 3). *Inter alia*, L-ascorbic acid (peak 4a) increased very rapidly after reaction with homocysteine, and peaks 4 and 6 represent monomeric forms of DHAA.

The complete data set and analysis is available in Kennedy *et al.* (1989).



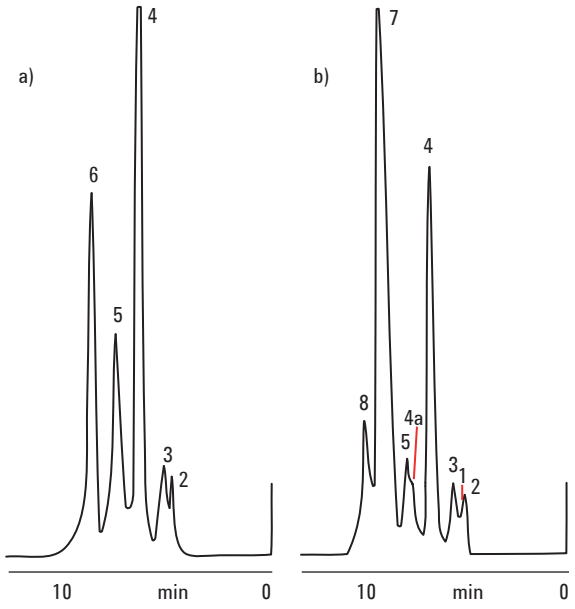
Peak identification
2. oxalic acid
3. L-threonic acid
3a. L-2,3-diketoglutaric acid
4. DHAA monomer in 1,4-lactone form
5. unstable component of DHAA
6. DHAA bicyclic hydrated monomer

Figure 1. HPLC separation of a) L-dehydroascorbic acid solution spiked with b) potassium L-2,3-diketoglutaric acid and c) calcium threonate reference materials. d) is L-dehydroascorbic acid produced by Dietz's method, at 220 nm (0.2 AUFS).



Peak identification
1. oxalic acid
2. DHAA monomer in the 1,4-lactone form
3. L-ascorbic acid
4. Hcys 1
5. Hcys 2

Figure 2. HPLC separation of a) bromine-oxidized L-ascorbic acid, at 220 nm (0.2 AUFS), and b) after addition of homocysteine (1:1), at 220 nm (0.1 AUFS) and c) at 268 nm (0.02 AUFS).



Peak identification
1. unidentified
2. oxalic acid
3. threonic acid
4. DHAA monomer in the 1,4-lactone form
4a. L-ascorbic acid
5. unidentified
6. DHAA monomer in the bicyclic form
7. Hcys 1
8. Hcys 8

Figure 3. HPLC separation of L-dehydroascorbic acid solution a) before and b) after reduction reaction with homocysteine, at 220 nm (0.05 AUFS), using optimized conditions.

Conclusion

PLRP-S columns successfully revealed the identities of some degradation products of vitamin C in the development of a qualitative and quantitative HPLC method for the fast analysis of L-ascorbic acid.

Reference

Kennedy, JF, White, CA, Warner, FP, Lloyd, LL and Rivera, ZS (1989) The identification and analysis of the oxidation products of L-ascorbic acid by HPLC C. *J. Micronut. Anal.*, 5, 91-109.

www.agilent.com/chem

Published in UK, May 20, 2011
5990-8217EN

Agilent Technologies

Evaluation of a novel nebulizer using an inductively coupled plasma optical emission spectrometer

Application note

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Abstract

The OneNeb nebulizer for inductively coupled plasma optical emission spectrometry (ICP-OES) features unique Flow Blurring technology. Compared to previous nebulizers, this universal nebulizer provides improved sensitivity, greater tolerance to dissolved salts and strong acids such as HF, resistance to most common organic solvents and efficient operation over a much wider flow rate range.

This application note demonstrates the superior performance of the OneNeb nebulizer compared to commercially available glass concentric nebulizers usually provided with ICP-OES instruments. Detection limits and reproducibility were better in a range of analytes and liquids.

Agilent Technologies

Introduction

The OneNeb nebulizer for use with an inductively coupled plasma optical emission spectrometer (ICP-OES) is a novel nebulizer that uses Flow Blurring technology. It is designed as a universal nebulizer offering a unique alternative to a variety of nebulizers by providing improved sensitivity, greater tolerance to dissolved salts and strong acids such as HF, resistance to most common organic solvents and efficient operation over a much wider flow rate range than existing nebulizers.

In this application note we will compare the performance of the OneNeb nebulizer to the commercially available glass concentric nebulizer normally fitted, using a range of performance criteria such as limits of detection and reproducibility using a range of analytes and liquids.

Description

The OneNeb nebulizer (Agilent part number 2010126900, Figure 1) is made completely from inert polymeric materials. It is physically robust and can withstand physical shocks that usually damage a glass concentric nebulizer.

Figure 1. OneNeb nebulizer

The capillary tubing extends nearly to the tip. The geometry at the tip, is carefully dimensioned to allow the carrier gas (in this case, argon) to mix with the sample liquid.

The OneNeb nebulizer uses Flow Blurring technology to mix argon with the sample to efficiently create an aerosol of smaller droplets with a narrower size distribution than conventional concentric nebulizers. Smaller droplets with narrow size distribution are more efficiently desolvated and excited in the plasma, ensuring better analytical precision and improved sensitivity.

By using Flow Blurring principles instead of the venturi effect for nebulization, the OneNeb is ideal for samples with high dissolved salts.

Other nebulizer designs

Concentric glass nebulizers (Figure 2) are the most common nebulizer type used in ICP-OES. The design features two concentric glass tubes with liquid pumped through the narrow inner capillary and argon forced through the gap between the inner sample capillary and outer quartz tube. A venturi effect creates an aerosol of relatively narrow droplet distribution, resulting in a nebulizer that provides good analytical RSD and detection limits. However, the narrow sample capillary is prone to blockages and precipitates forming on the end of the capillary that can affect nebulizer efficiency over time. Nebulizers using the venturi effect are not well suited for use with high dissolved salts because of this tendency to block.

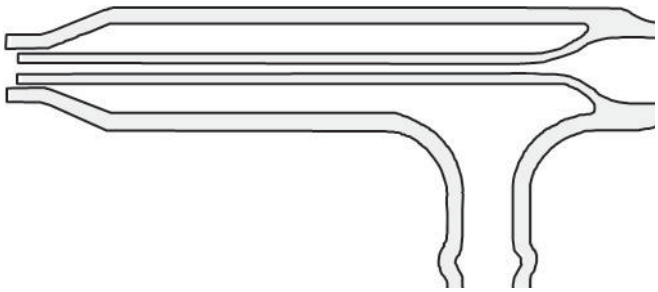

Figure 2. Concentric glass nebulizer

Nebulizers designed for samples with high total dissolved solids (TDS) such as the V-Groove nebulizer and cross-flow nebulizer do not rely on the venturi effect of the concentric glass nebulizer and are therefore more tolerant to dissolved salts. However, typically these nebulizers generate an aerosol with a wide range of droplet sizes resulting in higher analytical relative standard deviation and poorer detection limits.

Experimental

Instrumentation

An Agilent 725 ICP-OES with radially-viewed plasma and SPS 3 Sample Preparation System was used for this work.

The 725 ICP-OES features a custom-designed CCD detector, which provides true simultaneous measurement and full wavelength coverage from 167 to 785 nm. The CCD detector contains continuous angled arrays that are matched exactly to the two-dimensional image from the echelle optics. The thermally-stabilized optical system contains no moving parts, ensuring excellent long-term stability.

Operating parameters

- RF power: 1.3 kW
- Plasma gas flow: 15 L/min
- Auxiliary gas flow: 2.25 L/min
- Spray chamber: Single-pass and double-pass glass cyclonic
- Torch: Standard demountable with 0.38 mm quartz injection tube.
- Nebulizer flow: 0.7 L/min
- Replicate read time (for determining limits of detection): 30 s
- Number of replicates (for limits of detection): 10
- Stabilization time (for limits of detection): 30 s
- Replicate read time (for stability): 10 s
- Number of replicates (for stability): 6

Pump tubing

Two cases of pump tubing were used:

- Instrument: Orange-green (0.38 mm ID), of materials matched to the solvent being studied.
- Waste: Orange-orange (0.89 mm ID) Marprene for organic solutions.
- Instrument: Black-black (0.76 mm ID) for aqueous only.
- Waste: Blue-blue (1.65 mm ID) for aqueous only.

Results and discussion

The transport efficiency of the OneNeb at conventional flows is equivalent to a high-efficiency concentric glass nebulizer (Table1). As shown in Table 2, the OneNeb is capable of operating with even higher transport efficiency at very low sample flow rates, which a conventional concentric glass nebulizer is not capable of. Typically, for operation with low sample uptake rates, a specialized low flow nebulizer is required. The very high transport efficiency of the OneNeb at low flow rates makes it an ideal nebulizer for precious samples or samples with limited volumes, such as biological fluids.

Table 1. Transport efficiency at conventional ICP-OES uptake rates

Nebulizer	Solvent	Spray chamber	TE (%)
Glass concentric	Water	Double-pass	6.1
OneNeb	Water	Double-pass	6.6
OneNeb	Water	Single-pass	3.8–12.8

Table 2. Transport efficiency of OneNeb at very low uptake rates

Solvent	Spray chamber	TE (%)
Water (2–6% HNO_3)	Double-pass	12.5–18.79
Water (2–6% HNO_3)	Single-pass	17.7–31.4
ShellSol	Single-pass	44.0–48.7
Diisobutyl ketone	Single-pass	49.0

With organic solvents commonly used in ICP-OES analysis such as diisobutyl ketone and ShellSol, the OneNeb nebulizer provided excellent stability (Figures 3 and 4) over long-term runs, demonstrating excellent chemical resistance.

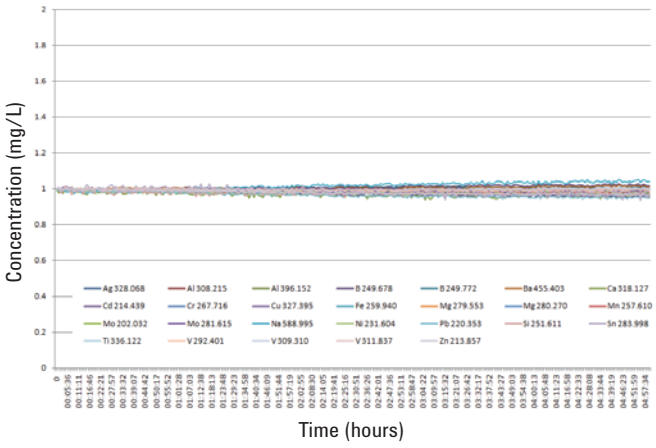

Figure 3. Long-term stability of the OneNeb nebulizer with diisobutyl ketone

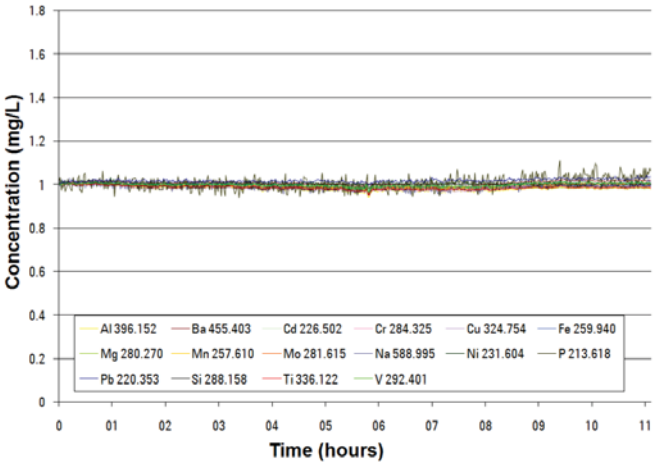

Figure 4. Long-term stability of the OneNeb nebulizer with ShellSol

The OneNeb nebulizer provided superior (>100% ratio) detection limits compared to the high performance concentric glass nebulizer for all elements analyzed, except for silver and zinc, which exhibited equivalent detection limits (Table 3).

Table 3. Comparison of 30 second detection limits (DLs) between concentric glass nebulizer (CGN) and OneNeb nebulizer

Element	CGN DL	OneNeb DL	DL ratio (%)
Ag 328.068	0.61	0.61	100
Al 167.019	1.94	1.53	127
As 188.980	12	9.84	122
Ba 455.403	0.07	0.05	162
Be 313.042	0.01	0.01	193
Ca 396.847	0.09	0.07	121
Cd 214.439	1.27	0.91	139
Co 238.892	1.9	1.7	110
Cr 267.716	0.86	0.70	123
Cu 327.395	1.76	0.96	183
Fe 238.204	0.90	0.68	132
K 766.491	59	38	154
Mg 279.553	0.05	0.05	107
Mn 257.610	0.19	0.15	131
Na 589.592	2	1.04	197
Ni 231.604	5	5	108
Pb 220.353	12	10	113
Se 196.026	17	13	133
Tl 190.794	15	12	129
V 292.401	1.24	0.96	129
Zn 213.857	0.50	0.49	101

Conclusion

The OneNeb nebulizer with Flow Blurring technology demonstrated excellent tolerance to samples with high TDS. Over weeks of extended testing of these high TDS samples, the OneNeb nebulizer proved virtually unblockable. This was in stark contrast to the regular failure of the glass concentric nebulizer due to blocking.

In terms of detection limits and tolerance to organic solvents, the OneNeb nebulizer proved superior to a high performance concentric glass nebulizer. Its resistance to strong acids such as HF proved similar to inert polymeric nebulizers. Tolerance to high TDS samples by the OneNeb nebulizer ranked it equal to nebulizers dedicated to handling high TDS such as V-groove nebulizers, without the deterioration in precision or detection limits in aqueous solutions.

The OneNeb nebulizer proved to be a genuinely universal nebulizer that is mechanically rugged and durable. It is competitive in price with a high performance concentric glass nebulizer. The OneNeb is capable of replacing many different types of nebulizers typically required to analyze the range of samples an ICP-OES is called upon to measure, without compromising performance. A universal nebulizer also simplifies method development and day-to-day operation by eliminating the need to decide which nebulizer is best for which sample, and reducing the need for many different nebulizers. It operates with very high nebulization efficiency at sample uptake rates from 40 µL/min, potentially allowing the analysis of volume limited samples.

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Published June 24, 2011
Publication number: 5990-8340EN

Agilent Technologies

Using a Dual LTM Series II System with Flow Modulated Comprehensive GCxGC

Application Note

Application Area Identifier

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Abstract

A comprehensive GCxGC system based on differential flow modulation is described that uses three independent programmable ovens. The first dimension separation occurs in the 7890A air bath oven while two simultaneous second dimension separations occur on 5 inch LTM Series II modules. All columns operate in constant flow mode. Oven temperature programs can be customized independently for each column. Typically the two LTM columns will be of different polarities and phase ratios to maximize the information that can be gathered from the sample. A typical column configuration consists of a 20 m x 0.18 mm x 0.25 µm DB5ms for the first dimension, a 7 m × 0.25 mm × 0.2 µm HP-INNOWax for LTM module 1 and a 5 m × 0.25 mm × 0.15 µm DB17HT for LTM module 2. Many other column combinations are possible.

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Introduction

Conventional flow modulated GCxGC usually consists of one first dimension column and one second dimension column where both are subjected to the same temperature program. The basic one-oven system has been described previously [1,2]. Flow modulation also has the distinct advantage of not requiring cryo fluids for operation, rather it relies on a high flow differential between 1st and 2nd dimensions for operation.

Careful matching of the retention factors (k) between the first and second column is necessary in a one-oven system in order to produce meaningful 2D data and avoid the wrap around effect. The wrap around effect occurs when analytes injected onto the second column do not elute in one modulation cycle. However, the single oven system is in widespread use for a variety of applications and works well if k's are matched appropriately.

Flow modulated GCxGC works best when all columns are operated in constant flow mode. The Low Thermal Mass (LTM) Series II system is fully integrated into the GC and MSD ChemStations and Agilent 7890A firmware allowing control of all parameters. Since this integration enables LTM to operate in constant flow, the system can be easily interfaced to a flow modulated GCxGC 7890 system.

Experimental

A diagram of the system is shown in Figure 1. A Capillary Flow Technology (CFT) splitter is used to direct the out flow from the CTF modulator to two LTM column modules for a simultaneous dual channel GCxGC analysis. Each column operates with its own independent temperature program.

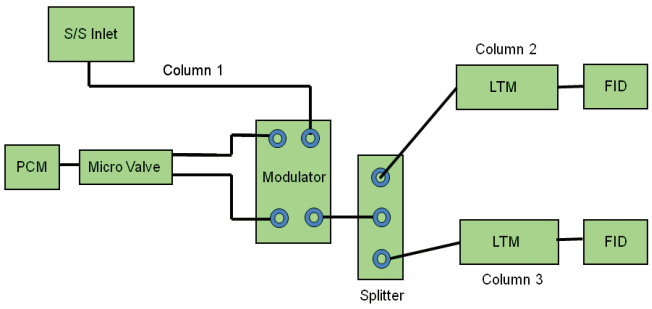

Figure 1. Diagram of the dual LTM GCxGC system.

The operation scheme of the flow modulator showing both the load and inject states is shown in Figure 2. Effluent for the first column fills the collection channel, and before significant diffusion or overfill occurs the three way valve is switched and a high flow (21 mL/min) controlled by the PCM injects the channel contents into the two second dimension columns. The modulation cycle then repeats based on the user set collect and inject times.

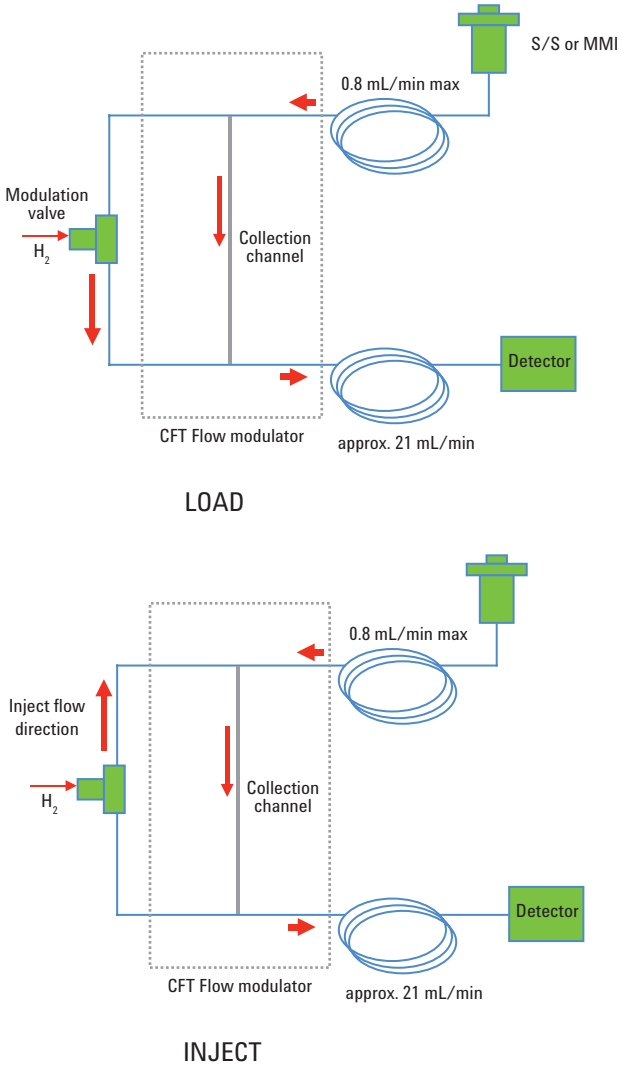

Figure 2. Operational detail of the flow modulator showing load and inject states.

Column 1 flow rate depends on column dimensions, but cannot exceed 0.8 mL/min. Figure 3 shows the relationship between modulation period and Column 1 flow rate.

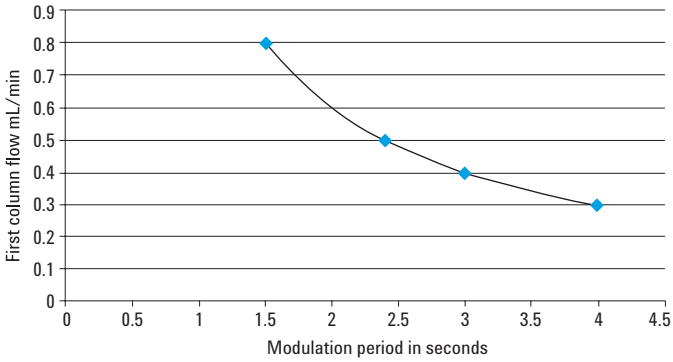

Figure 3. Relationship between modulation period and first dimension column flow rate.

Referring again to Figure 1, since LTM column flow rate is controlled by a single PCM, column flow will be the same in both modules provided they are of the same dimension. If this is not the case, the column configuration (in Chemstation) should set the PCM to control the longer or more restrictive column at 21 mL/min or greater. The second LTM column will then operate at a higher flow. Therefore, it is advisable that the two LTM columns do not differ greatly in length. Also, it is best to keep the second dimension columns at 0.25 mm ID. For this work, LTM column pairs were either both 5 meter or 5 and 7 meter. An example column configuration Chemstation pane for the system is shown in Figure 4.

	Column	Calibration Results	Inlet	Outlet	Heated By
1	Agilent 19091J-413: 400 °C: 7 m x 250 µm x 0.25 µm Additional Segments: inSeg Heated By Oven: 0.5 m x 250 µm x 0 µm outSeg Heated By Oven: 0.5 m x 250 µm x 0 µm HP-5 5% Phenyl Methyl Siloxan: <Not Inventoried>	Uncalibrated	PCM A-1	Front Detector	LTM-II
2	J&W Custom LTM 5M: 320 °C: 5 m x 250 µm x 0.15 µm Additional Segments: inSeg Heated By Oven: 0.3 m x 250 µm x 0 µm outSeg Heated By Oven: 0.6 m x 250 µm x 0 µm LTM 5M x 0.25 x 0.25: <Not Inventoried>	Uncalibrated	PCM A-1	Back Detector	LTM-II
3	450 °C: 20 m x 180 µm x 0.18 µm restrictor: <Not Inventoried>	Uncalibrated	Front Inlet	PCM A-1	Oven

Figure 4. Column configuration pane from the GC Chemstation showing set up of all three columns.

Hardware

Agilent 7890A GC with S/S inlet and dual FID's

Flow modulator	G3440A option887, and G3487A
If adding to existing GC	G3486A
CFT un-purged splitter	Kit: G3181-64010
LTM Series II	G6680A, 2-channel, 5-inch system, two power supplies

Firmware and Chemstation

Agilent 7890A firmware	A.01.12.1 or greater
ChemStation	B.04.03 DSP1, includes LTM II software

Typical Parameters

Carrier gas	Hydrogen
Primary column	20 m × 0.18 mm × 0.18 µm HP-1
LTM Module 1	7m × 0.25 mm × 0.25 µm HP- INNOWax, or 5 m × 0.25 mm × 0.15 µm HP- INNOWax
LTM Module 2	5 m × 0.25 mm × 0.15 µm DB17HT
Primary column flow	0.35 mL/min, 27.6 psi starting pressure
LTM 1	20 mL/min, 25.6 psi starting pressure (7 m column)
LTM 2	29 mL/min
Inlet	Split/splitless, 280 °C, 200-600 to 1 split
Primary oven program	35 °C (2 min) to 280 °C @ 3 °C/min
LTM 1 program	55 °C (3 min) to 270 °C @ 5 °C/min
LTM 2 program	60 °C (5 min) to 300 °C @ 3 °C/min
LTM InSeq retention gaps	0.5 m × 0.25 mm
LTM OutSeg retention gaps	0.5 m × 0.25 mm
Detectors	dual FID's at 300 °C

GCxGC Parameters

Load time	2.700 sec
Inject time	0.090 sec
Modulation period	2.799 sec

GCxGC Data Processing Software

GC Image, Version 2.1b4

Results and Discussion

In flow modulated GCxGC, greater flexibility in optimizing methods may be achieved by use of independent ovens for the first and second dimension columns. Correct matching of the retention factors between the 1st and 2nd dimension columns is critical for achieving the best performance with flow modulated GCxGC. If retention on the 2nd D column is too high, analytes injected during one modulation cycle may not elute completely before the next modulation begins.

When a second independent oven is available for the 2nd dimension column, more column choices are available in terms of phase ratio and length. Using a temperature offset, (2nd column starts at higher temp compared to 1st) may allow more retentive columns to be used. Then fine tuning the temperature ramp rate becomes an additional tool to help achieve a difficult separation throughout a 2D chromatographic run or in a particular section of a run. Employing an LTM module for the second dimension makes this possible.

The system can be further enhanced by inserting a CFT unpurged splitter between the modulator and the 2nd dimension. This allows two completely independent 2nd dimension LTM modules (with different stationary phase polarities) to be used which will yield two sets of 2D data for each run.

In figure 5a, a lower phase ratio 7 m INNOWax column is used for the analysis of a jet fuel. When both 1st and 2nd dimension columns are in the air bath oven, the standard 5 m × 0.25 mm × 0.15 µm column must be used to avoid wrap around at low oven ramp rates. With the second column configured as an LTM, longer, thicker film columns can be used to achieve better group separation while ensuring that all compounds will elute from the 2nd column in one modulation cycle. Figure 5b shows the same jet fuel analyzed simultaneously on a less polar 5 m × 0.25 mm × 0.15 µm DB17HT. Both offer useful information and allow different levels of compound group determination when using GC Image.

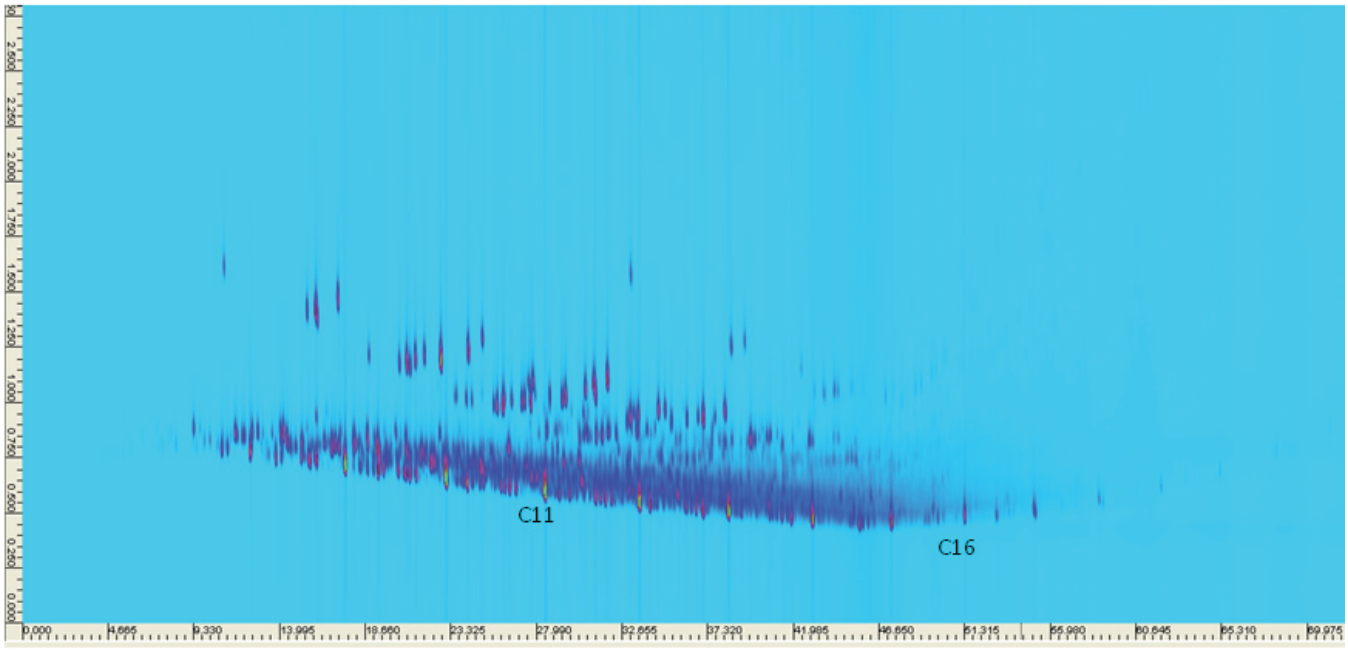

Figure 5a. Jet fuel 2D image. 7 m × 0.25 mm × 0.24 µm HP-INNOWax, LTM program: 55 °C (3 min) to 270 °C @ 5 °C/min. 7890A program: 35 °C (2 min) to 280 °C @ 3 °C/min.

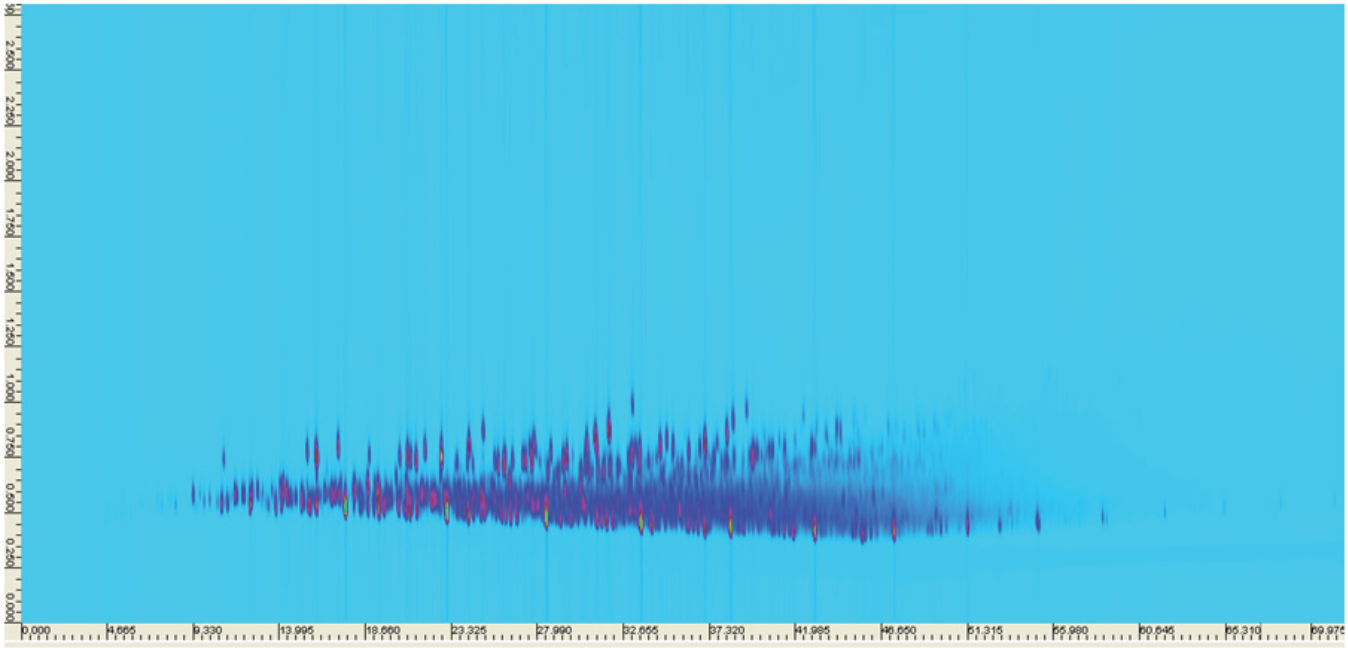

Figure 5b. Jet fuel on 5 m × 0.25 mm × 0.15 µm DB17HT, LTM program: 60 °C (5 min) to 300 °C @ 3 °C/min. 7890A program: 35 °C (2 min) to 280 °C @ 3 °C/min.

2D images of a fragrance additive used in detergents is shown in figures 6a and 6b, on the 7 m INNOWax and DB17HT LTM columns, respectively. Peak 3, 4-tert-butyl-cyuclohexyl acetate, shown on the wax column eluted on a second modulation cycle. However, it remains well separated from other components and does not complicate interpretation of the 2D image. Labeled compounds determined by a GC × GC - 5975C MSD system.

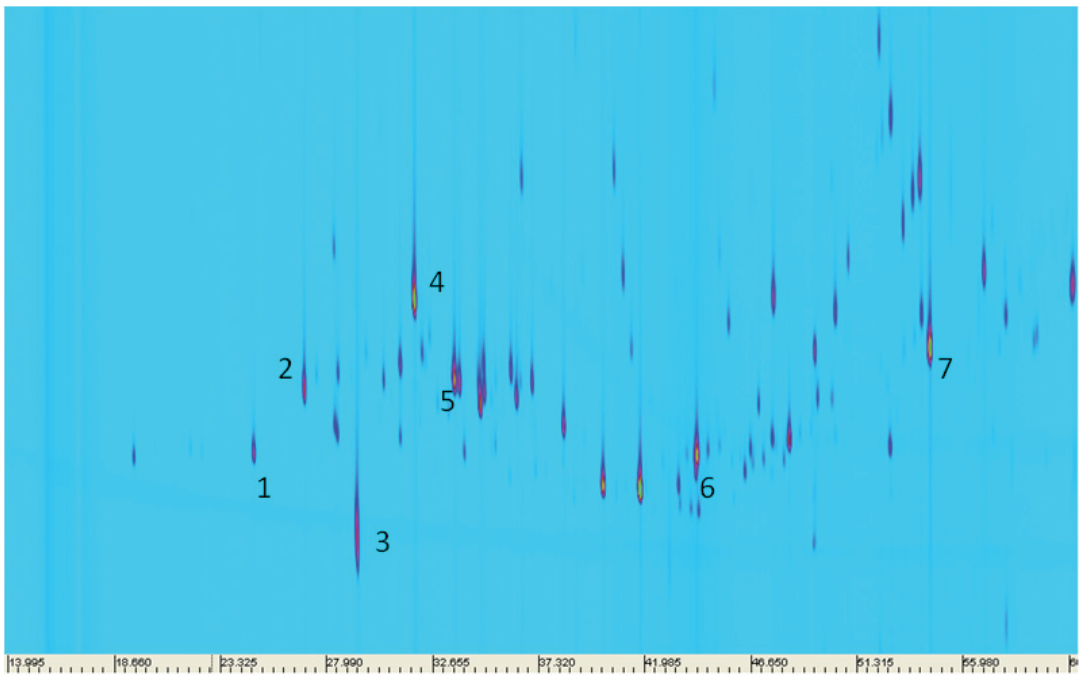

Figure 6a. Fragrance additive using 7 m INNOWax for 2nd dimension, LTM program: 55 °C (3 min) to 270 °C @ 5 °C/min. 7890A program: 35 °C (2 min) to 280 °C @ 3 °C/min. 1. Alpha Pinene, 2. Limonene, 3. 2,6 dimethyl 7-octen-2-ol, 4. Phenethyl acetate, 5. Terpenol, 6. Bicyclopentadiene, 7. 4-tert-butylcyclohexyl acetate.

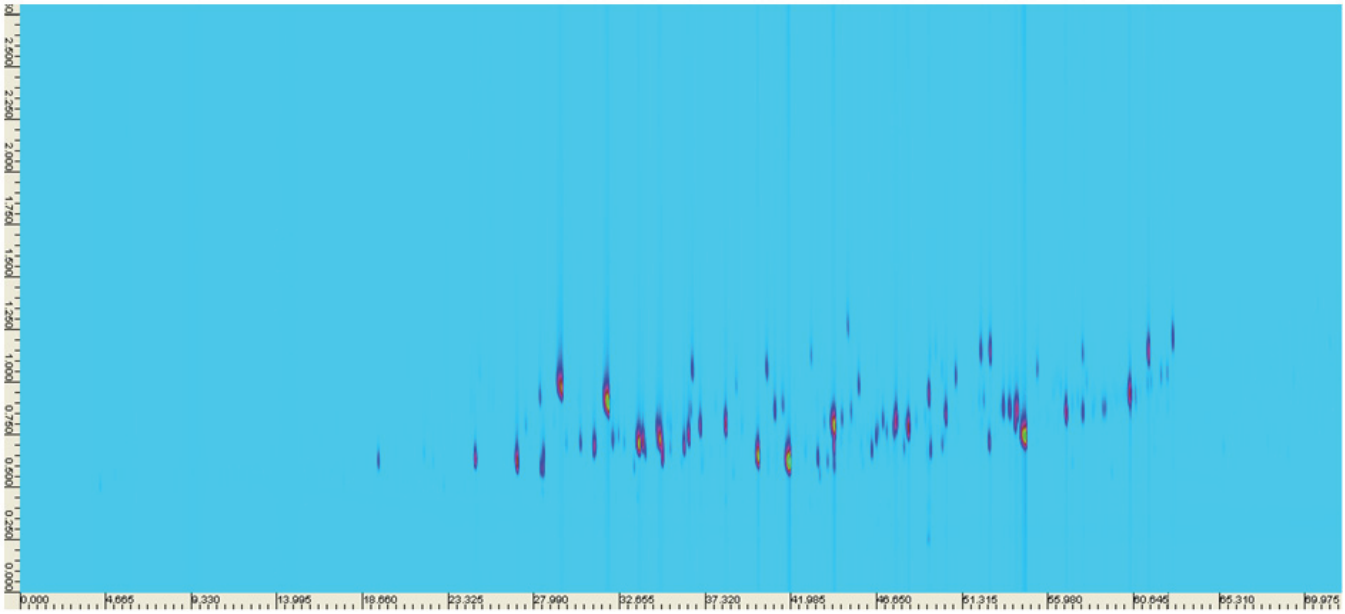

Figure 6b. Fragrance additive using 5m DB17HT for 2nd dimension separation. LTM program: 60 °C (5 min) to 300 °C @ 3 °C/min. 7890A program: 35 °C (2 min) to 280 °C @ 3 °C/min.

Lime oil images are shown in figures 7a and 7b. Only the regions around limonene are shown to highlight the separation differences on INNOWax and DB17HT. The 7M thicker film wax column separates minor components from dominate limonene. Compounds identified using a GC × GC - 5975C MSD system.

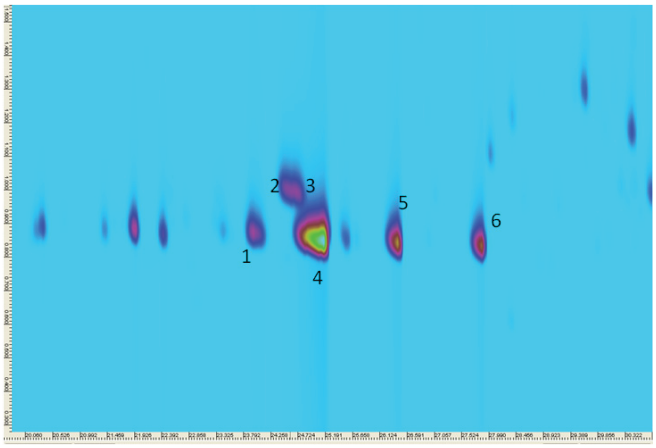

Figure 7a. Lime oil on the 7 m INNOWax. LTM program: 55 °C (3 min) to 270 °C @ 5 °C/min. 7890A program: 35 °C (2 min) to 280 °C @ 3 °C/min. 1. Alpha Pinene, 2. Limonene, 3. 2,6 dimethyl 7-octen-2-ol, 4. Phenethyl acetate, 5. Terpenol, 6. Bicyclopentadiene, 7. 4-tert-butylcyclohexyl acetate 1.beta pinene, 2. 1,4 Cineol, 3. m-cymene, 4. Limonene, 5. Terpinen, 6. Terpinolen

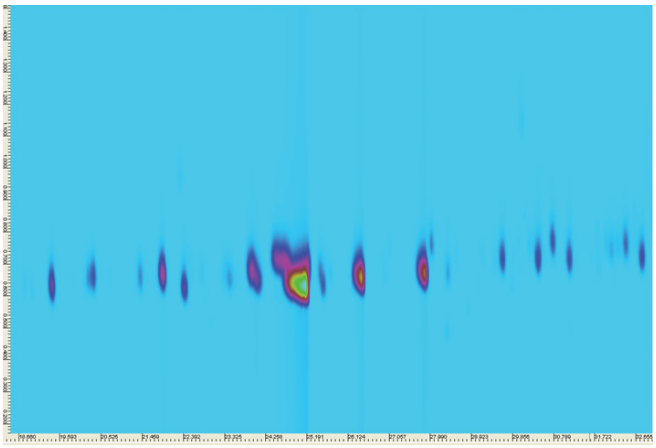

Figure 7b. Lime oil on the 5 m DB17HT. LTM program: 60 °C (5 min) to 300 °C @ 3 °C/min. 7890A program: 35 °C (2 min) to 280 °C @ 3 °C/min.

Finally, a 2D analysis of B20 (20% soy) biodiesel is shown in figure 8 using a 5 m × 0.25 mm × 0.15 µm INNOWax. Here, the LTM module and 7890 air oven are programmed at 3 °C/min. However the starting temperature of LTM is offset by minus 5 °C.

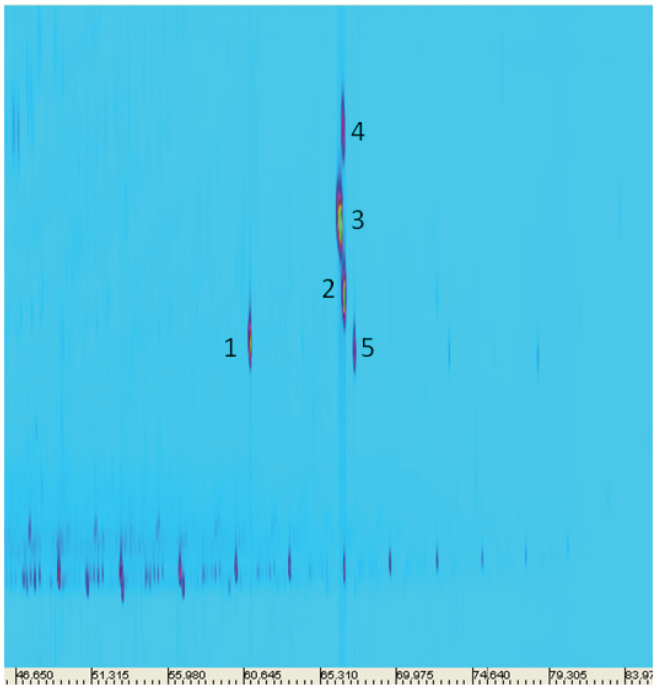

Figure 8. Separation of C16 and C18 fatty acid methyl esters in B20 biodiesel on a 5 m × 0.25 mm × 0.15 µm LTM INNOWax column in the 2nd dimension. LTM program: 30 °C (0 min) to 270 °C (5 min) @ 3 °C/min. 1. C16:0, 2. C18:1, 3. C18:3, 4. C18:3, 5. C18:0.

Conclusions

Comprehensive GCxGC is normally used when faced with a very difficult separation in a complex sample, perhaps a specific analyte determination. It is also a powerful tool for group determination, especially in fuels, and as a classification tool when used with chemometrics. The LTM series II system gives the analyst additional separation power and is easily interfaced to a flow modulated GCxGC system. Depending on how the system is configured, two or three independent temperatures programs can be used. This allows a wider range of column retention in the second dimension to be used.

This work is intended to illustrate some of the possibilities where comprehensive GC and LTM technology can be put to work. Only one combination of column stationary phases was tested (DB5ms-INNOWax-DB17HT). Many other combinations are possible. For example, some useful combinations to consider with the dual LTM system where different polarities are used include (INNOWax-DB1-DC200), and (DB1-DB200-DB35). Reversing polarities (most polar as 1st dimension) can be useful, i.e. (DB210-DB1-DB17) for problems where a few polar compounds must be separated from a complex non-polar matrix. When using LTM with GCxGC, appropriate matching of the retention factors of the 1st to 2nd dimension columns is still important; however LTM offers some additional flexibility to use lower phase ratio columns through temperature offsets and temperature ramps.

References

1. Comprehensive Flow Modulated Two-Dimensional Gas Chromatography, Roger L. Firor, Application Note 5989-6078EN, 2008
2. Comprehensive GC System Based on Flow Modulation for the 7890 GC, Roger L. Firor, Application Note 5989-8060EN, 2009

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Printed in the USA
June 20, 2011
5990-8391EN

Agilent Technologies

Fullerene Analysis using Agilent PLgel Columns and Gel Permeation Chromatography

Application Note

Materials Testing and Research, Polymers

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Introduction

Fullerenes are normally prepared by evaporating graphite rods in arc reactors under a helium atmosphere to produce soot that contains C_{60}, C_{70}, higher fullerenes, and uncharacterized insoluble material. The soluble fractions may be extracted using a suitable solvent and further purification may be carried out by gel permeation chromatography (GPC). Low pore size Agilent PLgel 50Å GPC columns are most appropriate for these applications. As an analytical tool, high efficiency columns can be used to screen fullerene compounds. The behavior of preparative separations can be predicted by running an analytical separation using the same column packing.

Analysis of Fullerene

One of the difficulties in fullerene chromatography is the relatively poor solubility of the compounds. However, toluene exhibits favorable properties as a GPC solvent since it provides reasonable solubility (6 to 8 mg/mL), good chromatographic selectivity, and is amenable to solvent removal and recovery by distillation in preparative separations.

Figure 1 illustrates the separation achieved for a material whose basic structure was believed to be that indicated in Figure 2.

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Conditions for Figure 1

Columns	2 × Agilent PLgel 5 µm 50Å, 7.5 × 300 mm (p/n PL1110-6515)
Eluent	Toluene
Flow rate	1.0 mL/min
Loading	2 mg/mL, 200 µL
Detector	RI
System	Agilent PL-GPC 50

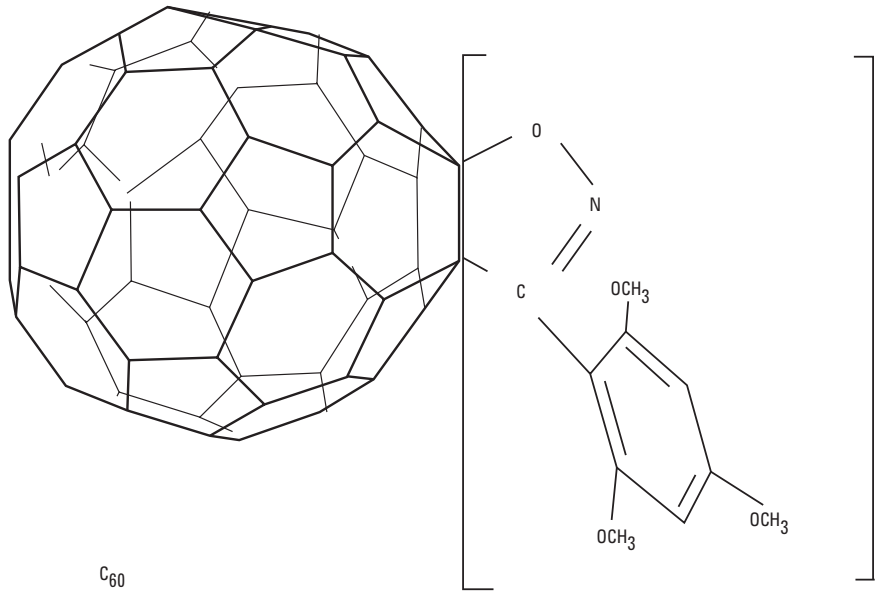

Figure 1. Separation of a fullerene on an Agilent PLgel two-column set.

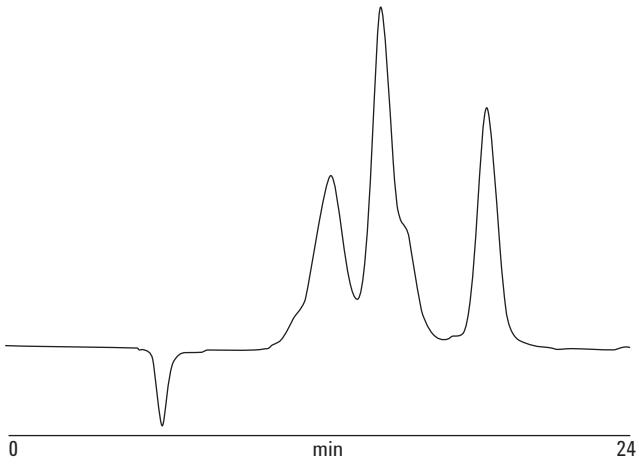

Figure 2. Suspected structure of the fullerene under investigation.

In preparative work, columns packed with larger particle size packings are preferred for increased loading. Preparative column separations can easily be predicted by running samples on analytical columns packed with the same type of beads. Figure 3 illustrates this approach; if this separation was scaled up to a preparative system it would require 25-mm id columns with a subsequent tenfold increase in flow rate and loading.

Conditions for Figure 3

Columns	4 × Agilent PLgel 10 µm 50Å, 7.5 × 300 mm (p/n PL1110-6115)
Eluent	Toluene
Flow rate	1.0 mL/min
Loading	2 mg/mL, 200 µL
Detector	RI
System	PL-GPC 50

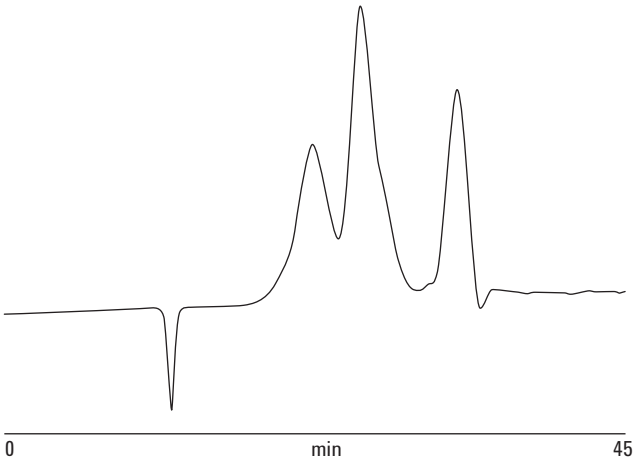

Figure 3. Separation of fullerene using an Agilent PLgel 10 µm analytical packing to predict a preparative scale separation using the same packing.

Conclusion

Soluble fullerene fractions extracted using a suitable solvent can be further purified by gel permeation chromatography using low pore size Agilent PLgel 5 µm columns. The behavior of preparative separations can be predicted by first running an analytical separation with larger pore sized Agilent PLgel 10 µm columns, using the same column packing.

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Printed in the USA
June 29, 2011
5990-8492EN

Agilent Technologies

Permanent Gas Analysis – Separation of Helium, Neon and Hydrogen a MolSieve 5A column using the Agilent 490 Micro GC

Application Note

Micro Gas Chromatography, Permanent Gas Analysis

Author

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Agilent Technologies, Inc.

Middelburg

The Netherlands

Introduction

This application note shows an example of the permanent gas analysis in a sample with high % level of Oxygen and Nitrogen (Air) on an Agilent 490 Micro GC, including the separation of Helium, Neon, and Hydrogen (ppm level). The separation of these compounds is done on a 10 m MolSieve 5A column and requires the use of Argon as carrier gas to detect all potential other carrier gases like Helium, Hydrogen, and Nitrogen.

The advantage of the Agilent 490 Micro GC, is the ease-of-use and the speed of analysis, resulting in a total analysis time of less than 40 seconds.

The Agilent 490 Micro GC is a rugged, compact, and portable lab-quality gas analysis platform. When the composition of gas mixtures is critical, count on this fifth generation micro gas chromatography.

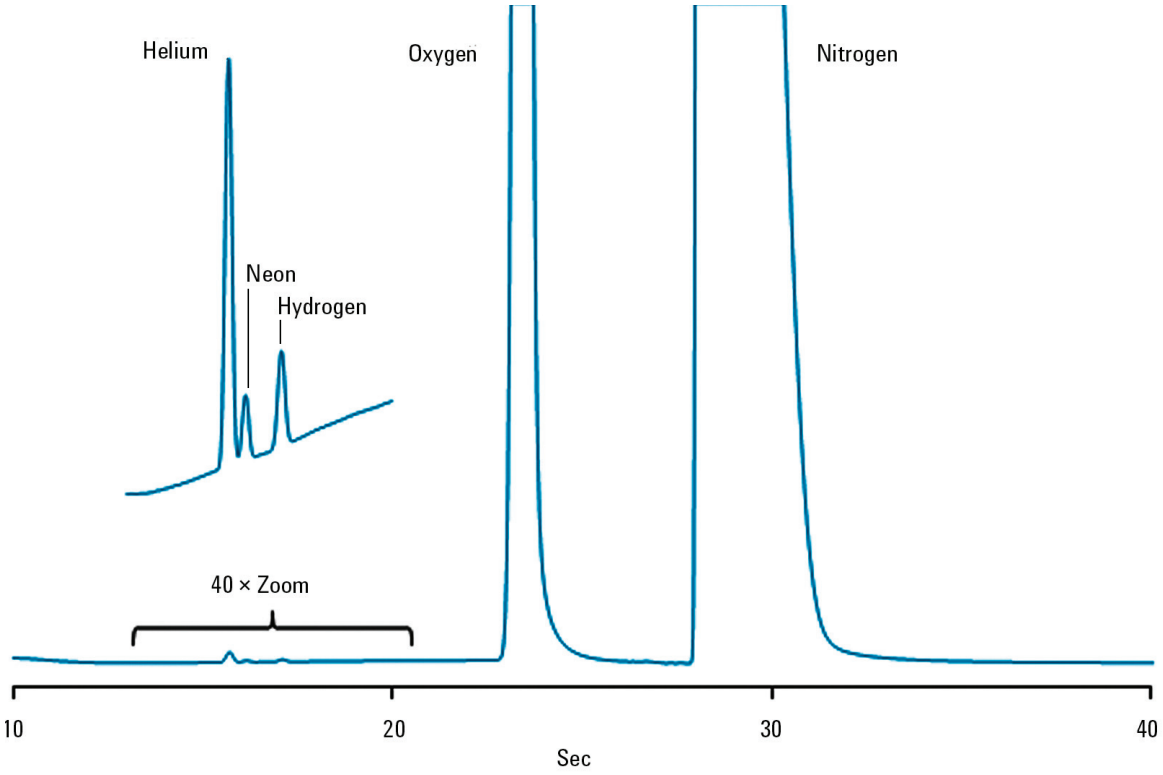
Agilent Technologies

Instrumentation

Instrument	Agilent 490 Micro GC (G3581A)
Column channel	MolSieve 5A, 10 m
Column temperature	80 °C
Carrier gas	Argon, 240 kPa
Injector temperature	60 °C
Injection time	60 msec

Sample information

Helium	ppm level
Neon	ppm level
Hydrogen	ppm level
Oxygen	high % level
Nitrogen	high % level



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Printed in the USA
July 5, 2011
5990-8527EN

Agilent Technologies

Fast Separation of Oxygen and Nitrogen on a MolSieve 5A Channel Using the Agilent 490 Micro GC

Application Note

Micro Gas Chromatography, Permanent Gas Analysis

Authors

Mohamed Bajja and Remko van Loon

Agilent Technologies, Inc.

Middelburg

The Netherlands

Introduction

When a really fast separation of Oxygen and Nitrogen is required, the Agilent 490 Micro GC, equipped with a short MolSieve 5A column channel, delivers the speed you need.

This application note shows the fast separation of Oxygen and Nitrogen using a 4 m MolSieve 5A column channel instead of using the standard 10 m MolSieve 5A column channel. The advantage of the Agilent 490 Micro GC, in combination with this 4 m MolSieve 5A column channel, is the ease-of-use and the speed of analysis. Nitrogen will elute in less than 20 s.

Argon and Oxygen will not be separated on the 4 m MolSieve 5A column. These compounds will coelute. The separation of Argon and Oxygen requires the use of a 20 m MolSieve 5A column channel on a low temperature.

The Agilent 490 Micro GC is a rugged, compact and portable lab-quality gas analysis platform. When the composition of gas mixtures is critical, count on this fifth generation micro gas chromatography.

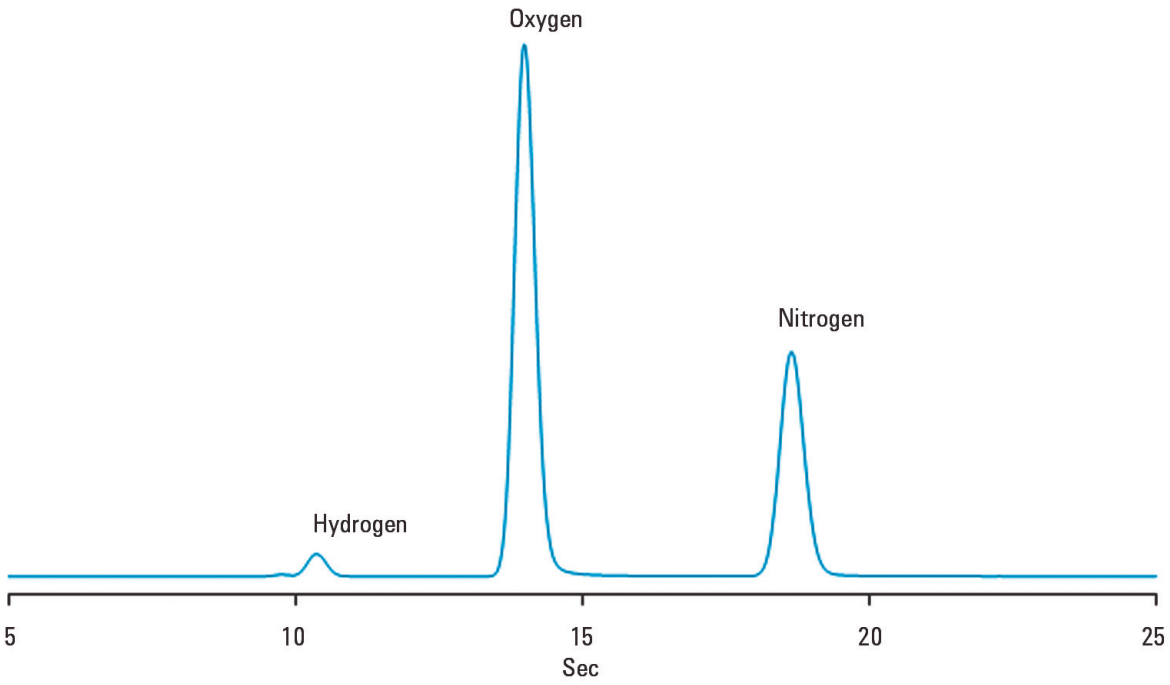
Agilent Technologies

Instrumentation

Instrument	Agilent 490 Micro GC (G3581A)
Column channel	MolSieve 5A, 4 m
Column temperature	100 °C
Carrier gas	Helium, 100 kPa
Injection time	40 msec

Sample information

Hydrogen	1.0%
Oxygen	0.4%
Nitrogen	0.2%



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Printed in the USA
June 28, 2011
5990-8529EN

Agilent Technologies

New methodology for determination of gold and precious metals using the Agilent 4100 MP-AES

Application note

Geochemistry, metals and mining

Author

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Melbourne, Australia

Introduction

The accurate and precise determination of gold and other precious metals (PMs) is a vital task for companies involved in PM production and the contract or service laboratories that support them. This might involve the processing of hundreds of samples typical of exploration and mining activities, or supplying the quality control assay of the final product from PM producers or recyclers. PMs are also produced as a by-product of copper or nickel processing.

Today's analysts have access to a range of elemental analytical techniques including flame and graphite furnace atomic absorption spectroscopy (AA), inductively coupled plasma optical emission spectroscopy (ICP-OES) and ICP mass spectroscopy (ICP-MS). The choice of method will depend on a number of factors, for example, the technology that is available, the skill of the operator, sample type, cost of analysis, sample throughput requirements and the objective of the analytical result.

Agilent has identified an increased need for multi-element determinations over a wide dynamic range at an attractive overall cost of analysis, taking into account instrument supplies, consumables, power and labor, and has developed a new instrument to meet this need.

The Agilent 4100 Microwave Plasma-Atomic Emission Spectrometer (MP-AES) (Figure 1) is a low-cost, highly automated technique that is suitable for the trace analysis of PMs in samples typically found in mining and exploration activities. As MP-AES relies on the generation of a microwave plasma using nitrogen, no flammable gases such as acetylene are required. This reduces running costs and improves lab safety. Nitrogen can be supplied from bottled gas or the Agilent 4107 Nitrogen Generator. This alleviates the difficulty and costs in sourcing gases such as acetylene, especially in remote locations.

Figure 1. Agilent 4100 MP-AES

This application note describes the analysis of PM samples prepared by fire assay using the Agilent 4100 MP-AES.

Experimental

Instrumentation

The Agilent 4100 MP-AES is a fast sequential multi-element analytical technique that has a microwave-induced nitrogen plasma at its heart. As a result running costs are significantly reduced as only nitrogen is required for plasma operation.

The 4100 MP-AES uses Agilent's unique Microwave Excitation Assembly to create a concentrated axial magnetic field around a conventional torch. This focuses and contains the microwave energy where it is needed to produce a toroidal plasma with a cooler central channel that is suitable for stable introduction of liquid samples using a conventional sample introduction system.

Samples and sample preparation

A series of samples that are normally analyzed by flame AA were prepared using fire assay. A 30 g rock sample was heated with flux to over 1,000 °C. The process yields a small silver sphere, which was then dissolved in 4 mL of 25% aqua regia. The 4100 MP-AES operating parameters were then optimized, as shown in Table 1.

Table 1. Agilent 4100 MP-AES operating parameters

Instrument parameter	Setting
Analytes (wavelength)	Au (267.595), Pt (265.945), Pd (363.470)
Nebulizer pressure	140–240 kPa
Read time	3 s
No. of replicates	3
Sample uptake delay	10 s
Stabilization time	5 s
Background correction	Auto

Method detection limits

Method Detection Limits (MDLs) for gold, platinum and palladium were determined by measuring two sets of ten method blanks twice, on non-consecutive days, using the conditions as defined in the analytical method. The MDL was calculated as the 3 sigma standard deviation of the twenty concentration results.

The MDLs listed in Table 2 are sufficiently low for this type of analysis.

Table 2. MDLs for Au, Pt and Pd in fire assay samples

Analyte	Wavelength (nm)	MDL (µg/L)
Au	267.595	4
Pt	265.945	13
Pd	363.470	0.7

Linear range

The concentration or working range of an analytical technique is the range of concentrations that can be measured accurately without the need to recalibrate or dilute the sample. The linear range for Au, Pt and Pd was investigated using the 4100 MP-AES. A series of standards was prepared at concentrations of 2, 7, 70, 90, 100, 110 and 120 mg/L, and analyzed using the 4100 MP-AES. The calibration graphs obtained are shown in Figures 2, 3 and 4. These show that the linearity for all three analytes was acceptable up to a concentration of 120 mg/L, which exceeds the requirements of the application.

Sample volume

The analysis of PMs is volume-sensitive. The typical total sample volume available for analysis is around 4 mL. By using an Agilent SPS 3 Sample Preparation System connected to the 4100 MP-AES, the method cycle time (sample to sample) was 55 s and the sample volume consumed during analysis was 1.8 mL.

Accuracy

In order to test the ability of the 4100 MP-AES to analyze PMs at variable concentrations, a batch of Certified Reference Materials (CRMs) was analyzed.

Figure 2 Calibration graph for Au at the 267.595 nm wavelength

Figure 3. Calibration graph for Pt at the 265.945 nm wavelength

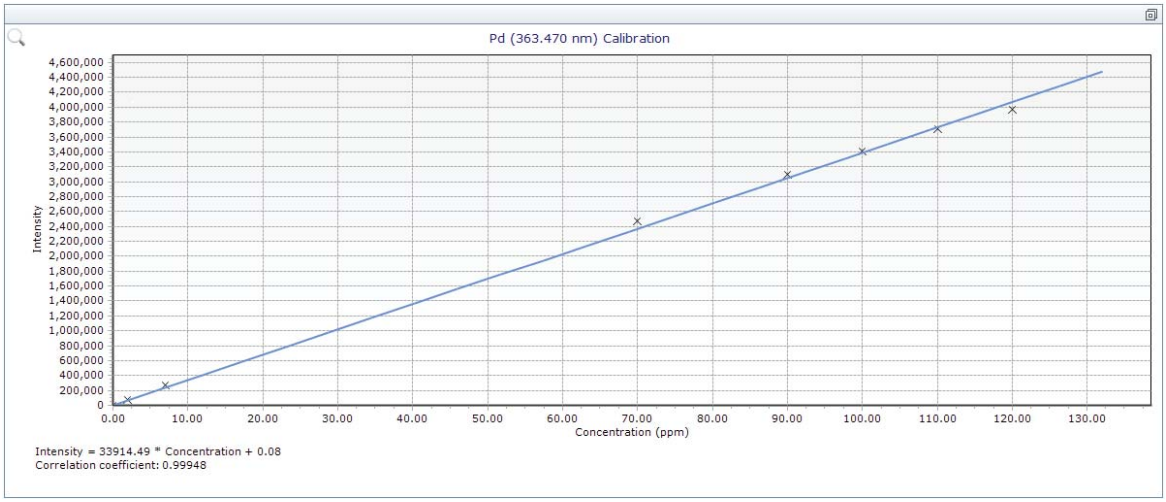

Figure 4. Calibration graph for Pd at the 363.469 nm wavelength

These were custom CRMs that had been professionally prepared from solid ore samples and certified through a round robin test process. They are not commercially available. The results listed in Table 4 show excellent agreement (accuracy) between the 4100 MP-AES measured results and the certified values.

Table 4. Results for Au, Pt and Pd obtained using the 4100 MP-AES compared to certified reference values

Gold	CRM certified value (mg/L)	MP-AES result (mg/L)
CRM 1	19.8	19.3
CRM 2	7.9	7.4
CRM 3	23.1	22.7
CRM 4	5.6	5.7
CRM 5	57.8	55.3
CRM 6	3.1	3.3
CRM 7	35.9	35.4
CRM 8	8.4	8.9

Platinum	CRM certified value (mg/L)	MP-AES result (mg/L)
CRM 6	0.74	0.75
CRM 7	35.6	35.9
CRM 8	9.0	9.5

Palladium	CRM certified value (mg/L)	MP-AES result (mg/L)
CRM 6	3.21	3.4
CRM 7	44.4	44.0
CRM 8	35.0	36.5

Recovery of unknown samples

A batch of unknown samples was analyzed for gold content using the 4100 MP-AES, and the results compared with the data obtained from analysis using conventional flame AA. The comparison can be seen in Table 5.

A typical spectrum of a sample containing approximately 40 ppm of gold can be seen in Figure 5. This demonstrates excellent signal to noise ratio with the flat baseline and the narrow emission peak confirming there are no spectral interferences.

Table 5. Results for gold in unknown samples, comparing the 4100 MP-AES with flame AA

Sample	MP-AES result (mg/L)	Flame AA result (mg/L)	Agreement with AA result (%)
1	0.09	0.09	100
2	0.85	0.84	101
3	5.3	5.1	104
4	13.7	14.4	95
5	20.8	21.8	95
6	4.3	4.1	105
7	1.0	1.0	100

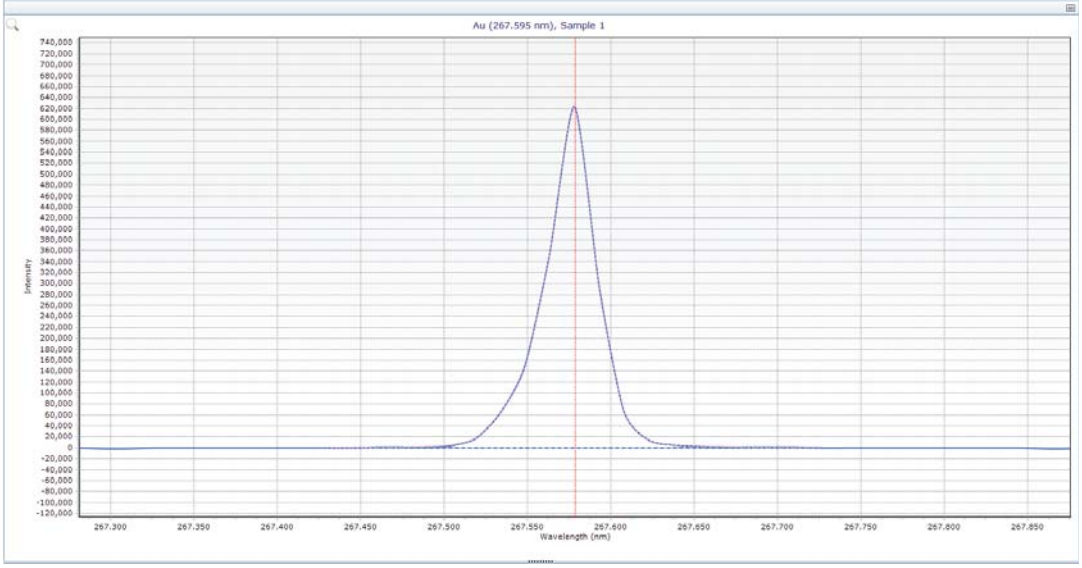

Figure 5. A typical PM sample spectrum for Au at the 267.595 nm wavelength

Conclusions

Following a thorough evaluation of the performance of the Agilent 4100 MP-AES, it is apparent that the 4100 MP-AES offers many advantages for the analysis of gold and other precious metals compared to conventional analysis techniques such as flame AA. The 4100 MP-AES offers superior sensitivity, an increased linear dynamic range and improved speed of analysis. In addition, the 4100 MP-AES more than doubles the measurement speed of conventional AA systems. It also offers exceptional accuracy as demonstrated by the excellent agreement with the certified values for several CRMs and real samples. Furthermore, the 4100 MP-AES has the lowest running costs of all of today's atomic spectroscopy techniques, due to reduced gas costs. Its use of non-combustible nitrogen also ensures that, unlike flame AA, the 4100 MP-AES can provide safer, multi-element unattended overnight operation.

The 4100 MP-AES also offers the option of installation at remote locations. This enables laboratories to analyze samples at the source rather than shipping the samples to a central laboratory for analysis, as is the current practice. In other instances where remote analysis is already performed using flame AA, the MP-AES provides the user with capability to analyze the samples in a safer environment, without the need for flammable gases such as acetylene.

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Published September 1, 2011
Publication number: 5990-8597EN

Agilent Technologies

Permanent Gas Analysis – Separation of Argon and Oxygen on a MolSieve 5A Column using the Agilent 490 Micro GC

Application Note

Micro Gas Chromatography, Permanent Gas Analysis

Authors

Mohamed Bajja and Remko van Loon

Agilent Technologies, Inc.

Middelburg

The Netherlands

Introduction

This application note shows an example of the analysis of permanent gases, including the separation of Argon and Oxygen, using the Agilent 490 Micro GC. For the separation of Argon and Oxygen, a High Resolution 20 m MolSieve 5A column is used.

The advantage of the Agilent 490 Micro GC is speed of analysis. Even with the 20 m HR MolSieve 5A column, you get the results fast. Total analysis time for the permanent gases until Nitrogen is approximately 3 minutes. The Agilent 490 Micro GC delivers lab-quality separations in an ultra-compact, portable instrument.

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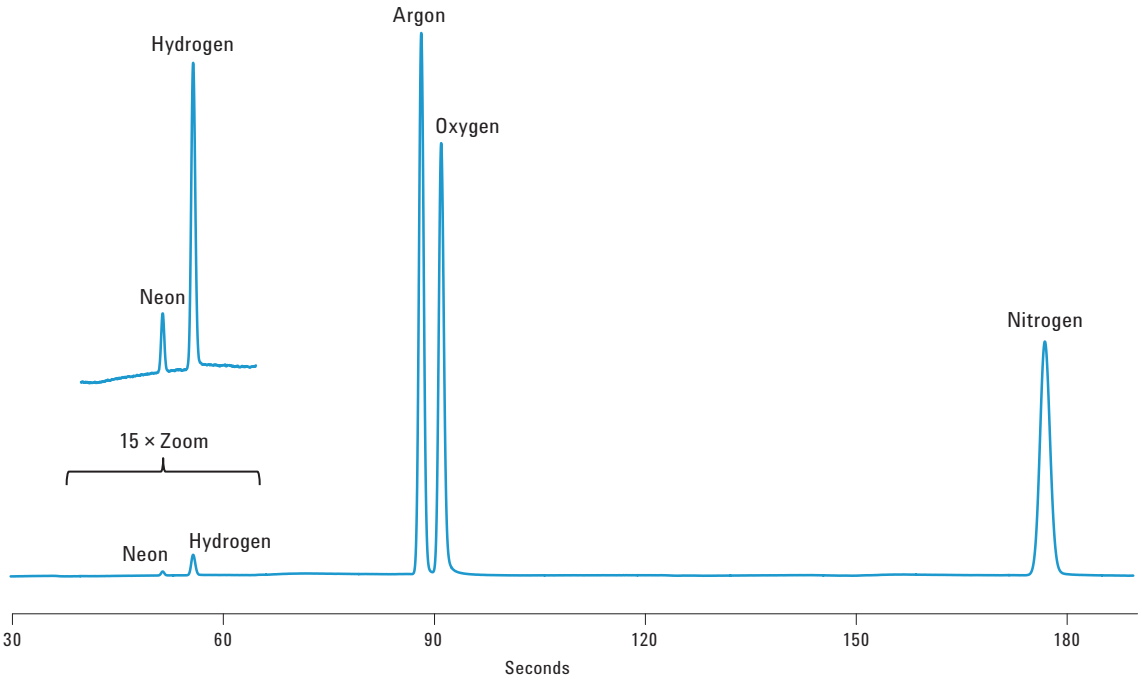
Instrumentation

Instrument	Agilent 490 Micro GC (G3581A)
Column channel	20 m MolSieve 5A
Column temperature	40 °C
Carrier gas	Helium, 200 kPa
Injection time	40 msec

Sample information

Neon	18 ppm
Hydrogen	1.0 %
Argon	0.2 %
Oxygen	0.2 %
Nitrogen	0.2 %
Helium	matrix



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Printed in the USA
July 27, 2011
5990-8700EN

Agilent Technologies

The Analysis of Cement

Application Note

Atomic Absorption

Author

P. E. Thomas

Introduction

The atomic absorption technique may be used for the determination of a wide range of elements in various samples of cement.

Several methods can be used for the preparation of sample solutions, which are suitable for analysis, by atomic absorption spectrophotometry.

When silicon has to be determined the cement samples can be fused with an alkali metal salt, but a hydrochloric acid leach is adequate when the silicon content is not required.

Both of the above procedures for obtaining cement sample solutions have been tested and are employed at Agilent Technologies, Inc.

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Experimental

Fusion Technique

Fuse 0.5 g of the sample of cement with 2 g of either NaOH or Na_2O_2 in either a platinum or a zirconium crucible over a Meker burner.

If a platinum crucible is used do not raise the temperature above a dull red heat or prolong the fusion unnecessarily.

Cool the melt and dissolve the fused cake in 100 mL of warm distilled water and acidify by the addition of 15 mL of conc. HCl, and finally add 5 mL of a 3% H_2O_2 solution. Do not boil the solution.

Transfer the solution quantitatively to a 250 mL volumetric flask and make up to volume with distilled water.

This sample solution is suitable for the determination of the following elements:

Silicon, Aluminium, Magnesium and Calcium.

Notes on the Individual Determinations

Silicon

In the determination of silicon it is necessary to match standards with samples by the addition of both sodium and aluminium, since both of these metals enhance silicon absorption additively by approximately 10%. The standards should also contain an equivalent amount of HCl to that used in the sample preparation.

It should be noted that silica does not precipitate readily from acid solutions, provided that the normality does not exceed 3N and provided that the acidic solution is not boiled.

The atomic absorption measurements are made at the 2516.1 Å resonance line, using a fuel-rich (reducing) N_2O-C_2H_2 flame.

Aluminium

In the determination of aluminium the standards are matched with sample solutions for sodium, calcium and silicon. Both sodium and calcium enhance aluminium absorption, while silicon depresses it.

The atomic absorption measurements are made at the 3092.7 Å, 3092.8 Å doublet, using a N_2O-C_2H_2 flame.

Magnesium

In the determination of magnesium the standards are matched with sample solutions with respect to their sodium content, since sodium acts as an ionization suppressant and hence its presence overcomes the slight ionization interference. If an air-acetylene flame is used the standards are also matched for aluminium and silicon content.

The atomic absorption measurements are made at the 30 times less sensitive 2025 Å resonance line rather than at the more sensitive 2852.1 Å line to avoid time-consuming dilutions, and in this case it is recommended that the lamp is operated at 8 mA.

Generally, the air-C_2H_2 flame is used in magnesium determinations, although the N_2O-C_2H_2 flame is especially recommended if aluminium and/ or silicon is present. However, in these types of matrices, the N_2O-propane flame (~2400 °C) also appears to be quite satisfactory, from the point of view of ionization and interferences.

Calcium

As with magnesium, so with calcium, the standards are matched with the sample solutions with respect to their sodium content. If an air- acetylene flame is used they should also be matched for aluminium and silicon content.

The atomic absorption measurements are made at the 2398.6 Å resonance line, rather than at the 200 times more sensitive 4226.7 Å line, using a N_2O-C_2H_2 flame. It is recommended that the lamp should be operated at 10 mA since the 2398.6 Å line is weak in signal intensity.

Acid Leaching Technique

Digest in a tall beaker 2 g of a finely divided cement sample with 10 mL of 1:1 v/v HCl at just below the boiling point for 5–10 minutes. Transfer the contents of the beaker quantitatively to a 100-mL volumetric flask and make up to volume with distilled water.

Allow the sediment to settle and sample the supernatant liquid, or either filter or centrifuge the solution prior to making measurements upon the solution.

The following metals may be determined in this solution:

Iron, Sodium and Potassium (as well as Nickel or Chromium if desired).

Notes on the Individual Determinations

Iron

The atomic absorption measurements are carried out on a diluted solution (~10 times) at the 3719.9 Å line, rather than the 9 times more sensitive 2483.3 Å line, using an air-C_2H_2 flame.

The sample solution obtained from the fusion procedure could also be used. However, it is then necessary to match standards and samples for both sodium and HCl content.

Sodium

The atomic absorption measurements are made on a diluted solution (~10 times) at the 5890 Å resonance line, using the air-C_2H_2 flame and the burner rotated at 90 degrees to the optical path in order to reduce the analytical sensitivity by a factor of about fifteen.

Dilution may be avoided by the use of the much less sensitive (550 times) 3302.3 Å, 3303.0 Å doublet without burner rotation. In either case the cooler air-propane flame may also be used.

Potassium

The atomic absorption measurements are made on a diluted solution (~10 times) at the 7664.9 Å resonance line, using an air-C_2H_2 flame together with a very small rotation of the burner. Rotation may be avoided by using an air-acetylene flame burning at the 5 cm nitrous oxide-acetylene burner. This is quite safe.

Some Typical Results

Table 1. Typical Results

	Sample A		Sample B	
Component	**Supplied analysis%**	**A. A. S.%**	**Supplied analysis%**	**A. A. S.%**
SiO_2	24.17	24.20	21.03	20.59
Al_2O_3	3. 3	3.10	5.38	5.02
Fe_2O_3	3.07	3.05	2.07	2.11
CaO	64.43	65.5	66.6	66.8
MgO	1.39	1.37	1.12	1.12
Na_2O	0.2	0.17	0.08	0.07
K_2O	0.32	0.32	0.26	0.26

Conclusion

An estimated time of analysis for all seven elements in two cement samples, in a routine laboratory, is approximately 2½ hours from the time of receipt of the samples. The efficiency can be greatly increased by carrying out the atomic absorption measurements on a larger number of samples.

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Printed in the USA
November 1, 2010
A-AA01

Agilent Technologies

The Determination of Selenium in Steels

Application Note

Atomic Absorption

Authors

B. J. Lowings

S. Skujins

Introduction

The determination of selenium by atomic absorption spectroscopy [1,2] and also by atomic fluorescence spectroscopy [3] has been reported by several workers. Improvements in detection limits have been achieved both with the use of an argon-hydrogen-entrained air flame [4], as well as with a nitrogen separated air-acetylene flame [5].

The relatively poor sensitivity and detection limit for selenium together with the large chemical interference from iron and acids make the accurate determination of the very low concentrations of selenium, found in steels, very difficult.

In the procedure described below interferences are largely overcome by carrying out a separation of the selenium from steel, as well as maintaining a closer control over the final acidity of the resultant sample solutions.

Experimental

Instrumental

Standard selenium solutions are prepared, each containing 15% vol./vol. $HClO_4$ (S.G. 1.54).

The atomic absorption measurements are made at the selenium 1960.3 Å resonance line using a spectral band pass of 6.6 Å and the hollow cathode lamp operating at 8 mA. An argon-hydrogen entrained-air flame together with scale expansion are employed. Other flames may also be employed, for example, nitrogen-hydrogen entrained-air, air-hydrogen, and air-acetylene flames.

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Samples

Approximately 2 g of the steel sample are weighed out accurately, transferred to a 250-mL conical flask and dissolved in 30 ml of a 2 vol. $HClO_4$ (S. G. 1.54) -2 vol. HNO_3 (S. G. 1.42) - 1 vol. H_3PO_4 (S. G. 1.75) acid mixture.

This mixture is gently heated until all of the sample has dissolved and is then evaporated to fumes.

The solution is allowed to fume gently for 2 minutes, cooled and 20 mL of HCl (S. G. 1.16) are added and then boiled for 5 minutes.

The solution is cooled once more to 20 °C and 10 mL of a 35% $SnCl_2$ solution are added and the whole mixture is allowed to stand for 15 minutes.

Note: The stannous chloride solution is prepared by dissolving 35 g of $SnCl_2$ in 50 mL HCl (S.G. 1.16) with beating, followed by cooling and dilution to 100 mL with distilled water. The addition of $SnCl_2$ reduces the selenium to the metallic state thus enabling its separation from iron to take place.

The solution is filtered on a tight pulp pad and the precipitate is washed with 50% HCl until it is free from iron.

The precipitate and the pad are transferred to a beaker, 20 mL of HNO_3 and 15 mL of $HClO_4$ are added and the whole evaporated to fumes in order to destroy the organic matter. The solution is then cooled and diluted to exactly 100 mL with distilled water.

2 g of high purity iron are treated in exactly the same manner in order to obtain a selenium free blank solution.

Typical Results

Component		% Selenium	
		By atomic absorption	By colorimetric method
MAC 89 Steel	(a)	0.018	0.013
	(b)	0.0175	0.013
MAC 103 Steel	(a)	0.0385	0.039
	(b)	0.0390	0.039

Conclusion

For the MAC 103 steel the atomic absorption results are in excellent agreement with the colorimetric method results.

The high results obtained for MAC 89 steel may be due to chemical interference from tellurium (0.018% Te in MAC 89). Chakrabarti 2 has shown that 160 ppm Te cause the absorbance of a 1 ppm Se solution to be enhanced by about 15%. Thus, in order to compensate for this enhancement both the sample and standard solution should contain approximately equal amounts of tellurium.

This method for the determination of selenium in steels is readily adaptable to the determination of tellurium in steels, using the 2142.8 Å tellurium resonance line.

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Printed in the USA
November 1, 2010
A-AA02

Agilent Technologies

The Analysis of Iron Ores

Application Note

Atomic Absorption

Authors

J. B. Sanders

P. E. Thomas

Introduction

The atomic absorption technique is ideally suited for the rapid analysis of a wide range of elements in ores. A description is given of the methods employed at Agilent Technologies in the analysis of iron ore samples for fifteen different elements.

Agilent Technologies

Experimental

Fe, Al and Si Sample Solutions

Fuse 0. 5 g of the finely ground sample of iron ore with 2 g of NaOH pellets in a zirconium crucible.

Cool the melt and dissolve it in a solution of 100 mL of distilled water plus 2 mL of 10 vol. H_2O_2 and 9.5 mL of conc. HNO_3. Boil until a clear solution is obtained and then cool.

Transfer the solution quantitatively into a 200 mL volumetric flask and make up to volume with distilled water.

Aluminium and silicon can be determined directly on this solution.

For the determination of iron the above solution must be diluted 50-fold.

Notes on the Individual Determinations

Aluminium

No serious interferences occur provided that the standard and the sample solutions are matched with respect to their Na and Fe content.

Standard solutions are prepared to cover the range 0–150 ppm Al, for example, for Al concentrations of less than 6% in the original ore sample.

The atomic absorption measurements are made at the 3092.7, 3092.8 Å doublet, using the N_20-C_2H_2 flame.

Silicon

Standard solutions are prepared to cover the range 0–100 ppm Si, containing similar concentrations of Na and Fe as are found in the sample solution.

A standard Si stock solution containing 1000 ppm Si may be prepared by fusing 1.069 g of ignited SiO_2 with 2 g of NaOH, dissolving and making up to 500 mL with distilled water.

It should be noted that silica does not precipitate readily from acid solutions, provided that the normality does not exceed 3N and provided that the acidic solution is not boiled.

The atomic absorption measurements are made at the 2516.1 Å resonance line, using a slightly fuel-rich (reducing) N_2O-C_2H_2 flame.

Iron

The use of the less sensitive (9 times) 3719.9 Å line is recommended because of the high concentration of iron in solution and because of its much higher intensity than the more commonly used 2483.3 Å line, resulting in a better signal to noise ratio and thus improved precision.

Care should be taken to ensure, when any free acids are present (particularly HCl and $HClO_4$), that a fuel-lean flame is used in order to overcome the suppressing effect of these acids. It is advisable to match the standard and sample solutions with respect to their acid content.

- **Trace Metals**
  The relatively low concentrations of other elements generally found in iron ores necessitate the use of sample solutions which contain approximately 2 g of ore per 25 mL of solution. At such high concentrations (~8%) severe blockage of both the nebulizer and the burner can occur. In order to overcome this problem the bulk of the iron is first removed by extraction into iso-butyl acetate.

- **Extraction Procedure**
  2 g of finely ground ore are digested with 25 mL of cone. HCL The mixture is evaporated to dryness and the residue is redissolved in a minimum amount of 50% v/v HCl

  This acidic solution is filtered and the residue (A) is washed with minimum amounts of hot 20% v/v HCl and distilled water.

- **Treatment of Filtrate (Plus Washings)**
  The filtrate is evaporated down to a paste and redissolved in 20 mL of conc. HCl

  This solution is oxidized by the dropwise addition of cone. HNO_3. After cooling it is washed into a 100 mL separating funnel with conc. HCl, giving a final total volume of 40 mL.

  50 mL of iso-butyl acetate are added and the mixture is shaken for 30 seconds. The lower aqueous layer is run into a second separating funnel.

  The residual organic layer is washed with 5 mL of cone. HCl and the washings added to the second funnel.

  The above extraction procedure, using 30 mL of iso-butyl acetate followed by a 5 mL conc. HCl wash, is repeated on the aqueous acid solution in the second separating funnel.

  The combined aqueous layer and washings are evaporated almost to dryness and the paste is redissolved in 5 mL of 50 % v/v HNO_3 (Solution B).

- **Treatment of Residue (A)**
  The residue (A) is ignited and then treated with a mixture of cone. HF (5 mL) and conc. H_2SO_4 (2–3 drops) in order to remove SiO_2.

  The residue is ignited once more before fusion with 4 times its weight of anhydrous sodium carbonate. The cooled melt is extracted with 10 % v/v $HN0_3$ and combined with solution (B).

  Finally, the combined solutions are transferred to a 25 mL graduated flask and made up to volume with distilled water.

  The atomic absorption determination of each element by comparison with prepared standard solutions is carried out, using the conditions mentioned below.

Magnesium

The atomic absorption measurements are made at the 2852.1 Å line, using the air-C_2H_2 flame.

It has been reported that Si causes a depression of the Mg absorption in the air-C_2H_2 flame. It is recommended that either the hotter N_2O-C_2H_2 be used (together with excess Na as an ionization suppressant) or that when the air-C_2H_2 flame is used that both the standard and sample solutions are matched with respect to the Si content.

Calcium

In the determination of Ca it is recommended that the N_2O-C_2H_2 flame, rather than the cooler air-C_2H_2 flame, be used. This flame gives an increase in sensitivity and virtually eliminates interferences.

Sufficient Na is available from the fusion step to suppress Ca ionization in this flame.

The atomic absorption measurements are made at the 4226.7 Å line.

Sodium

It is obvious that Na cannot be determined on this solution. Treatment of another sample must be carried out in which the Na fusion of residue (A) is omitted. The residue (A) is dissolved in 5 mL of 10% v/v HNO_3. A blank should be run with the sample to compensate for sodium pick-up from reagents and glass-ware.

The atomic absorption measurements are made at the 5890.0 Å line, using either an air-C_2H_2 or an air-propane flame.

Chromium

The atomic absorption measurements are made at the 3578.7 Å line, using either a fuel-rich air-C_2H_2 or a N_2O-C_2H_2 flame.

All the other elements are determined in an air-C_2H_2 flame.

Element	Resonance line
Pb	2170.0 Å
Ni	2320.0 Å
Zn	2138.6 Å
Cu	3247.5 Å
K	7664.9 Å
Bi	2230.6 Å
Mn	2794.8 Å
Co	2407.3 Å

Other metals which may also be determined in such sample solutions are: As, Mo, Sb, Sn, Ti, V.

Some typical results

Element	Concentration	Unit
Fe	60.0	%
Si	3.50	%
Al	2.31	%
Bi	3.0	ppm
Ca	41	ppm
Co	4.5	ppm
Cr	35	ppm
Cu	18	ppm
K	40	ppm
Mg	10	ppm
Mn	39	ppm
Na	110	ppm
Ni	3.5	ppm
Pb	20	ppm
Zn	28	ppm

Conclusion

It is estimated that a suitably equipped laboratory set up for routine analysis of iron, silicon and aluminium could carry out the determination, of these elements within 3 hours of receipt of the sample. For twelve elements the time required would be approximately 6 to 8 hours.

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Printed in the USA
November 1, 2010
A-AA03

Agilent Technologies

Determination of Low Levels of Arsenic using Flame AAS and Agilent UltrAA Lamps

Application Note

Atomic Absorption

Author

Dr. Jon Hall

Introduction

Nickel ore can be profitable to mine and process depending on the prevailing market value of the nickel. It can also be profitable depending on the levels of more valuable base metals such as cobalt that may be present and that can be extracted by the processing method. The value of the ore may be decreased however depending on the levels of "penalty" elements. Arsenic presents a unique case in that it is an unwanted impurity and at high levels (generally greater than 200 ppm in ore) may inhibit the efficiency of modern processing technologies.

Rapid screening of the ore is therefore required. Base metals such as iron, nickel, cobalt, copper, zinc can be quickly and easily determined by flame AAS. Arsenic presents a unique challenge because it is usually present at levels that make it difficult to determine by flame AAS.

The matrix to be analyzed and the relative slowness of both hydride and graphite atomizer techniques make them unattractive for this application. The UltrAA boosted discharge hollow cathode lamps allow arsenic to be determined by flame to the low levels required for the screening application in the presence of high levels of the other base metals, without the need to resort to separate extraction procedures for arsenic or the use of a different technique. That is, all elements – high level base metals and low level arsenic – can be determined from a single extracted solution by a single analytical technique.

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Experimental

Instrumentation

The following were used for this study:

- Agilent SpectrAA 55B Atomic Absorption Spectrometer, connected to a PC
- Agilent UltrAA lamp control module
- Arsenic UltrAA lamp
- Sample Introduction Pump System accessory (SIPS) for actual samples

The instrument parameters for sample measurement are listed in Table 1.

Table 1. Instrument Parameters Used for Sample Measurements

Parameter	Value
Instrument mode	Absorbance
Sampling mode	Manual
Calibration mode	Concentration
Measurement mode	Integrate
Replicates standard	10
Replicates sample	10
Wavelength	189.0 nm
Slit width	1.0 nm
Lamp current	10.0 mA
Background correction	BC On
SIPS	On
Number SIPS standards	4
Measurement time	1.0 s
Pre-read delay	5 s
Flame type	N_20/Acetylene
N_20 flow	9.00 L/min
Acetylene flow	6.80 L/min

Reagents and Solutions

Standards used were diluted from 1000 ppm AA standards supplied by BDH (Poole, England).

All acids used were AR grade supplied by BDH (Poole, England).

Sample Preparation

The general procedure is that the milled ore sample (1 g) is treated with a mixed acid reagent (nitric acid, hydrochloric acid, hydrofluoric acid, perchloric acid) with hydrofluoric acid being used to remove silicates and the perchloric used to dissolve sulfates.

Warning: Observe all precautions when using hydrofluoric acid or perchloric acids.

The digest solution is taken to dryness, re-dissolved in 10 mL of 10% hydrochloric acid and made up to 100 mL with distilled water.

Calibration

A study was carried out to determine the best resonance line to use. Arsenic has three resonance lines, 189.0 nm, 193.7 nm and 197.2 nm. The UltrAA lamp boosts all three lines; the intensities as measured (without flame) by the relative instrument gain are similar (Table 2). The respective calibration graphs for each of the resonance lines using air-acetylene flame are compared in Figure 1.

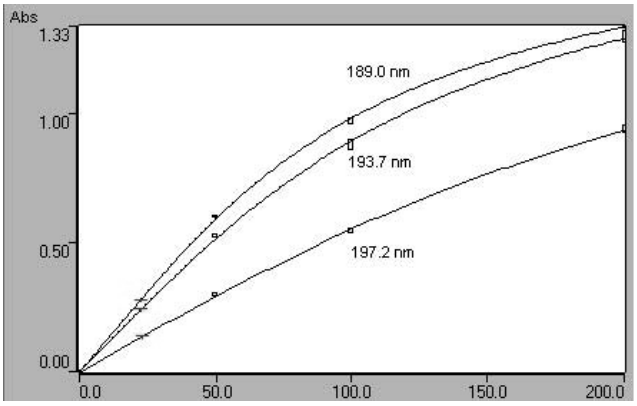

Figure 1: Calibration graphs at the three resonance lines for arsenic using air-acetylene flame.

The respective calibration graphs for each of the resonance lines using nitrous oxide-acetylene flame are compared in Figure 2.

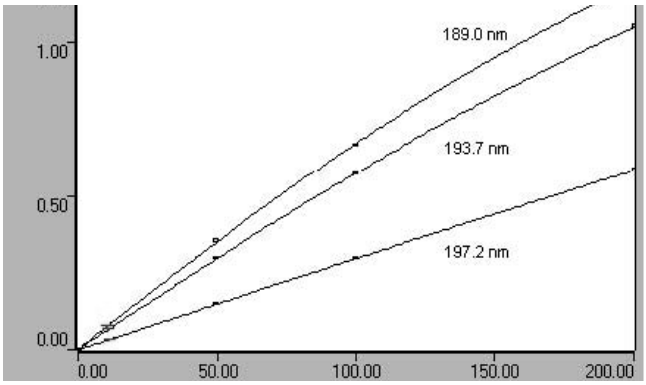

Figure 2. Calibration graphs at the three resonance lines for arsenic using nitrous oxide-acetylene flame.

The characteristic concentrations (C.C.) for each resonance line using air-acetylene and nitrous oxide-acetylene flames were determined and are reported in Table 2.

Table 2. Characteristic Concentrations of Arsenic at Different Wavelengths and Different Flame Conditions

Wavelength (nm)	189.0	193.7	197.2
Gains (%)	54	52	47
C.C. mg/L (air-C_2H_2)	0.37	0.42	0.74
C.C. mg/L (N_2O-C_2H_2)	0.62	0.74	1.47

Although the most sensitive conditions are in the air-acetylene flame at 189.0 nm, the nitrous oxide-acetylene flame is generally preferred for the nickel ore samples. The nitrous oxide-acetylene flame provides the normal advantages of reduced interferences from the sample matrix because of its hotter temperature. Additionally, the nitrous oxide-acetylene flame tends to be more transparent than air-acetylene flame in the extreme ultra-violet region of the spectrum.

A variety of high nickel ores which contained low levels of arsenic (10–2500 ppm in ore) were analyzed (using SIPS). The instrument parameters are shown in Table 1. The results are shown in Table 3.

Table 3: Extract of Report for 26 Samples

Sample id	Concentration mg/L	Mean Abs	Recovery
Cal zero	0.0	-0.0033	
Standard	25.0	0.1717	
Standard	50.0	0.3342	
Standard	75.0	0.4805	
Standard	100.0	0.6186	
50 ppm Standard 2	49.1	0.3281	
Blank	0.2	0.0017	
Sample 001	13.3	0.0916	
Sample 001 dup	13.8	0.0950	104%
50ppm Standard 2	45.6	0.3064	91%
Sample 013	11.9	0.0816	
Sample 014	5.7	0.0395	
Sample 013 dup	11.5	0.0791	97%
Sample 014 dup	5.4	0.0372	95%
Sample 017	0.1	0.0007	
100 ppm Standard 4	92.6	0.5790	93%
Sample 023	23.4	0.1604	
Sample 024	12.2	0.0841	
Sample 025	0.6	0.0043	
Sample 026	0.9	0.0065	
100 ppm Standard 4	97.3	0.6029	97%
Sample 023 dup	23.6	0.1613	101%
Sample 024 dup	12.0	0.0828	98%
Sample 024 dup	11.6	0.0801	95%
Sample 023 dup	23.9	0.1634	102%
Blank	0.3	0.0019	

The limits of detection ranged from 0.05 to 0.14 mg/L in final solution. This equates to 5 to 14 ppm in ore.

Notable are the quality of the repeats, the recoveries of QCs and the ability to discriminate between samples with very low levels of analyte (< 1ppm in solution) without the need to modify the extraction procedure specifically for arsenic or to have a second extraction procedure for this element.

Conclusion

The combination of nitrous oxide-acetylene flame and UltrAA lamps with any SpectrAA instrument allows rapid and simple determination of low levels of arsenic in nickel ores on extractions that are common for the determination of the base metals. The high intensity UltrAA lamp allows full use of the more sensitive 189.0 nm and 193.7 nm lines not normally available on other systems.

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Printed in the USA
November 1, 2010
A-AA11

Agilent Technologies

Obtaining Optimum Performance When Using the SIPS Accessory

Application Note

Atomic Absorption

Introduction

The SIPS accessory, which was introduced in December 1994, was the first practical dilution system for flame AA to provide calibration from a single standard and fast, on-line dilution of over range samples. A few simple procedures, outlined in this information sheet, ensure reliable and productive operation of this accessory.

The Agilent SIPS pump tubing is manufactured from a composite material known as Santoprene. The pump tubing commonly used on VGA and ICP pumps is a single-mix polymer. All types of pump tubing, but especially composite tube materials, can sometimes show signs of "spalling" under normal operation. This is a variable effect in which very small particles of the tubing material break away. If severe spalling occurs, these particles can stick together and cause blockage of the nebulizer.

Spalling occurs in various degrees with all peristaltic pump tubing manufactured from composite materials. It is not unique to SIPS.

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The Effect of Spalling

The symptom of severe spalling is an initial increase in the absorbance followed by a decrease as the nebulizer capillary becomes increasingly blocked. A totally blocked nebulizer will cause the sample to be pumped into the diluent bottle thus contaminating the diluent. Sometimes the blockage may clear without intervention.

The extent of the blockage can depend on the nature of the solutions being pumped. It has been found that very dilute solutions are more likely to induce spalling and block the nebulizer than are concentrated solutions.

Why Use Composite Materials?

Composite materials produce long-wearing tubes that have consistent performance. Spalling usually has no noticeable effect. Some formulations, however, display a higher level of spalling. Naturally these are not recommended for use with SIPS.

Achieving Reliable SIPS Operation

There are four easy steps required to minimize spalling effects and to achieve reliable operation. These are:

1. Use only Agilent-supplied SIPS pump tubing
2. Determine, and use the correct arm pressure for each unit
3. Condition new pump tubes, and re-condition (used) tubes before a run
4. Add a detergent to the diluent

A brief summary of these procedures follow. The complete procedures are outlined in publication no. 85-101710-00, which is supplied with all batches of pump tubes.

Use Only Agilent-Supplied SIPS Pump Tubing

It is recommended that SIPS users obtain their pump tubing from Agilent only. Agilent supplied pump tubing is guaranteed to achieve our specified performance and this minimizes batch to batch variations. As with graphite tubes, individual batches of pump tubes are tested to ensure satisfactory operation. Only those batches passing our tests are accepted. Stretching and other problems have been noted with tube batches sampled from a range of vendors.

Determine the Correct Arm Pressure

When the SIPS is first installed, the user must determine the optimum arm pressure setting for that particular unit. This setting does vary from one SIPS unit to another. By optimizing the arm pressure setting, tube life is maximized and the optimum pumping efficiency is achieved.

In practice, this calibration does not have to be repeated when new tubes are installed as there is little variation from one batch of tubes to another.

The procedure need only be repeated if the SIPS unit is repaired or changed (for example, if a SIPS-10 is upgraded to a dual pump SIPS-20).

Condition the Pump Tubing

Before each use of a new pump tube, the pump tubing should be cleaned and conditioned, using the following procedure. Briefly, a dilute detergent solution (such as a 1% solution (mass/volume) of Triton X-100) is pumped through the tube for 15 minutes. Then distilled water is pumped for 30 minutes to rinse it. Once this time has elapsed, the SIPS unit is ready for regular operation.

If the pump tubing has been used previously, it is recommended that before use of the SIPS, the pump tubing is reconditioned. This is achieved by pumping a solution of 0.01 % Triton X-100 (mass/volume) through the tube for 15 minutes. This procedure can be completed while waiting for the hollow cathode lamp and the burner to warm-up and stabilize. Once this time has elapsed, the SIPS unit is ready for regular operation.

Add a Detergent

To minimize nebulizer blockage from spalling, it is recommended that all SIPS users add Triton X-100 (a readily available laboratory detergent) at a concentration of 0.01% (mass/volume) to the Rinse and Make-up (Diluent) solutions. The Triton X-100 evidently alters the surface of the particles so that the particles do not stick together, but pass through the nebulizer and disappear in the flame.

Summary

The SIPS accessory offers real time-saving and cost-saving benefits to users. Completing the simple procedures described above ensures users can achieve the best performance and the maximum benefit from their SIPS.

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Printed in the USA
November 1, 2010
A-AA15

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Routine Maintenance for Atomic Absorption Spectrophotometers

Application Note

Atomic Absorption

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Introduction

Instruments in good operating condition are a necessity in any analytical laboratory. This level of integrity can be achieved by a regular maintenance schedule with minimal work. The four main areas of such a program for atomic absorption spectrophotometers include:

- General instrument maintenance
- Gas supply maintenance
- Flame component maintenance
- Furnace component maintenance

The benefits of routine maintenance include:

- Increased instrument lifetime
- Reduced downtime
- Overall improvement in instrument performance; giving the operator greater confidence in the validity of his analytical results

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General Instrument Maintenance

Dust and condensed vapors can accumulate on the instrument case, and corrosive liquids can be spilled on the instrument. To minimize damage, wipe off the instrument with a damp, soft cloth using water or a mild detergent solution. DO NOT USE ORGANIC SOLVENTS. The sample compartment windows and the lamp windows can accumulate dust or fingerprints. In such cases, clean the windows with a soft tissue moistened with a methanol or ethanol and water solution. If the windows are not clean, the operator will observe noisy lamp signals and non-reproducible analytical results.

The remaining optical components are sealed, but they should not be exposed to corrosive vapors or a dusty atmosphere. In laboratories where high concentrations of dust or vapors are unavoidable, schedule a yearly check by a service engineer to maintain the efficiency of optical light transmission in the instrument. There is no need for an operator to clean the sealed optical components.

Gas Supply Maintenance

Three gases are suitable for flame M. Air and nitrous oxide are used as combustion support gases (oxidants). Acetylene is used as the fuel gas. Each gas is supplied to the instrument through piped supply systems and rubber hoses. Copper or copper alloy tubing may be used for the oxidant gases. Acetylene should only be supplied through stainless steel or black iron pipe. Check connections regularly between the supply and instrument for leaks, especially when tanks are changed using a soap solution or commercial leak detector. Check the rubber hoses connected to the instrument for fraying and cracking. In addition, each time a tank is changed, check the regulators and valves for proper operation.

Because potentially toxic gases are used or produced in the flame, it is necessary to use a suitable exhaust system with a minimum capacity of 6 m^3/min (200 cfm). A simple smoke test will indicate if it is functioning properly.

Compressed Air Supply

Air may be supplied to the instrument from cylinders, a house air system, or small compressor. Cylinders are the most expensive source of air, particularly where large amounts are consumed and cylinders must be changed frequently. If compressed air from an in-house supply is used, a filter/regulator assembly must be installed in the input line to the instrument. An acceptable “Air Service Unit” (Part No. 01 102093 00) may be ordered from any Agilent sales office.

Whatever source is used, the supply must be continuous and have a delivery pressure of 420 kPa (60 psi). The air must be clean, dry and oil free. Approximately 50% of all gas unit failures are caused by moisture or other impurities inthe air supply.

Excessive noise in the readout has also been attributed to contaminated air. An air filter assembly is therefore an essential component of the atomic absorption spectrophotometer, and its inclusion in the air supply installation is mandatory. Weekly, check the air filter for particle and moisture accumulation. When necessary, dismantle the air filter assembly and clean the filter element, bowl, and drain valve components. Use the following procedure for dismantling and cleaning the air filters supplied with the instrument.

1. Shut off the air supply and allow the system pressure to bleed off.
2. Unscrew the filter bowl, complete with automatic drain valve.
3. Unscrew the retaining ring and push the drain valve back into the bowl.
4. Unscrew the baffle carefully, and remove the filter and filter shield.
5. Clean the filter bowl, drain valve components, baffle, and filter shield by washing in a solution of soap and water. DO NOT USE ORGANIC SOLVENTS AS THEY WILL DESTROY THE BOWL AND VALVE COMPONENTS. Rinse thoroughly in fresh water.
6. Clean the filter element by washing in ethyl alcohol or similar solvent.
7. Ensure that all components are properly dried before reassembly.

Nitrous Oxide Supply

The nitrous oxide used for atomic absorption spectrophotometry must be oil free. If a heated regulator is not used, loss of regulation can occur due to the expansion cooling effect encountered when nitrous oxide is drawn from a cylinder. This can lead to erratic results and create a potential flashback situation with manual gas control units: An acceptable heated regulator may be ordered from any Agilent sales office. The consumption rate is dependent on the application, but is usually 10–20 liters per minute.

Acetylene Supply

Acetylene is the only combustible gas which is normally used in MS. The gas must be supplied packed in acetone. Some companies supply acetylene packed in proprietary solvents, but unfortunately the disadvantages outweigh the advantages. The major disadvantage is that the solvent may be carried over into the instrument and corrode the internal tubing, causing a potential explosion hazard. Ensure that the acetylene is at least 99.6% pure "M Grade" and packed in acetone.

The delivery pressure must be regulated and never exceed 105 kPa (15 psi). Check the instrument operation manual for the correct delivery pressure for the particular instrument being used. In addition, check the acetylene cylinder pressure daily, and maintain in excess of 700 kPa (100 psi) to prevent acetone from entering the gas line and degrading analytical results or causing damage to the instrument.

Flame Component Maintenance

The flame component section of the instrument can be divided into three areas; the nebulizer, spray chamber and burner. Each requires routine maintenance to assure optimum performance.

Nebulizer

The nebulizer area of the flame component consists of the capillary tubing and the nebulizer body. Always ensure that the plastic capillary tubing used for aspirating solutions is correctly fitted to the nebulizer capillary. Any leakage of air, tight bends, or kinks will cause unsteady, non-reproducible readings.

At times the plastic capillary tubing can become clogged and it will be necessary to cut off the clogged section or fit a new piece of capillary tubing (about 15 cm long). in any event, make sure the plastic capillary tubing fits tightly on the nebulizer capillary. The nebulizer capillary can also become clogged. If this occurs, proceed as follows:

1. TURN THE FLAME OFF.
2. Remove the plastic capillary tubing from the nebulizer.
3. Remove the nebulizer from the bung.
4. Dismantle the nebulizer as described in the instrument operation manual or the instruction manual supplied with the nebulizer.
5. Place the nebulizer in an ultrasonic cleaner containing 0.5% liquid soap solution such as Triton X-100 for 5 to 10 minutes. If the ultrasonic bath fails to clear the blockage, pass a burr-free nebulizer wire CAREFULLY through the nebulizer and then repeat the ultrasonic cleaning procedure.
6. Re-assemble the nebulizer in accordance with the instructions.
7. Install the cleaned nebulizer.

   Replace the plastic capillary tubing.

   If blockages are allowed to build up and are not removed, the analytical signal will steadily drop until no absorbance is observed.
8. Check the nebulizer body, capillary, and venturi occasionally for corrosion. Nebulizer problems can be minimized by taking care to always aspirate 50–500 mL of distilled water at the end of each working day.

Spray Chamber

As the sample leaves the nebulizer it strikes the glass bead and breaks into an aerosol of fine droplets. The efficiency of the glass bead can be degraded by surface cracks, pitting and the accumulation of solid material. The reduction in bead efficiency can cause lower absorbance readings and noisy signals. When removing the nebulizer for inspection, always check the glass bead. Look for pitting, cracks, breakage, ensure that the adjusting mechanism operates properly and that the bead is correctly positioned over the nebulizer outlet (venturi).

While the nebulizer and glass bead are removed from the instrument for inspection, the spray chamber and liquid trap should be removed, dismantled, and cleaned. Discard the liquid in the liquid trap and wash both the spray chamber and liquid trap thoroughly with laboratory detergent and warm water. Rinse completely with distilled water and dry all components. Refill the liquid trap and reassemble the spray chamber, checking for any distortion of O-rings or blockages in the gas inlets. Reconnect the drain hose. If a bottle or jug is used to collect the waste solutions, check that the hose is not below the level of the waste. If the hose is below that level, absorbance readings will steadily decrease with occasional abrupt increases as intermittent drainage of the spray chamber occurs. Therefore, it is necessary to daily check the level of the waste and to dispose of it frequently. This is imperative when using organic solvents because of the potential hazards introduced by flammable liquids. Only wide necked, plastic containers can safely be used to collect the waste solutions.

Burner

The final area of concern in the flame component is the burner. During aspiration of certain solutions, carbon and/or salt deposits can build up on the burner causing changes in

the fuel/oxidant ratio and flame profile, potential clipping of the optical beam, and degradation of the analytical signal. To minimize the accumulation of salts, a dilute solution of acid (HNO_3) may be aspirated between samples. However, if salts continue to build up, turn off the flame and use the brass cleaning strip supplied with the instrument. Insert the strip in the burner slot and move it back and forth through the slot. This should dislodge any particles which will then be carried away once the flame is lit and water aspirated.

DO NOT USE SHARP OBJECTS such as razors to clean the burner as they can nick the slot and form areas where salt and carbon can accumulate at an accelerated rate.

If this type of cleaning is inadequate, remove the burner, invert, and soak it in warm soapy water. A scrub brush will facilitate cleaning. Soaking may also be done in dilute acid (0.5% HNO_3). Ultrasonic cleaners containing dilute non-ionic detergent only are another alternative for cleaning. After cleaning, thoroughly rinse the burner with distilled water and dry before installing in the instrument. NEVER DISASSEMBLE THE BURNER FOR CLEANING. IMPROPERLY RE-ASSEMBLED BURNERS WILL LEAK COMBUSTIBLE GAS MIXTURES, POTENTIALLY CAUSING EXPLOSIONS.

Each day after all analyses are completed, 50–100 mL of distilled water should be aspirated to clean the nebulizer, spray chamber, and burner. This is even more important after aspirating solutions containing high concentrations of Cu, Ag, and Hg, since these elements can form explosive acetylides. The entire burner/nebulizer assembly should be disassembled and thoroughly cleaned after analyzing these types of solutions. The burner should be removed weekly, scrubbed with a laboratory detergent, and rinsed with distilled water.

Furnace Component Maintenance

The graphite furnace accessory maintenance can be divided into three major areas; the gas and water supplies, the workhead, and the autosampler. Each plays an important role in obtaining valid analytical results. The following general maintenance program refers to the GTA-95.

Gas and Water Supplies

Normally the gases used in FAAS are inert gases such as N_2 and Ar. Either one may be used, but must be clean, dry, and of high purity. The regulated pressure should be 100–340 kPa (15–50 psi). At times the incorporation of air may be useful to fully ash a sample. However, air should not be used at ash temperatures higher than 500 °C because of the accelerated rate of graphite component deterioration at elevated temperatures.

The water supply, used to cool the furnace, may be supplied either from a laboratory tap or a cooling-recirculating pump. If a recirculating pump is used the water must be kept below 40 °C. The water used must be clean and free of corrosive contamination. The flow should be 1.5–2 liters/minute. Maximum permissible pressure is 200 kPa (30 psi).

Workhead

The workhead is a closed assembly with quartz windows on either end. Before starting an analysis, check the windows for dust or fingerprints. If needed, clean both sides of the quartz windows with a soft tissue moistened with an alcohol/water solution. Never use coarse cloths or abrasive cleaning agents. While the windows are removed, inspect the gas inlets on the window mountings. If the graphite components have deteriorated extensively, graphite particulates may have dropped into the gas inlets, blocking the proper flow of gas. This will cause further graphite deterioration at an accelerated rate and lead to poor analytical performance. To clean, carefully blow out the particulates with a supply of air. Inspect the inside of the window mountings and clean off any sample residue which may have deposited over time.

In the center of the workhead are the graphite components. At frequent, regular intervals, remove the graphite tube atomizer and inspect the inside of the graphite shield. Ensure that the bore and the injector hole area are free of loose carbon or sample residue. Check the electrodes on either end of the graphite shield for proper tapering. If the tapering is worn or burnt, the electrodes will not make the correct contact with the graphite tubing, causing fluctuations in applied power resulting in irreproducibility. The electrodes also have a series of gas inlets which must be free of loose carbon or sample residue.

Above the graphite shield is the titanium chimney. Injected sample or sample residue from the ash/atomize cycles may deposit in this area. A cotton swab soaked with alcohol can be used to clean both the inside and outside of the chimney. Alternatively, the titanium chimney may be soaked in dilute acid to remove deposits.

Autosampler

The components of the autosampler requiring routine maintenance are the rinse bottle, syringe, and capillary tubing, the proper care of which will minimize contamination and improve reproducibility of analytical results.

Regularly remove the rinse bottle for cleaning. This involves soaking the bottle in 20% HNO_3 followed by rinsing with distilled-deionized water. Refill the bottle with a solution of 0.01–0.05% HNO_3 in distilled-deionized water. The solution

may also include 0.005% v/v Triton X-100 R. The Triton helps maintain the sample capillary in clean condition and assists in obtaining good precision.

At times, graphite particulates may accumulate on the capillary tip and should be carefully removed with a tissue. If these particulates are not removed, the dispensing characteristics of the capillary may change. Contamination of the capillary may become a problem when using some matrix modifiers. In such cases, direct the capillary to a vial containing 20% HNO_3, draw up 70 µL, and stop the autosampler while the capillary is in the vial. After a period of a few minutes, the autosampler RESET should be utilized to rinse out the acid solution. This will clean the internal and external areas of the capillary. Similarly, organic residues can be removed by directing the capillary to a vial of acetone and repeating the above procedure. The PTFE capillary should be treated carefully during cleaning and operation. If bends or kinks appear, it can take time to reshape, and while doing so the repeatability of injection may be degraded. If the capillary tip is damaged, the damaged portion should be cut off at a 90° angle with a sharp scalpel or razor blade.

The final area of the autosampler maintenance schedule is the syringe. Daily, check for bubbles in both the capillary and syringe. Any bubbles in the system can cause dispensing errors and lead to erroneous results. Follow the instructions in the operating manual to free the system of bubbles. If the bubbles continue to cling to the syringe, it may need cleaning. The syringe can be washed with a mild detergent solution and thoroughly rinsed with deionized water. Ensure that contamination is not introduced through the syringe. Be particularly careful not to bend the plunger while washing the syringe.

Conclusion

Attached is a routine maintenance schedule for atomic absorption spectrophotometers (Figure 1). By adhering to this program, the overall integrity of the atomic absorption spectrophotometer can be maintained and the laboratory analyst will reap the benefits of increased instrument lifetime, reduced downtime, and gain greater confidence in the analytical results.

Maintenance Schedule (Flame AA)	
Daily	**Completed**
1. Check Gas	
2. Check Exhaust system with smoke test	
3. Empty the drain receptacle	
4. Clean lamp and sample compartment windows	
5. Rinse spray chamber with 50-100 mL of distilled water	
Weekly	
1. Disassemble spray chamber	
(a) Check glassbead	
(b) Check nebulizer components	
(c) Wash the spray chamber and liquid trap	
(d) Scrub the burner	
(e) Change the liquid in the liquid trap	
(f) Check the O-rings	
2. Check air filter assembly	
3. Wipe off instrument	
4. At Time of Gas Tank Change	
5. Check for leaks	
6. Check for operation of the regulators	
7. Check for operation of the shut off valves	
8. Check the gas supply hoses	
Yearly	
1. Schedule an Agilent service engineer to perform Preventive Maintenance	

Figure 1. Routine maintenance schedule for atomic absorption spectrophotometers.

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Printed in the USA
November 1, 2010
AA039

Agilent Technologies

Guidelines for Using Non-Aqueous Solvents in Atomic Absorption Spectrometry

Application Note

Atomic Absorption

Author

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Introduction

Much of our environment consists of water. Therefore the bulk of AA methodology deals with water as a solvent. The use of water also has advantages:

- Restricted density range
- Relatively constant viscosity
- Constant specific heat
- Nonflammable
- Transparent in UV and visible region

The relatively constant physical properties allow optimized design of nebulizers, spray-chamber and burner. Background correction is not necessary for many applications.

Some disadvantages of water as a solvent include:

- Potentially corrosive action towards metal
- Dissolved solids levels can be very high
- Flame characteristics affected by cooling

The first can be controlled by careful selection of instrument construction materials. Correct instrument setup (such as glass bead adjustment) can substantially minimize flame perturbation caused by the last two.

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The use of non-aqueous (mainly organic) solvents for AA is necessary for certain applications. These include:

- Solvent extraction of metal chelates
- Direct analysis of petroleum products like oil
- Direct analysis of edible oil products
- Direct analysis of pharmaceuticals

The use of organic solvents introduces many complicating aspects including:

- Wide range of densities
- Differing viscosities
- Flammability
- Major effect on flame stoichiometry
- Relatively low flashpoints
- Effect on plastics
- Irritating and noxious fumes
- Increased care required for safe disposal

This wide range of physical and chemical properties (Table 1) makes it difficult to anticipate all the requirements of a particular application. An instrument used with organic solvents must be more flexible than one used for aqueous solvents. The operator also requires more training, especially with the safety aspects. Materials used to protect an instrument from corrosive aqueous solutions are often attacked by organic solvents. Sometimes expensive alternative materials must be used in instrument construction.

Safety Aspects

Organic solvents generally used in AA include the following:

- Hydrocarbon (kerosene, white spirit, xylene)
- Ketone (MIBK, DIBK)
- Alcohol (butanol)
- Ester (isobutylacetate)

The most widely used solvents are usually either a hydrocarbon or a ketone. Further information may be found in Table 1.

Table 1. Physical Properties of Some Organic Solvents

Solvent	Flash point °C	Boiling point °C	Specific gravity
4-Methylpentan-2-one (MIBK)	22	118	0.79
2-Methylpropan-2-ol	23	148	0.83
m-Xylene	29	139	0.86
Cyclohexanone	34	155	0.95
Kerosene (Jet-A1)	39-74	175-325	0.78
3-Heptanone	46	148	0.82
Shellsol T	50	186-214	0.75
White spirit (Pegasol)	55	179-194	0.76
2,6-Dimethylheptan-4-one (DIBK)	60	166	0.81
Cyclohexanol	68	161	0.96
Tetrahydronapthalene (Tetralin)	71	207	0.76

Note: The flash point is the lowest temperature at which the liquid gives sufficient vapor to form an ignitable mixture with air and to produce a flame when an ignition source is brought near the surface of the liquid.

To varying degrees, all organic solvents are both flammable and toxic. The use of organic solvents requires great care.

Organic solvents should be kept in glass bottles. The bottles should be stored in a metal cabinet or in a separate storage area well away from flames and other ignition sources. When using solvents only a relatively small quantity (less than 2 L) should be open to the atmosphere at any one time. In addition most countries have legislation which applies to the storage and handling of flammable liquids. These legal aspects must also be considered.

Prolonged exposure to organic solvent fumes is a health risk. All work with them should be carried out in a fume cupboard which has adequate venting. Samples not being analyzed should be covered. If a sampler is used, it should be placed in an venting system which removes the vapors from the area.

There is always a risk of fire from fumes reaching the flame and adequate ventilation must be provided for the instrument itself. These vapors also absorb ultraviolet radiation and if present in the sample beam light path, can cause a significant background signal.

The plastics materials and paints used in the instrument and its accessories should be protected from direct contact with any solvents. Nearly all plastics except fluorinated plastics are affected to some degree by organic solvents and will swell and distort. Instrument parts are made to close tolerances and such changes may cause malfunctions. Generally if allowed to dry thoroughly these parts will return to their original shape.

A plastic waste container must be used for the instrument wastes. A flashback may shatter a glass waste container with potentially dangerous results. The waste container must be emptied often. All wastes including those from the instrument must be stored in approved containers. Legislation should be consulted for proper disposal of all waste liquids.

The following should never be used as solvents for AA (especially flame):

- Halogenated hydrocarbons (chloroform, Freon)
- Very low boiling point hydrocarbons (petroleum spirit)
- Ethers and acetone
- Tetramethylfuran (TMF)
- Dimethylsulphoxide (DMSO)

Halogenated hydrocarbons are toxic. If aspirated into a flame, even more dangerous gases (phosgene is the most common) are produced.

The other solvents in the list are extremely hazardous in the vicinity of a naked flame because they are volatile. Some are so flammable that they could support a spectrometer flame without acetylene.

Standards

Atomic absorption spectrometric measurement and calibration is based on comparison. Care is needed in preparing standards to obtain accurate results. The amount of care and time needed depends on how accurate the results must be.

Aqueous standard solutions are not generally suitable to calibrate an instrument for organic work. Hydrated metal cations in water have different physical and chemical properties to metallo-organic compounds in an organic solvent.

Metal compounds soluble in organic solvents are commercially available. These can either be dry powders or else dissolved in a matrix oil.

The oil-based standards are easy to use. Single element standards can be weighed out and blended together. This multi-element standard can then be weighed into a clean base matrix. If it is not known whether the base matrix is free of the analyte of interest, then the calibration should be treated as a standard additions calibration. This prepared standard is then diluted by an organic solvent to give a working standard to calibrate the instrument. This approach allows the matrix and concentration range to be adapted to specific requirements. Companies such as Conostan (Ponca City, OK USA) and National Spectrographic Laboratories (Cleveland, OH USA) offer a range of single and multi-element standards that only need dilution to the required levels. Most countries have agents who represent these companies.

The dry standards are typically the cyclobutyrate salts of most metals. The powders are stable and can be stored for long periods. Dissolving the powders can be time consuming and may require two or three liquids. Once dissolved, they may be used in the same way as the oil-based standards. Chemical companies supplying atomic absorption standards also offer the dry powder standards.

Some ways of checking standards accuracy and instrument calibration are:

- Recovery studies
- Measure reference materials
- Inter-laboratory studies

A recovery study is done by spiking a sample with a known amount of standard. The absorption of the sample and spiked sample are measured and the respective concentration calibrated. Percent recovery is calculated by the following equation (US EPA abbreviations are used):

$$\% \text{ Recovery} = (SSR - SR)/SA \times 100$$

where:
SSR = spiked sample result
SR = sample result
SA = spike added

Reference materials are check samples which have accurately known compositions. There are organizations which supply reference materials. A list of these is given in later in this document. Consult their catalogs for further information. Reference materials should be treated in the same way as the other samples. A measured result should be within experimental error of the certified result. These materials could also be used as calibration standards. This is not recommended for two reasons:

- Cost is very high
- Calibration standards and quality control (QC) samples should have different sources to reduce systematic errors

Inter-laboratory studies require the cooperation of laboratories doing the same type of analyses. A sample is divided among the laboratories and measured. The results are all collated and compared. When done as a long term project, this method can monitor a laboratory's performance and allows any necessary remedial action to be taken.

Calculations

Units

Concentration of oil standards are generally expressed as µg/g or ppm (mass).

For solutions presented to the instrument for aspiration, the range is generally in mg/L or ppm (volume).

The term ppm (parts per million) in particular must be very carefully defined. An oil standard may contain 500 µg/g of the element of interest. If diluted 1:10, the solution contains 50 mg/L. To allow direct comparison of oil samples, the concentration of the standard can be entered as 500 in the instrument software. However, when comparing absorbances with other studies, it must be remembered that the solution concentration is 50 mg/L. The unit part per million (ppm) is therefore somewhat ambiguous and will not be used in this discussion.

Dilution

Very often organic samples cannot be presented directly to an instrument's nebulizer. For example an oil sample is too viscous to be aspirated directly without dilution. A gasoline sample is too flammable to be used with a flame instrument. These must be diluted in a suitable miscible liquid. Dilution must be done to allow meaningful measurement of the analyte in question. A 1:5 or 1:10 dilution is usually appropriate for the determination of copper or iron in used oil analysis. The determination of zinc or sodium may require a greater dilution and/or selection of a suitably sensitive resonance line. Burner rotation may also be necessary to reduce sensitivity.

Remember that when the sample has been diluted, the analyte concentration must be carefully defined. It must be very clearly stated whether the concentration refers to the analyte in the original sample or in the diluted solution.

Some examples of typical dilutions are given below.

Case 1: Preparation of oil standards using an oil-soluble metallo-organic salt.

Mass (in grams) of salt to be weighed out, m, can be calculated by equation 1.

$$\text{mass salt} = \frac{MC}{10{,}000\ P}\ \text{grams} \qquad (1)$$

where M is mass of oil standard required (g)
C is concentration of analyte in oil (µg/g))
P is percent analyte in salt

Example 1: Prepare a 500 µg/g Si standard in 100 g oil. The silicon was assayed at 14.29% in the salt. Using equation 1,

$$\text{mass salt} = \frac{100 \times 500}{10{,}000 \times 14.29} = 0.3499\ \text{g}$$

Method: Weigh out 0.3499 g salt. Dissolve in xylene and organic solubilizers (refer to the instructions provided by the chemical supplier) with warming. Add 80–90 g warm base oil with stirring. Cool. Make up to 100.00 g.

Case 2: Preparation of an oil standard using an oil dissolved standard and clean base oil.

Mass of oil standard (in grams) to be weighed out, m, can be calculated by equation 2.

$$\text{mass oil standard} = \frac{M\ C}{S}\ \text{grams} \qquad (2)$$

where M = mass of standard to be prepared
C = concentration of analyte required
S = stock oil concentration

Example 2: Prepare 10 g of multi-element oil containing 120 µg/g Cu and 300 µg/g Al starting with 5000 µg/g standards.

Using equation 2,

$$\text{Cu}\quad m = \frac{10 \times 120}{5000} = 0.2400\ \text{g} \qquad \text{Al}\quad m = \frac{10 \times 300}{5000} = 0.6000\ \text{g}$$

Method: Weigh out 0.2400 g of the copper standard and 0.6000 g of the aluminium standard. Dissolve in about 8–9 g of warm base oil. Cool. Make up to 10.000 g.

Case 3: Prepare 20 g of a standard to analyze an oil sample with less than or equal to 1.5% Zn.

In this case, there are two possible methods. One method is to make up a standard from the cyclobutyrate salt (assayed at 16.18% Zn) as shown in Case 1.

Method 1: 1.5% Zn = 1.5 × 10,000 µg/g Zn
From equation 1: $m = \frac{20 \times 1.5 \times 10\ 000}{10{,}000 \times 16.18} = 1.854$ g

Dissolve the salt in xylene and organic solubilizer as recommended by the chemical supplier. Add about 18 g warmed clean base oil with stirring. Make up to 20.000 g.

To reduce the amount of diluent required, the 307.6 nm resonance line could be used in this analysis. A 1:5 or 1:10 dilution

would be sufficient. Note that the signal to noise ratio for the 307.6 line is not as good as the 213.9 line, but would still give acceptable results.

Another method is to use a variation of Case 2 and make up a standard from a more easily handled oil-based standard. However the sample (15 000 µg/g) is more concentrated than the standard (usually 5 000 µg/ g). So this method uses a different dilution for the sample compared to that for the standard. If the very sensitive 213.9 nm zinc line is used, then a 1:10 000 dilution of sample is necessary to obtain about 1.5 mg/L. Such a large dilution would mean that the sample solution would have almost the same physical properties as the solvent.

If a 5000 µg/g standard is used, a 150 µg/g working standard can be made which only has to be diluted 1:100. At a 1:100 dilution the physical properties of the standard solution would also be similar to the solvent.

Method 2:

From equation 2 $m = \frac{20 \times 150}{5000} = 0.600$ g

Weigh out the oil standard. Add about 12 g warm clean base oil with stirring. Cool. Make up to 20.000 g.

Dilute the sample by weighing out 1.000 g and dissolving in 100 mL solvent solution. Pipette out 1 mL of the solution and make up to 100 mL. This is the solution to be analyzed.

Dilute the standard by weighing out 1.000 g and dissolve in 100 mL solvent solution. This standard is equivalent to 1.5% Zn in the original oil sample.

Ionic Suppression

A nitrous oxide-acetylene flame is recommended for the measurement of the Group II elements (magnesium, calcium, strontium, barium). Under these conditions, the analytes are partially ionized and require the use of an ionization suppressant for their accurate measurement. An organic soluble potassium or sodium salt is added to the standards and samples to give a final concentration of 2000–5000 ppm. The salts are either napthenates, sulphonates or cyclobutyrates.

A branched capillary to aspirate an ionization suppressant and sample simultaneously has been described [1] and it has been claimed to work with organic samples. This has not yet seen wide application.

Hardware

Spraychamber: Check that the components are resistant to solvent attack and do not distort. Removable components should be checked to ensure they are not binding or tight.

O-Rings: Inspect these frequently. KALREZ O-rings are resistant to solvent attack and are available as sets.

Liquid Trap: This should be filled with the liquid being aspirated or a liquid miscible with the solvent being aspirated.

It is recommended that the spraychamber and liquid trap be dismantled and cleaned at the end of each working day. Wash with hot water and detergent or acetone and allow to dry. Reassemble while checking the O-Rings.

Nebulizer: An adjustable nebulizer which allows control of the uptake rate is necessary. The uptake can be continuously varied from zero up to about 10 mL/min.

An adjustable nebulizer does not have a thimble like the standard preset nebulizer. Instead it has a housing with an uptake control. Refer to the instructions on initial setup.

Setting the correct uptake rate should be done using an air-acetylene flame and the selected solvent:

1. Check nebulizer is set for zero uptake rate
2. Light flame and adjust gas flows to give a very lean flame
3. Place capillary in solvent
4. Slowly rotate uptake control clockwise until flame is beginning to become fuel-rich (some yellow may be seen)
5. Measure and record uptake

   Generally, MIBK, DIBK and xylene - 2 mL/min white spirit, kerosene - 4 mL/min. The nitrous oxide-acetylene flame can tolerate higher uptake rates (MIBK - 6 mL/min).

   A high uptake rate is not desirable for a number of reasons: the flame may be extinguished between samples because of insufficient fuel; the risk of background and inter-element interferences is increased; the gains in signal are usually not significant enough.

Burner: An air-acetylene burner should only require periodic cleaning. The use of organic solvents however increases the possibility of carbon buildup with the nitrous oxide-acetylene flame. More frequent cleaning of the nitrous oxide-acetylene burner may be needed.

A carefully cleaned burner gives the best performance and

reduces salt blocking and carbon build-up. The use of a brass strip is no longer recommended. Studies revealed that a metal strip does not clean sufficiently well and that it does not polish the jaws [2]. For optimum performance, any burner should be cleaned as follows:

1. Use a card (for example, business card) and a brass polish (for example, "Brasso")
2. Wet card on both sides with polish
3. Slide card into slot
4. Move card up and down to polish inside of burner jaws
5. Rub card along top of slot
6. Scrub with a soft nylon brush (for example, toothbrush) using hot water and detergent
7. Use ultrasonic bath if available
8. Rinse with hot running water
9. Rinse with distilled water
10. Allow to dry or use a card to remove water from inside slot

Background correction: The organic nature of the matrix means that UV absorption is significant. Background correction is more likely to be required for most elements. Background studies are recommended to determine if correction is needed.

Programmable Gas Box: The sample uptake rate affects the flow of oxidant through the nebulizer into the spraychamber. At low sample uptake rates in the air-acetylene flame, the oxidant flow must be set somewhat higher than the default 13.0 L/min. It is suggested the flow should be about 19 L/min.

Graphite Furnace Operation

Many of the practical precautions of flame are not needed for graphite furnace operation. For example the fire potential is greatly reduced because there is no naked flame and the volumes involved are very small. However some precautions are still necessary. Guidelines for handling, storing and disposing organic solvents must still be observed.

The chemical nature of the metallo-organic compounds means that organic standards may still be required for calibration.

The solvent used for dilution should not be too volatile. A furnace run can take a long time. The solution concentrations could be affected because of evaporation. The ketones (MIBK and DIBK) are probably the most suitable general purpose solvents for furnace work. They are miscible with many organic compounds and solvents. DIBK is also immiscible with water.

The organic phase is very mobile. When injected into a furnace, this mobility may cause more spreading than is desirable. To control droplet spreading in the furnace, a partition graphite tube should be used. Some analyzes of volatile elements like lead and cadmium may require the use of a platform [3]. The platform controls droplet spreading provided no more than about 20 mL is injected. For both types of atomization (wall and platform), the hot injection facility can also be used to control spreading. For example, using DIBK as a solvent the inject temperature on the sampler page can be set to 130 °C and the injection rate slowed down to 5. This facility also helps shorten the time needed to dry the injected solution and allows faster furnace cycles [4].

The solution in the rinse bottle of the sampler does not have to be organic. The rinse solution can be distilled water with 0.01% nitric acid and 0.1% Triton X-100 (a non-ionic detergent)3. If the samples are such that the dispenser tip is not being cleaned, a slightly higher concentration of Triton X-100 may be tried. A small amount (0.5 - 1%) of propan-2-ol in the rinse solution as well can assist with keeping the tip free of grease and oil.

Safety Checkpoints

Choose a Suitable Solvent Which Has the Following Properties

- Miscible with sample
- Uitably high flashpoint
- Density greater than 0.75
- No toxic by-products formed

Handling Solvents

- Use small volumes near instrument
- Keep solutions covered when not in use
- Do not inhale vapors
- Empty waste vessel often
- Use fume cupboard for solution preparation
- Dispose of all wastes carefully and responsibly
- Do not mix with nitric or perchloric acids or wastes

Instrument

- Fill liquid trap with suitable solvent before starting
- Attach tube to spraychamber vent and allow other end to vent safely away from flame
- Install an efficient exhaust system above instrument
- Keep burner clean
- Do not clean burner while flame is on
- Drain liquid trap at the end of each day
- Wash spraychamber and allow to dry overnight; check condition of O-rings often

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Printed in the USA
November 1, 2010
AA100

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Agilent Oil Analyzer: customizing analysis methods

Application Note

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Introduction

Traditionally, the analysis of used oils has been conducted by physical and wet chemical methods. FTIR spectroscopy has become a routinely used technique to analyze used oils, providing the following major advantages[1]:

- Ability to simultaneously determine several parameters from a single experiment
- Increase in speed of analysis
- More cost effective than traditional techniques
- Mobility and portability allowing remote on-site analysis

The Agilent FTIR Oil Analyzer is designed to meet the requirements of the US Department of Defense Joint Oil Analysis Program (JOAP)[2] for use in their condition monitoring program as well as commercial applications. It is optimized for monitoring relative changes in various indicators of oil conditions (oil failure symptoms) using a standardized protocol developed by the Joint Oil Analysis Program Technical Support Center (JOAP-TSC). This protocol sets the data extraction algorithm for several types of petroleum and synthetic-based lubricants and hydraulic fluids, and eliminates the need for reference samples as spectral subtraction is no longer required.

The Agilent Oil Analyzer software allows users to readily customize existing methods as well as create new methods to measure other parameters and properties of lubricants defined by the user. The methods can be easily adjusted for performing analysis of samples where spectral subtraction is required.

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This application note describes the tools available with the Agilent FTIR Oil Analyzer and procedures that a user should follow to customize analysis methods, while reinforcing the importance of reliable calibration in quantitative spectral analysis.

Analysis methods

The sampling and analyzing procedures available in the Agilent FTIR Oil Analyzer conform to the ASTM E 2412–04 "Standard practice for condition monitoring of used lubricants by trending analysis using Fourier Transform Infrared (FTIR) Spectrometry"[3]. These methods provide a generalized protocol for condition monitoring of contaminants and breakdown products in used lubricants including water, ethylene glycol, fuels, incorrect oil, soot, oxidation, nitration and sulfonation. The methods are based on calculating trends and distributions from mid-IR absorption measurements, and encompass both direct and differential (spectral subtraction) trend analysis approaches.

The Agilent Oil Analyzer software is configured to run twelve predefined analysis methods that correspond to different classes of lubricating oils or hydraulic fluids, and their applications with differing limits. The methods are:

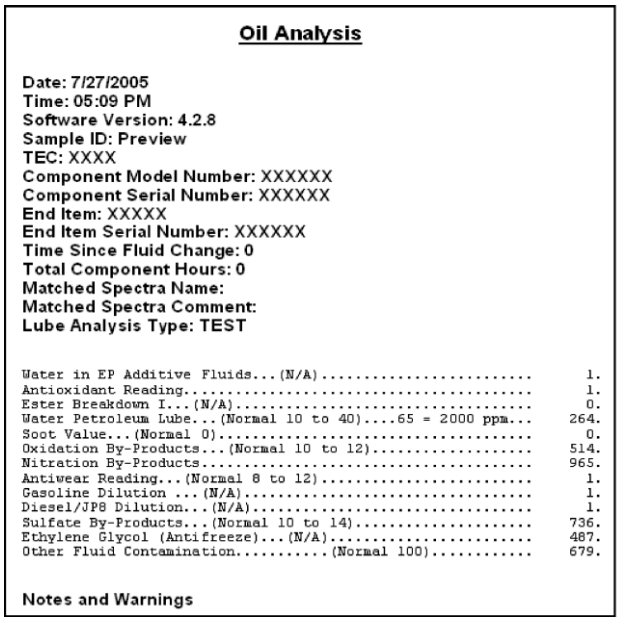
- Aircraft hydraulic (Mil-H-83282)
- Aircraft hydraulic (Mil-H-83282_350 ppm limit for water)
- Dextron transmission fluid
- Engine crankcase (Diesel_gasoline_natural gas)
- Fire retardant hydraulic (Mil-H-46170)
- Gas turbine or Helo Gbx (Mil-L-23699)
- Ground equipment hydraulic (Mil-L-2104_10W)
- Ground equipment synthetic hydraulic (Mil-H-5606)
- Marine diesel crankcase (Mil-L-9000)
- Conostan IR OTS fluid
- Steam turbine (Mil-L-17331)
- Generic or undetermined (Unknown lubricant type)

Each of the methods measures numerical indicators (parameters) that are related to the oil's condition. The software then generates a report that contains thirteen measurement parameters, as listed below:

- Water in EP fluids
- Antioxidant reading
- Ester breakdown
- Water in petroleum
- Soot value
- Oxidation by-products
- Nitration by-products
- Antiwear reading
- Gasoline dilution
- Diesel/JP8 dilution
- Sulfate by-products
- Ethylene glycol
- Other fluid contamination

Additionally, a separate procedure for predicting Total Base Number (TBN) is available and can be integrated into existing methods.

The parameters are reported in the units of spectral absorbance (peak areas or heights) rather than in physical concentrations, such as ppm, wt.% or mg of KOH. Figure 1 shows an example of a typical standard Oil Analysis report.



Oil Analysis

Date: 7/27/2005
Time: 05:09 PM
Software Version: 4.2.8
Sample ID: Preview
TEC: XXXX
Component Model Number: XXXXXX
Component Serial Number: XXXXXX
End Item: XXXXX
End Item Serial Number: XXXXXX
Time Since Fluid Change: 0
Total Component Hours: 0
Matched Spectra Name:
Matched Spectra Comment:
Lube Analysis Type: TEST

Water in EP Additive Fluids...(N/A)	1.
Antioxidant Reading	1.
Ester Breakdown I...(N/A)	0.
Water Petroleum Lube...(Normal 10 to 40)....65 = 2000 ppm...	264.
Soot Value...(Normal 0)	0.
Oxidation By-Products...(Normal 10 to 12)	514.
Nitration By-Products	965.
Antiwear Reading...(Normal 8 to 12)	1.
Gasoline Dilution ...(N/A)	1.
Diesel/JP8 Dilution...(N/A)	1.
Sulfate By-Products...(Normal 10 to 14)	736.
Ethylene Glycol (Antifreeze)...(N/A)	487.
Other Fluid Contamination.....(Normal 100)	679.

Notes and Warnings

Figure 1. Typical standard Oil Analysis report

Calibration

All analysis methods in the Agilent FTIR Oil Analyzer consist of a set of calibration models (procedures) in the form of corresponding files with an indication of the calibration model's type (univariate, or multivariate, or a combination). The analysis method may be composed of one or several calibration files.

The construction of calibration models in quantitative spectral analysis is a two-step procedure: calibration and validation. In the calibration step, indirect instrumental measurements (spectra) are obtained from standard samples in which the value of the parameter of interest has been determined by a standard reference method (an accurate direct measurement method). The set of spectra and results from the reference method, referred to as the calibration set or training set, is used to construct a model that relates parameter values to the spectra. Before the calibration model is accepted and used for prediction, it should be validated by a set of independent (not used in the calibration set) samples of known parameter concentrations (validation set). If parameters from the validation set fall within acceptable accuracy limits using the model derived from the calibration set, an acceptable model has been constructed that can be used to predict for new "unknown" samples.

To build a univariate calibration model, it is necessary to specify a single measurement from a spectrum, such as peak area or height that demonstrates the most distinctive spectral response for the parameter of interest. The univariate calibration and prediction procedures are available as a standard part of Resolutions/Resolutions Pro software and are defined as a simple quantitative analysis. The analysis is described in detail in the Resolutions online help and the corresponding system reference manuals for previous software versions (Win-IR Pro and Merlin). The user must generate a quantitative calibration document and save it as *.BSQ file using Resolutions/Resolutions Pro (Win-IR Pro or Merlin) software.

Where spectral responses attributed to different parameters overlap and the selective spectral measurements for the parameter of interest is very difficult, univariate models may not be reliable. Multivariate methods such as Principal Component Regression (PCR) and Partial Least Squares (PLS) allow multiple responses at the selected wavenumbers to be used. These methods are better suited to extracting spectral information where bands overlap and it is difficult to discern the relevant spectral regions attributable to a particular parameter. The main advantage of multivariate methods is the ability to calibrate for a parameter of interest when it correlates in a complicated (non-specific) way with multiple spectral regions, while minimizing background matrix interferences in the lubricants.

The Agilent Oil Analysis software allows multivariate calibration models created with the use of third party software to be incorporated in analysis methods. The PLSplus IQ package available as an additional application in the Galactic GRAMS/AI (GRAMS/32) software suite must be used. The "PLSplus IQ User's guide" gives step-by-step instructions on how to construct and validate a multivariate calibration model

as well as theory of advanced statistical analysis in spectroscopic quantitative analysis. The user must build an accurate calibration model and save it into a *.CAL file using PLSplus IQ.

The validity of empirically-built calibration models depends heavily on how well the standard samples (calibration set) represents the unknown samples to be analyzed (prediction set). In all cases, the selection of standard samples to be used for calibration must adequately cover the expected range of measurement parameters in the prediction set. This means that the expected extreme values for each parameter of interest in unknown samples must be included in the calibration set, as extrapolation outside the calibrated value range can be unreliable. It is important to ensure that any phenomena that influence the spectral measurements (e.g., not only the total amount of soot but its particle size distribution) also vary in the calibration set over ranges that span the levels of the phenomena occurring in the prediction set. It is also very important to minimize the errors in the standard sample parameters that are used to construct the empirical calibration model, as any calibration model can only be as accurate as the reference measurements from which it was constructed.

Many conditions can affect the results obtained from FTIR lubricant monitoring such as lubricant type, engine type, operational conditions, environmental conditions, etc. When the conditions are changed significantly, new calibration models and methods may be required to ensure accurate prediction of oil properties. For instance, new calibrations may be required when a new oil type with a different base stock and additive chemistries comes for the analysis.

Care must be taken when measuring overall oil quality parameters such as Total Acid Number (TAN) and Total Base Number (TBN) using FTIR spectroscopy. The secondary formation of acidic products in lubricants is characterized by TAN or indirectly by TBN, which assesses the consumption of basic reserve additives in the oil. While the various acids or bases present in a lubricant could, in principle, be individually quantified based on their characteristic absorption bands, no unique absorption bands can be directly related to TAN or TBN. Thus, only indirect FTIR spectroscopic methods for TAN and TBN have been standardized to date. In addition, there is a large discrepancy in new lubricant TAN values, from less than 0.1 mg KOH/g for R&O type oils to 9 or higher for some synthetic oils in industrial applications. On the other hand, the incremental decrease in TBN used to indicate that a product is failing, varies in broad ranges: some oils may have a new TBN value of 12, but rapidly decrease to a value of 3, whereas other synthetic oils may have the beginning TBN of 40.

A calibration model for TBN is currently available in Agilent Oil Analyzer. The calibration is intended for prediction of the values in gasoline and diesel engine oils having typical baseline numbers not higher than 12 mg KOH/g.

Note that in many individual cases, in order to estimate TAN and TBN satisfactorily the user needs to construct a multivariate calibration model that would cover the higher range of values as well as take into account any other factors that could influence the accuracy and the reproducibility of spectral measurements.

Method editor

Once the univariate or multivariate calibration models are built, the corresponding *.BSQ or *CAL files must be moved or copied into the directory C [Local Disk]:\ Program Files\Varian\Resolutions\Oil Analyzer\Methods. This is the storage location for the available calibration and method files. Then, log in as Administrator to the Agilent Oil Analysis software and enter the Method editor. Follow the Chapter 11 “Method Editor” in “Agilent Oil Analyzer operational manual” to incorporate the calibrations to an existing method or to develop a new method.

Note that spectral subtraction is available in the Agilent Oil Analyzer but was not utilized in JOAP protocol. It is not considered to be practical in view of the deployability aspect of many JOAP laboratories and that the required sample volume would increase because of the necessity of new oil samples to act as references. In order to apply the spectral subtraction procedure, the user needs to select "Use spectral subtraction" option in the Sampling method group in the General option dialog and edit the relevant analysis method, by clearing the "Zero less than Zero" check box in all the associated calibration models. Refer to Chapter 4 "General Options–Setup" and Chapter 11 "Method Editor" of "Agilent Oil Analyzer operational manual" for more information.

Conclusion

FTIR spectroscopy has been gaining increased acceptance as a method of choice for used oil analysis. Designed and optimized as a complete system for predictive maintenance programs, according to JOAP standards, the Agilent FTIR Oil Analyzer combines specific capabilities with the flexibility to be successfully used in any oil analysis laboratory.

The Oil Analyzer software allows new and improved analysis methods to be built and ensures that new types of lubricating oils and fluids used in a variety of different machinery are timely and reliably monitored and tested.

The software allows the user to include PCR/PLS methods to measure oil parameters and convert the units of spectral absorbance into physical results (ppm, wt.%, cSt, mg KOH/g oil, etc.) applying spectral subtraction if needed.

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www.agilent.com/chem

Published March, 2011
Publication Number ftir127

Agilent Technologies

AA or ICP – Which Do You Choose?

Application Note

Inductively Coupled Plasma-Optical Emission Spectrometers

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Introduction

For many analysts Atomic Absorption Spectrometry (AAS) is a well established and understood technique. However, even though Inductively Coupled Plasma Emission Spectrometry (ICP-ES) instrumentation has been commercially available for over a decade, the technique has proven to be more complex. This article discusses the main differences between the two techniques.

AAS Versus ICP

The basic difference between the two techniques is that one relies upon an atomic absorption process while the other is an atomic/ionic emission spectroscopic technique. The next essential difference is the means by which the atomic or ionic species are generated. A combustion flame or graphite furnace is typically used for AA while ICP-ES uses a plasma.

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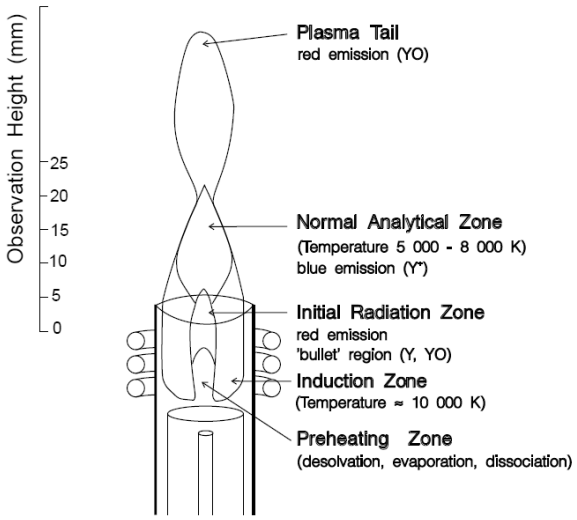

Figure 1. A plasma used for emission spectrometry. The regions refer to those seen when a Yttrium solution is introduced.

The typical maximum temperature for an air/acetylene flame is 2300 °C while for nitrous oxide acetylene, it is 2900 °C. Temperatures as high as 10,000 K can be reached in an argon plasma.

Detection Limits

The comparison of detection limits in Table 1 highlights the following differences:

- Furnace AA detection limits are generally better in all cases where the element can be atomized.
- Detection limits for Group I elements (for example, Na, K) are generally better by flame AAS than by ICP.
- Detection limits for refractory elements (for example, B, Ti, V, Al) are better by ICP than by flame AAS.
- Non metals such as sulfur, nitrogen, carbon, and the halogens (for example, I, Cl, Br) can only be determined by ICP.

While it is possible to determine phosphorous by AAS, its detection limit by ICP is more than three orders of magnitude better.

Optimum detection of non metals such as S, N and halogens by ICP-ES can only be achieved if a vacuum monochromator, with purged transfer optics, is used. The optics must be purged to exclude atmospheric oxygen and eliminating its absorption.

Sulfur can be measured at 180.73 nm by purging the monochromator. To detect the primary aluminium wavelength at 167.08 nm, the monochromator must first be evacuated, then purged with the inert plasma gas.

Note that a continuous flow vapor generation accessory can be used with either ICP-ES or AAS for improved detection limits for As, Se, Hg, Sb, Bi and Ge.

Sample Throughput

In ICP-ES, the rate at which samples may be determined depends on the type of instrument: both simultaneous and sequential ICP spectrometers are available. Most ICP spectrometers purchased are the sequential type, providing maximum flexibility of choice of element and analytical wavelength. Surveys have shown that most analysts are interested in 6–15 elements per sample and choose to pump the sample (which increases washout times) to improve precision and accuracy by minimizing viscosity effects. Simultaneous ICP spectrometers demonstrate an advantage in analytical speed over sequential ICP spectrometers when more than 6 elements/sample are measured.

If a "one off" sample is presented for a few elements, flame AAS is faster. However, with flame equilibration time, program recall and monochromator condition changes, the cross over point where sequential ICP becomes faster than AAS is approximately 6 elements/sample for routine analysis.

Unattended Operation

Flame AAS cannot be left completely unattended for safety reasons. An ICP-ES instrument or graphite furnace AA can be left to run overnight as no combustible gases are involved, effectively increasing the working day from 8 hours to 24 hours.

Linear Dynamic Range

The inductively coupled plasma is doughnut shaped (with a “hollow” center). The sample aerosol enters the base of the plasma via the injector tube. The “optical thinness” of the ICP results in little self absorption and is the main reason for the large linear dynamic range of about 10^5. For example, copper can be measured at the 324.75 nm wavelength from its detection limit of about 0.002 ppm to over 200 ppm. In ICP, extrapolation of two point calibrations can be accurately used to achieve orders of magnitude above the top standard. This compares to a linear dynamic range of typically 10^3 for AAS.

Interferences

Chemical
Chemical interferences are relatively common in AA, especially with graphite furnace AA, but may be minimized with chemical modifiers.

ICP-ES is almost free from chemical interferences. The chemical bonds that still exist at below 3000 °C are completely ruptured at above 6000 °C. The high temperatures reached in a plasma eliminate chemical interferences, which accounts (for the most part) for the better detection limits achieved for refractory elements.

Ionization
The ICP contains a large number of free electrons, so ionization interferences for most applications are virtually nonexistent. Ionization interferences can be encountered when determining elements in matrices that contain very high concentrations of Group I elements (for example, Na & K). However, these effects can be minimized by optimizing the plasma viewing height.

Ionization interferences may also be found in AAS, such as, when measuring certain Group II elements in a nitrous oxide flame. An ionization buffer such as Cs, Li or K can be added to both samples and standards to minimize this effect.

Spectral
The optical requirements of AAS are fairly simple. The monochromator only needs to distinguish a spectral line emitted from the hollow cathode lamp from other nearby lines. The lamp itself only emits a few spectral lines. Most elements require 0.5 nm resolution with only iron, nickel and cobalt of the common elements requiring 0.2 nm or better.

In ICP-ES, the rich spectra present in the plasma means that there is a greater possibility of spectral interference. Spectral resolutions of 0.010 nm or better are required to resolve nearby interfering lines from the atomic and ionic analytical emission signals of interest.

Spectral interference in sequential ICP spectrometers can, in most cases, be overcome by selecting a different elemental wavelength with similar detection limits. With simultaneous ICP spectrometers, the elements and the wavelengths which may be determined are fixed at the time of purchase, and an alternative line may not be available. In this case, inter-element correction may be used to minimize the spectral interference.

Physical
These interferences relate to the different properties of various samples and can affect sample transport and droplet formation. ICP tends to be more susceptible to such interference because of the smaller droplet size required and lower transport efficiency.

Precision
Precision can be termed short term (or within-run) and long term (over a period of one day). For AAS a precision of 0.1–1% is typical for the short term, but recalibration is required over a longer period. With ICP-ES the short term precision is typically 0.3–2%, but precisions of 2–5% are not uncommon over an 8 hour period without recalibration.

One technique used to eliminate backlash in the grating drive mechanism of ICP spectrometers is by scanning and measuring at the same time. This method of measurement can be termed as “measurement on the move” and effectively results in poor short term precision. A more recent method drives the grating to a wavelength near the analytical peak. A refractor scan is then performed over a smaller wavelength region in order to identify and locate the peak position. Finally the refractor plate is repositioned “at the peak” where the replicate measurements are then performed. This method offers better precision.

AAS v ICP – A quick guide

	ICP-OES	Flame AAS	Furnace AAS
Detection limits	Best for : Refractories Non metals P, S, B, Al V, Ba, Ti	Best for : Group I metals Na, K Volatile elements Pb, Zn Rare Earths	Best for : All elements except : B,W,U, Refractories, for example P, S Halogens
Sample throughput	Best if more than 6 elements/sample	Best if less than 6 elements/sample	Slow (typically 4 mins/element)
Linear dynamic range	10^5	10^3	10^2
Precision Short term Long term (over 8 hrs)	0.3 – 2% Less than 5%	0.1 – 1%	0.5 – 5%
Interferences			
Spectral	Many	Virtually none	Minimal
Chemical	Virtually none	Some	Many
Ionization	Minimal	Some	Minimal
Operating costs	High	Low	Relatively high
Combustible gases	No	Yes	No

Table 1. Guide to ICP/AAS Analytical Values

				ICP	Flame AA			Zeeman Furnace AA			
Element		AA λ (nm)	ICP λ (nm)	Detection limit µg/L	Characteristic conc µg/L	Detection limit µg/L	Flame type	Characteristic** conc µg/L	Mass pg	MSR %	EI
Silver	Ag	328.1	328.068	3	30	2	Air	0.035	0.7	97	Ag
Aluminium	Al	309.3	167.081	1.5	800	30	N_2O	0.25	5	100	Al
Arsenic	As	193.7	188.985	12	500	300	N_2O	0.5	10*	86	As
Gold	Au	242.8	267.595	5.5	100	10	Air	0.22	4.4	94	Au
Boron	B	249.8	249.773	1.5	8000	500	N_2O	43	855*	70	B
Barium	Ba	553.6	455.403	0.07	200	20	N_2O	0.85	17	100	Ba
Beryllium	Be	234.9	313.042	0.2	15	1	N_2O	0.025	0.5	64	Be
Bismuth	Bi	223.1	223.061	12	200	50	Air	0.45	9	88	Bi
Bromine	Br		163.340	6000							Br
Carbon	C		247.856	65						–	C
Calcium	Ca	422.7	393.366	0.03	10	1	N_2O	0.03	0.6	94	Ca
Cadmium	Cd	228.8	228.802	1.5	10	2	Air	0.01	0.2*	87	Cd
Cerium	Ce	520.0	418.660	7.5	100000	100000	N_2O			–	Ce
Chlorine	Cl		725.665	200000						–	Cl
Cobalt	Co	240.7	228.616	5	50	5	Air	0.21	4.2	98	Co
Chromium	Cr	357.9	267.716	4	50	6	N_2O	0.075	1.5	100	Cr
Cesium	Cs	852.1	455.531	3200	20	4	Air	0.55	11	58	Cs
Copper	Cu	324.7	324.754	2	30	3	Air	0.3	6	84	Cu
Dysprosium	Dy	421.2	353.170	0.3	600	30	N_2O	2.3	45	100	Dy
Erbium	Er	400.8	337.271	0.7	500	50	N_2O	5	100	100	Er
Europium	Eu	459.4	381.967	0.3	300	1.5	N_2O	1.3	25	100	Eu
Iron	Fe	248.3	259.940	1.5	50	6	Air	0.06	1.2	97	Fe
Gallium	Ga	294.4	417.206	6.5	800	100	Air	0.23	4.5*	80	Ga
Gadolinium	Gd	368.4	342.247	2.5	20000	2000	N_2O			–	Gd
Germanium	Ge	265.1	265.118	13	1000	200	N_2O	0.45	9*	100	Ge
Hafnium	Hf	307.3	264.141	4	10000	2000	N_2O			–	Hf
Mercury	Hg	253.7	184.950	8.5	1500	200	Air	7.5	150*	69	Hg

*Modifier used to obtain these results.

**20 µL injection

***The Characteristic Masses listed were determined in aqueous solution using maximum heating rate in argon with zero gas flow during atomization.

Table 1. Guide to ICP/AAS Analytical Values (continued)

Element		AA λ (nm)	ICP λ (nm)	ICP Detection limit µg/L	Flame AA Characteristic conc µg/L	Flame AA Detection limit µg/L	Flame type	Zeeman Furnace AA Characteristic** conc µg/L	Zeeman Furnace AA Characteristic*** Mass pg	MSR %	EI
Holmium	Ho	410.4	345.600	0.5	700	40	N_2O			–	Ho
Iodine	I		178.276	60							I
Indium	In	303.9	325.609	18	150	40	Air	0.35	7.0*	100	In
Iridium	Ir	208.9	224.268	3.5	800	500	Air	6.8	135	97	Ir
Potassium	K	766.5	766.490	10	7	3	Air	0.02	0.4	90	K
Lanthanum	La	550.1	379.478	0.02	40000	2000	N_2O			–	La
Lithium	Li	670.8	670.784	0.6	20	2	Air	0.2	4	49	Li
Lutetium	Lu	336.0	261.542	0.05	7000	300	N_2O			–	Lu
Magnesium	Mg	285.2	279.553	0.1	3	0.3	Air	0.01	0.2	75	Mg
Manganese	Mn	279.5	257.610	0.3	20	2	Air	0.03	0.6	92	Mn
Molybdenum	Mo	313.3	202.030	4	300	20	N_2O	0.35	7	96	Mo
Nitrogen	N		174.272	50 000							N
Sodium	Na	589.0	588.995	1	3	0.2	Air	0.005	0.1	92	Na
Niobium	Nb	334.9	309.418	4	20000	2000	N_2O			–	Nb
Neodymium	Nd	492.5	401.225	2	6000	1000	N_2O			–	Nd
Nickel	Ni	232.0	231.604	5.5	70	10	Air	0.24	4.8	98	Ni
Osmium	Os	290.9	225.585	5	1000	100	N_2O			–	Os
Phosphorous	P	213.6	177.499	18	120000	40000	N_2O	110	2200*	69	P
Lead	Pb	217.0	220.353	14	100	10	Air	0.28	5.5	92	Pb
Palladium	Pd	244.8	340.458	7	50	10	Air	0.43	8.6	100	Pd
Praseodymium	Pr	495.1	417.939	0.8	20000	10000	N_2O			–	Pr
Platinum	Pt	265.9	265.945	20	1000	100	Air	3.5	70	82	Pt
Rubidium	Rb	780.0	780.023	35	50	10	Air	0.05	1	90	Rb
Rhenium	Re	346.1	227.525	11	8000	1000	N_2O			–	Re
Rhodium	Rh	343.5	343.489	5	100	5	Air	0.4	8	95	Rh
Ruthenium	Ru	349.9	267.876	5.5	400	100	Air	0.75	15	100	Ru
Sulphur	S		180.734	20						–	S
Antimony	Sb	217.6	217.581	18	300	40	Air	0.5	10	96	Sb
Scandium	Sc	391.2	361.384	0.4	300	50	N_2O			–	Sc
Selenium	Se	196.0	196.026	37	1000	500	N_2O	0.7	14*	92	Se
Silicon	Si	251.6	251.611	5	1500	300	N_2O	0.75	15	100	Si
Samarium	Sm	429.7	442.434	7	6000	1000	N_2O			–	Sm
Tin	Sn	235.5	242.949	15	700	100	N_2O	0.5	10*	93	Sn
Strontium	Sr	460.7	407.771	0.02	40	2	N_2O	0.1	2	94	Sr
Tantalum	Ta	271.5	268.517	9	10000	2000	N_2O			–	Ta
Terbium	Tb	432.7	350.917	5	7000	700	N_2O	0.18	3.5	90	Tb
Tellurium	Te	214.3	214.281	27	200	30	Air	0.45	9*	93	Te
Thorium	Th		274.716	17						–	Th
Titanium	Ti	364.3	334.941	0.6	1000	100	N_2O	2.5	50	100	Ti
Thallium	Tl	276.8	351.924	16	200	20	Air	0.75	15	63	Tl
Thulium	Tm	371.8	346.220	1.5	300	20	N_2O			–	Tm
Uranium	U	358.5	385.958	18	100000	40000	N_2O			–	U
Vanadium	V	318.5	309.311	2	700	100	N_2O	1.1	22	79	V
Tungsten	W	255.1	239.709	17	5000	1000	N_2O			–	W
Yttrium	Y	410.2	371.030	0.2	2000	200	N_2O			–	Y
Ytterbium	Yb	398.8	328.937	0.3	60	4	N_2O	0.15	3	97	Yb
Zinc	Zn	213.9	213.856	0.9	8	1.0	Air	0.0075	0.15	92	Zn
Zirconium	Zr	360.1	339.198	1.5	9000	1000	N_2O			–	Zr

*Modifier used to obtain these results.

** 20 µL injection

*** The Characteristic Masses listed were determined in aqueous solution using maximum heating rate in argon with zero gas flow during atomization.

Analytical Requirements

Before deciding which technique is appropriate, the chemist must define both present and future analytical requirements. That is:

- Number of samples/week?
- What matrices need to be analyzed? For example, steels, bronzes, effluents, soils.
- How many elements need to be determined for each sample type?
- What are the typical sample volumes?
- What elements need to be determined?
- What concentration ranges are present in the matrices?
- Would an Internal Standard be useful? For example, where the samples may change in viscosity from sample to sample, for example, battery acid analysis.
- What expertise do the operators have?
- How much money is available to purchase or lease costs/month?
- Cost of ownership and running costs. Can the user afford an automated AAS or ICP-ES, or is a simple AAS sufficient?

The answers to these questions will help you to decide which is the preferred technique. Sometimes the answer is further complicated by the fact that neither flame AAS nor ICP-ES will satisfy all requirements. You may find, as many do, that both an ICP-ES and a furnace AAS will be necessary to meet the analytical requirements.

For Deuterium Furnace systems, the equivalent Characteristic Concentration and Characteristic Mass is easily calculated using the following conversion:

CMn = CMz X MSR (%)/100 CCn = CCz X MSR (%)/100

where:

CMn = Characteristic Mass for Deuterium Furnace Systems

CMz = Characteristic Mass for Zeeman Furnace Systems (from Table 1)

MSR = Magnetic Sensitivity Ratio (as % from Table 1)

CCn = Characteristic Concentration for Deuterium Furnace Systems

CCz = Characteristic Concentration for Zeeman Furnace Systems (from Table 1).

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Printed in the USA
November 1, 2010
ICPES003

Agilent Technologies

Improving Throughput for Oils Analysis by ICP-OES

Application Note

Inductively Coupled Plasma-Optical Emission Spectrometers

Author

Ingrid Szikla

Introduction

Trend analysis of wear metals in lubricating oils is a proven, cost-effective predictive maintenance technique. The presence and levels of various metal elements in lubricating oils gives an indication of the type of wear occurring in an engine. For example, an increase in the level of copper may indicate increased wear of bushings. Non-metals such as silicon, boron and phosphorus elements can also be determined. Monitoring the levels of wear metals and other elements in lubricating oils provides many benefits apart from predicting engine failure. For example, machinery can be kept up and running until maintenance becomes necessary, avoiding premature maintenance. Potential problems can be associated with specific components, eliminating complete teardowns.

The inductively coupled plasma optical emission spectroscopy (ICP-OES) technique for monitoring wear metals is the method of choice for trend analysis because it is fast and accurate. For the busy laboratory, not only is accuracy and long-term stability important; sample throughput is often a vital factor. The most significant contributor to the time taken for an analysis is the sample introduction system; the actual measurement time is most often less than one tenth of the total analysis time. This work shows that the use of a novel pump tubing arrangement can improve the speed of analysis. Using an improved sample introduction system, it was possible to accurately determine key wear metals and other elements in less than 50 seconds per sample using one simple method.

Agilent Technologies

Experimental

Instrumental

A Vista-PRO simultaneous ICP-OES with a radially viewed plasma was used. The radial plasma configuration is the accepted standard for the oils industry. The radial plasma orientation allows direct venting of combustion products, thereby reducing carbon build-up on the torch. The highly efficient 40 MHz free-running RF generator is easily able to cope with solvents to produce a stable, robust plasma with excellent long term stability. The instrument was fitted with a 3 channel peristaltic pump to allow a modified pump tubing configuration for faster sample uptake and washout. A glass concentric nebulizer with wide internal bore size was used to better handle particulates, and a glass double-pass spraychamber was used to prevent overloading the plasma with sample. Optimized instrument operating conditions are set out in Table 1.

Table 1. Instrument Operating Conditions

Parameter	Setting	Part number (where applicable)
Power	1.35 kW	
Plasma gas flow	15.0 L/min	
Auxilliary gas flow	2.25 L/min	
Nebulizer pressure or flow	110 kPa or 0.60 L/min	
Viewing height	10 mm	
Pump speed	12 rpm	
Sample uptake delay	15 s	
Stabilization time	5 s	
Rinse time	10 s	
Replicate read time	1 s	
Replicates	2	
Nebulizer type	Slurry glass concentric	20-100976-00
Torch type	Radial fully demountable torch kit (includes bracket and clamp)	99-101064-00
Spraychamber	Twister double pass	79-100437-00
Sample tubing to nebulizer	Grey/grey solvent flex	37-100352-00
Sample tubing to waste	Black/black solvent flex	37-100348-00
Tubing to waste from spraychamber	Solvent flex waste tubing	37-100354-00
Transfer tubing	Solvent flex transfer tubing ¼"internal diameter	37-100378-00
Drain tubing	Purple/black solvent flex	37-100470-00
Autosampler	AIM 1250*	

* Manufactured by A.I. Scientific, Scarborough, Qld, Australia

Standards and Reagents

Calibration solutions of 5, 10, 25, 50, 100, and 250 mg/L were prepared from Conostan S-21 certified standard, which contains 21 elements (Ag, Al, B, Ba, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Mo, Na, Ni, P, Pb, Si, Sn, Ti, V, Zn) at 500 mg/kg in oil. These calibration solutions were viscosity matched using Conostan base oil 75. Single element standards of Ca, Fe, Pb, P, and Zn were prepared from certified 5000 mg/kg Conostan standards (Conostan Division, Conoco Specialty Products Inc., Ponca City, OK, USA). The single element standard concentrations prepared were 10, 25, 50, 100, 250, 500, 1000 and 2500 mg/L. Jet-A1 kerosene (Mobil, Melbourne, Australia) was used as diluent.

Results

Detection Limits

In general, sensitive emission line wavelengths have lower detection limits than less sensitive emission line wavelengths for any given element. This is because sensitive emission lines produce a larger signal for a given concentration than less sensitive emission lines. Thus, low concentrations can be better detected using a sensitive emission line wavelength than an insensitive one. Frequently, detection limits improve with increasing read time because readout noise is reduced. The detection limits of various elements in kerosene are shown in Table 2. All detection limits in the table are below 1 mg/L, which easily allows trace levels of wear metals to be detected and a trend to be observed, even at low levels.

Table 2. Detection Limits of Elements in Kerosene at 2, 5 and 10 Seconds Integration Time

Element and emission line wavelength	3 σ Detection limits (mg/L) 1 s	2 s	3 s
Ag 328.068	0.006	0.003	0.002
Al 308.215	0.05	0.02	0.02
Al 396.152	0.05	0.02	0.01
B 249.772	0.021	0.007	0.005
Ba 455.403	0.003	0.002	0.001
Ba 493.408	0.0010	0.0007	0.0005
Ca 317.933	0.02	0.01	0.01
Ca 396.847	0.002	0.002	0.002
Cd 226.502	0.023	0.003	0.002
Cr 284.325	0.012	0.005	0.003
Cu 327.395	0.011	0.004	0.003
Fe 259.940	0.014	0.006	0.005
Fe 274.932	0.06	0.02	0.02
Mg 280.270	0.001	0.001	0.001
Mn 257.610	0.002	0.001	0.000
Mo 202.032	0.072	0.009	0.005
Na 589.592	0.004	0.002	0.002
Ni 230.299	0.08	0.02	0.01
P 213.618	0.26	0.03	0.02
Pb 220.353	0.39	0.05	0.03
Si 251.608	0.05	0.02	0.02
Sn 283.998	0.11	0.04	0.02
Ti 336.122	0.003	0.002	0.001
V 311.837	0.012	0.004	0.003
Z n 206.200	0.063	0.007	0.005
Zn 213.857	0.017	0.002	0.002

Linear Range

In general, the maximum accurately measurable concentration of an element is obtained by using a less sensitive emission line wavelength for that element. Although sensitive emission line wavelengths have lower detection limits than insensitive ones, insensitive emission line wavelengths can measure higher maximum concentrations. Some elements, such as calcium and phosphorus, may be present at high concentrations in oils, so a high maximum measurable concentration is desirable. The wavelengths chosen for analysis reflect a compromize between best detection limits and desired concentration range.

Table 3. Maximum Measurable Concentration of Selected Elements at Specified Emission Line Wavelengths

Element and emission line wavelength	Maximum concentration (mg/L)
Ag 328.068	250+
Al 308.215	250+
Al 396.192	100
B 249.772	250+
Ba 455.403	100
Ba 493.408	250+
Ca 317.933	2500
Ca 396.847	100
Cd 226.502	250+
Cr 284.325	250+
Cu 327.395	250+
Fe 259.940	250+
Fe 274.932	1000
Mg 280.270	100
Mn 257.610	250+
Mo 202.032	250+
Na 589.592	250+
Ni 230.299	250+
P 213.618	2500
Pb 220.353	1500
Si 251.608	250+
Sn 283.998	250+
Ti 336.122	250+
V 311.837	250+
Zn 206.200	2500
Zn 213.857	250

* Note that 250+ designates an accurately measurable concentration that may surpass 250 mg/L.

Modified Pump Tubing Setup

To speed up sample delivery to the plasma, the flow rate of sample through the autosampler probe was increased based on the "rapid flow" concept conceived by Shane Elliott and investigated as applied to organic solutions by Ross Ashdown (both from Agilent). The idea is to increase the flow rate of sample from the autosampler to the peristaltic pump. To increase the sample flow rate, a wider internal diameter peristaltic pump tubing could have been used, but this would overload the nebulizer, adversely affecting nebulization. Instead, an additional sample peristaltic pump tube was introduced to the system via a T-piece inserted between the end of the autosampler line and the start of the sample peristaltic pump tubing so that sample would flow through two sample perstaltic pump tubings instead of one. One of the peristaltic pump tubes was directed to the nebulizer, and the other to waste, which avoided overloading the nebulizer with sample. By having sample flow through two pump tubings, the sample flow rate through the autosampler probe up to the point where the T-piece was inserted was increased, thus reducing sample uptake time.

To measure sample uptake time, kerosene was introduced to the autosampler probe manually after aspirating air, and the time taken for the plasma to turn bright green (which indicates that organic solution is being aspirated into the plasma) was measured by stopwatch. Table 4 shows that using the modified pump tubing setup, the sample uptake time was decreased by approximately 10 seconds. An added benefit of decreasing sample uptake time is that the time taken to achieve a fixed degree of washout is also reduced.

Table 4. Time Saved Using Modified Pump Tubing Setup

Pump tubing configuration	Acutal sample uptake time (s)	Sample uptake time in method (s)
Standard	24	25
Modified	15	15

Washout

To determine the washout achieved in an autosampler run, an analysis was performed where a blank kerosene solution was measured immediately following a solution containing 1000 mg/L of Fe. These two solutions were then measured in pairs six times each. Table 5 shows that three orders of reduction in sample concentration was achieved in an autosampler run with a rinse time of 10 seconds. If a more thorough rinse was required, then SmartRinse could have been used. The SmartRinse feature of the ICP Expert software optimizes the rinse time for each sample, ensuring that the rinse time is only as long as required to return the signal to that of a blank for each wavelength in the analysis [1]. This means that high concentration samples will take longer to analyze than low concentration samples. For this work, a washout of three orders was acceptable, so a short, fixed rinse time was used.

Table 5. Blank Results After Measuring 1000 mg/L Iron. This Demonstrates that Three Orders of Washout is Achieved with a Rinse Time of 10 Seconds.

Kerosene blank measurement number	Measured Fe conc. (mg/L)
2	0.66
4	0.77
6	0.79
8	0.79
10	0.80
12	0.64

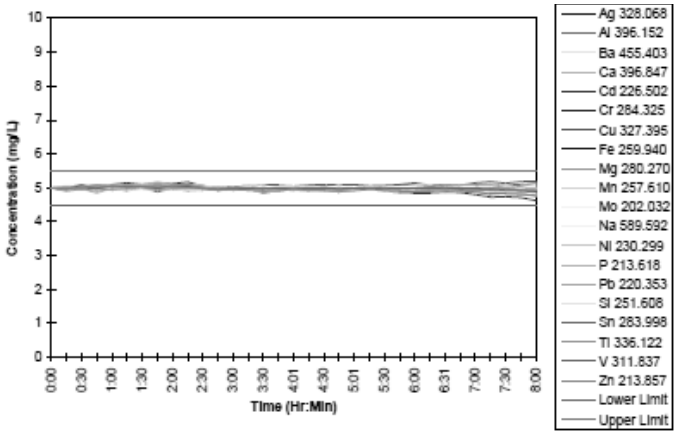

Figure 1. Stability of the Vista-PRO radial instrument over 8 hours. Results remained within ±10% for all elements in the 5 mg/L S21 kerosene solution without internal standardization or recalibration.

Long-Term Stability

A 5 mg/L solution of S21 elements in Jet-A1 kerosene was analysed continuously over an eight hour period. No recalibrations were performed, and no internal standard was used. Figure 1 shows that results remained within 10% of the true value over the entire 8 hours. Precision was typically better than 2 %RSD.

Conclusion

The Vista-PRO radial ICP-OES provides excellent throughput at 47 seconds per sample using a simple optimized sample introduction system. The detection limits and maximum measurable concentration of selected wavelengths allows typical oil samples to be analysed, while the excellent stability allows continuous running without recalibration, providing a saving on costs by reducing analysis time and the amount of standard solution used.

Acknowledgements

The author would like to thank Shane Elliott (Varian Australia) for the initial concept and his advice with alternative sample pump tubing configurations, Ross Ashdown (Varian U.K.) for his early work with fast throughput for organics, Barry Sturman, Alan Wiseman and Kate Pearson-Santiago (Varian Australia) for editing, and Glyn Russell (Varian Australia) for his input, encouragement and review of this work.

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Printed in the USA
November 1, 2010
ICPES031

Agilent Technologies

The Investigation of Fertilizer Analyses Using Microwave Digestion and the Agilent 720-ES

Application Note

Inductively Coupled Plasma-Optical Emission Spectrometers

Authors

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Introduction

Fertilizers play a vital role in sustaining crop yields by supplying essential plant nutrients such as macronutrients nitrogen (N), phosphorus (P_2O_5) and potassium (K_2O). The optimum "fertilizer ratio" of these three elements can vary according to the type of plant material being fertilized. Secondary nutrients such as calcium, magnesium and sulfur and micronutrients boron, copper, iron, manganese, molybdenum and zinc also play important roles in plant growth.

The purpose of these investigations was to evaluate microwave digestion procedures for two analyses of fertilizer samples. The experiment was divided into two phases.

Phase 1 was to evaluate using microwave digestion to prepare fertilizers for the determination of As, Ca, Cd, Cu, Cr, Fe, K, Na, Mg, Mn, P, Pb, Se and Zn by simultaneous ICP-OES.

Phase 2 was to evaluate using microwave digestion to prepare fertilizers for the determination of available phosphorus (P_2O_5) and potassium (K_2O) by simultaneous ICP-OES. Traditionally, the preparation of samples for phosphorus and potassium is done by extraction with ammonium citrate—EDTA. The samples are placed in a heated water bath and shaken for 1 hour. The reagents are added to warmed samples and the shaking must be continuous. The number of samples typically required for preparation is large and this process is extremely time-consuming.

In this field, phosphorus is traditionally determined using an auto-analyzer, which can be tedious to set up and run. Potassium is typically determined by flame photometry. To simplify sample preparation and analysis, the CEM MARS Xpress microwave digestion system with stirring option and Agilent 720-ES simultaneous ICP-OES were used for both analysis phases.

Agilent Technologies

Instrumentation

Agilent 720-ES

The Agilent 720-ES with axial torch configuration is a truly simultaneous ICP-OES with solid-state, Charge Couple Device (CCD) detection system. The custom-designed and patented CCD detector incorporates IMAP technology, whereby pixels are arranged in continuous angled arrays matched exactly to the image produced by the echelle optics. This provides true simultaneous measurement and full wavelength coverage from 167 nm to 785 nm.

Microwave Digestion System

CEM, Corp. MARSXpress is an ultra-high throughput microwave digestion system designed to make high-throughput sample preparation and research applications quick and easy.

Forty high-pressure vessels, available in 55 mL or 75 mL sizes, can be processed per run with temperature control of every vessel.

Sample Preparation

Phase 1

To verify the method, two Magruder fertilizer check standards (200204 and 200206), which form part of the association of American Plant Feed Control Officials (AAPFCO) round robin laboratory checks, and a certified reference material, Industrial Sludge (CRM-S-I) from High Purity Standards, were prepared for analysis. Approximately 0.5 g of sample was accurately weighed and transferred to 55 mL MARSXpress vessels. Then, 9 mL of HNO_3 and 1 mL of HCl was added to each sample vessel. Samples were digested in duplicate.

The microwave digestion method is summarized in Table 1.

Table 1. Microwave Digestion Settings

Stages	Max power (W)	% power	Ramp (min.)	Pressure (PSI)	Temp. (°C)	Hold (min)
1	1200	100	15:00	120	200	15:00

The total digestion time was 30 minutes. Figure 1 represents the average digestion temperature over time in the vessels during sample preparation.

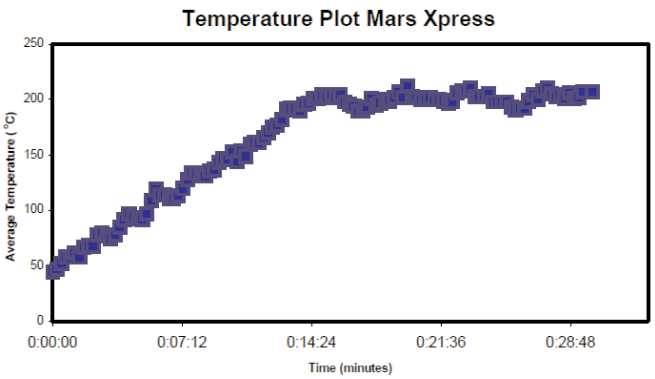

Figure 1. MarsXpress digestion temperature profile.

All samples were diluted to 50.00 mL in plastic disposable tubes and filtered with 2 micron Teflon FilterMate. This high dirt trapping FilterMate is especially suitable for trace level analysis and is supplied with lot certification for trace metals. The filtered samples were transferred directly to the Agilent SPS3 autosampler for analysis on the Agilent 720-ES.

Calibration Solution Preparation

The calibration summary for Phase 1 is listed in Table 2.

Table 2. Phase 1 Calibration Summary

Elements	Concentration (mg/L)
As, Cd, Cr, Cu, Mn, P, Pb, Se, Zn	0.5, 5, 10 and 50
Ca, Fe, K, Na, Mg	5, 10, 100 and 1000

Conditions

Instrument operating conditions are shown in Table 3.

Table 3. Instrument Operating Conditions

Parameter	Setting
Spraychamber type	Double-pass, glass cyclonic
Nebulizer type	SeaSpray
Nebulizer flow	0.75 L/min
RF Power	1.25 kW
Plasma gas flow	15 L/min
Auxiliary gas flow	1.5 L/min
Uptake delay	20 s
Stabilization delay	10 s
Rinse time	15 s
Internal standard	2 mg/L yttrium
Ionization buffer	0.4% caesium
Integration time*	60 s
Replicates	2
Total analysis time#	165 s

*If Se is not required at detection limit concentrations, a 5 s integration time is adequate. # 55 s for sample sets not requiring low concentrations of Se to be measured.

Results and Discussion

Phase 1

The results for Phase 1 are summarized in Table 4. Many of the samples encountered in feed and fertilizer laboratories consist of high concentrations of nutrients. Some of these elements cause ionization interferences while others cause spectral overlap problems, for example, iron. The use of an ionization buffer, for example, 0.4 % caesium minimized the ionization interferences. The spectral overlap can be overcome by the advanced background correction techniques of the ICP Expert II software, such as fitted background correction and FACT (Fast Automated Curve-Fitting Technique) spectral deconvolution.

Table 4. Results Summary

Sample labels units	As 188.980 mg/kg	Expected mg/kg	Ca 370.602 %	Expected %	Cd 214.439 mg/kg	Expected %	Cr 267.716 mg/kg	Expected mg/kg
Sludge B	141	141	0.0233	0.0242	0.64	NA	110	111
Magruder 4B	2.05	1.75	2.71	2.48	12.31	NA	125.2	132.6
Magruder 6B	5.75	5.66	4.93	5.94	1.51	NA	50.88	51.08

Sample labels units	Cu 327.395 %	Expected %	Fe 261.382 %	Expected %	K_2O 404.721 %	Expected % K_2O	Mg 279.078 %	Expected %
Sludge B	0.0407	0.0398	0.012	0.014	NA	NA	12.6	12.2
Magruder 4B	0.0461	0.0307	0.350	0.400	11.02	10.54	1.62	1.64
Magruder 6B	1.010	0.976	0.500	0.500	21.37	20.54	0.53	0.62

Sample labels units	Mn 294.921 %	Expected %	Na 589.592 %	Expected %	P 214.914 % P_2O_5	Expected % P_2O_5
Sludge B	0.51	0.48	0.94	0.94	0.51	0.50
Magruder 4B	0.036	0.039	0.31	0.29	8.1	9.1
Magruder 6B	0.014	0.015	0.57	0.58	9.1	9.9

Sample labels units	Pb 220.353 mg/kg	Expected mg/kg	Se 196.026 mg/kg	Expected mg/kg	Zn 213.857 %	Expected %
Sludge B	6.8	5.7	NA	NA	0.0244	0.0249
Magruder 4B	1.16	2.18	0.43	0.44	0.043	0.048
Magruder 6B	1.88	2.15	0.13	0.12	0.003	0.003

Phase 2 Available K_2O and P_2O_5

The extraction reagent preparation requires 325 g EDTA and 650 g dibasic ammonium citrate dissolved in 19.5 L distilled water. With mixing, 390 mL of a 1:1 solution of NH_4OH:H_20 is then added. When the solution is cooled to room temperature, the pH is carefully adjusted to 7.0 with additional 1:1 solution of NH_4OH:H_20 and the final solution diluted to 26.0 L.

The traditional method requires that 0.25 g of sample undergo extraction in 100 mL ammonium citrate reagent in a Wheaton bath stabilized to 65 °C (the extraction solution is added to the warmed sample). Upon completion of the extraction procedure, the solutions are cooled and diluted to 250 mL with ammonium citrate/EDTA reagent.

The samples are then typically run on the flame photometer for available K_2O and the auto-analyzer for P_2O_5.

The first part of the Phase 2 experiment was to determine if ICP-OES is a viable alternative technique for the determination of K and P. A set of ten fertilizer samples were extracted per the defined method and analyzed by the traditional techniques and ICP-OES.

With the ICP-OES technique, beryllium was selected as the internal standard and 0.8% caesium as an ionization buffer. Optimum instrument conditions were found to be at a power of 1.1 kW and a nebulizer flow of 0.65 L/min. The total sample analysis time was 55 s/sample.

A comparison of the results by technique for the determination of K and P are summarized in Table 5.

Table 5. Traditional Methodology Verses ICP–OES (1 Hour Water Bath Extraction) Results

Calibration solutions (units)	ICP-OES K_2O (769.897 nm) (% w/v)	Flame photometer K_2O (% w/v)	ICP-OES P_2O5 (214.914 nm) (% w/v)	Auto-analyzer P_2O_5 (% w/v)
Blank	0		0	
Std 1			0.1145	
Std 2	0.06023		0.4581	
Std 3			0.68709	
Std 4	0.24038		0.91612	
Std 5			1.3742	

Calibration solutions (units)	ICP-OES K_2O (769.897 nm) (% w/v)	Flame photometer K_2O (% w/v)	ICP-OES P_2O_5 (214.914 nm) (% w/v)	Auto-analyzer P_2O_5 (% w/v)
NIST SRM 200a	34.81	34.64	52.62	52.11
Sample 1	4.51	4.59	21.6	21.59
Sample 2	4.50	4.29, 5.11, 5.19	8.10	8.59
Sample 3	9.22	9.22, 9.30	10.04	10.09
Sample 4	9.89	9.80, 9.71	10.04	9.75, 9.95
Sample 5	1.80	1.48, 2.46, 2.16	19.87	20.07
Sample 6	2.50	2.53, 3.03, 3.15	22.47	22.49, 22.69
Sample 7	10.17	9.88	10.58	10.47
Sample 8	8.56	8.58	4.17	4.06
Sample 9	5.47	5.45	9.71	9.73
Sample 10	18.49	18.00	16.56	16.70, 16.45

Note: No internal standard for K_2O.

The final step of the Phase 2 study was to mimic the extraction process using microwave digestion. The microwave is not used for total digestion of the fertilizer sample, but as a way to consistently heat the extraction to 65 °C.

Three Magruder fertilizers and NIST SRM 200a potassium dihydrogen phosphate (KH_2PO_4) were carefully weighed to 0.1 g into HP 5000 vessels. Using the 75 mL vessels, 75 mL of ammonium citrate/EDTA extraction fluid was added. Stirring bars were added to each sample to simulate the shaking process and the contents were heated to 65 °C for 1 hour.

Table 6 summarizes the results collected by ICP-OES for available K_2O and P_2O_5.

Table 6. Summary of Results for Microwave Extraction and Determination of K_2O and P_2O_5 by ICP–OES

Calibration solutions(units)	K_2O 404.721 (% w/w)	K_2O 769.897 (% w/w)		P_2O_5 185.878 (% w/w)	P_2O_5 214.914 (% w/w)	Calibration
Blank	0	0		0	0	
Std 1				0.01139	0.01139	
Std 2		0.012				
Std 3		0.03038		0.05785	0.05785	
Std 4	0.06022	0.06022		0.11449	0.11449	
Std 5	0.1223	0.1223		0.22881	0.22881	
Std 6	0.2431	0.2431				
Std 7	0.49789					

Samples(units)	K_2O 404.721 (% w/w)	K_2O 769.897 (% w/w)	Expected (% w/w)	P_2O_5 185.878 (% w/w)	P_2O_5 214.914 (% w/w)	Expected (% w/w)
NIST_SRM 200a	34.42	34.58	34.61	52.44	52.56	52.11
NIST SRM duplicate	34.93	34.34		52.70	52.44	
MAG # 4	11.66	10.45	10.54	20.71	20.36	20.82
MAG # 4 duplicate	10.04	10.48		20.90	20.75	
MAG # 6	26.65	25.41	25.42	22.33	22.07	22.54
MAG # 6 duplicate	23.99	24.88		22.25	22.10	
MAG # 9	12.57	13.66	13.00	14.04	13.85	13.00
MAG # 9 duplicate	12.65	13.66		14.09	13.86	

Conclusion

The preparation of fertilizer samples by microwave digestion/extraction for the determination of macro, secondary and micro nutrients by simultaneous ICP-OES was evaluated and found to compare well with more traditional methods. The combination of microwave and ICP-OES techniques resulted in significantly faster and simpler sample preparation and analysis, requiring only a single analytical system to measure all elements of interest.

Acknowledgements

Elaine Hasty, CEM Corporation, Matthews, NC

Peter Kane, Office of Indiana State Chemist, West Lafayette, IN

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Printed in the USA
November 1, 2010
IO-037

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Determination of Mercury With On-line Addition of Stannous Chloride Using an Axial ICP-OES

Application Note

Inductively Coupled Plasma-Optical Emission Spectrometers

Author

Tran T. Nham

Introduction

Mercury is one of the most toxic heavy metals in the environment. It is therefore important to routinely monitor the Hg level in many types of samples. The US EPA approved methodology for the determination of Hg uses the cold vapor technique with stannous chloride as the reducing agent [1]. This methodology is applicable to Hg determinations in a range of waters (drinking, ground, surface, sea and brackish waters) plus domestic and industrial wastes. It allows mercury detection down to the sub-µg/L range.

While Hg can be determined with direct aspiration using the ICP-OES technique, the performance achieved is not sensitive enough for many environmental applications.

Ultra-trace level determination of Hg therefore requires the use of a vapor generation accessory such as the Agilent VGA-77. The detection limit of Hg achieved with this accessory using an axially-viewing ICP-OES is 0.02 µg/L [2]. However, if the required determination level of Hg is not so low, it is feasible to use on-line addition of reductant to achieve the required signal enhancement. This work demonstrates a simple and convenient way to determine Hg in the µg/L range using an axially-viewing ICP-OES.

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Instrumentation

All measurements were performed on an Agilent 720-ES axially-viewing ICP-OES. The Agilent 720-ES is a simultaneous ICP-OES featuring an Echelle polychromator incorporating a quartz prism and a custom-designed and patented CCD detector, which provides the benefit of simultaneous measurement and continuous wavelength coverage over the range from 167 to 785 nm. The system is available with a choice of sample introduction system; either a 3 or 4 channel peristaltic pump for sample introduction and mass flow control or manual pressure control of the nebulizer gas flow. For this application, the system was fitted with a mass flow controller and a four channel peristaltic pump. The four channel peristaltic pump allows the sample, $SnCl_2$ reductant and the waste to be simultaneously pumped. To enable on-line addition of the reductant to the sample, a Y-piece (Agilent p/n 1610132400) was used to combine the sample and reductant flows prior to the sample introduction system. The mixture was then nebulized into the plasma.

A conventional one piece axial torch was used. The sample introduction system consisted of a concentric glass nebulizer and a glass cyclonic chamber. Agilent ICP Expert II software was used for instrument operation. The operating parameters of the system are listed in Table 1.

Table 1. Instrument Operating Parameters

Conditions	Settings
Power	1.2 kW
Plasma gas flow	15 L/min
Auxiliary gas flow	1.5 L/min
Nebulizer flow	0.65 L/min
Pump speed	12 rpm
Pump tubing	White-white (inlet) for both sample and reductant (1.02 mm id)
	Blue-blue (outlet) (1.65 mm id)
Sample uptake rate	1.0 mL/min
Sample uptake rate	1.0 mL/min
Replicate read time	30 s
Sample uptake delay time	80 s
Fast pump	Off
Rinse time	120 s
Sampling mode	Manual
Background correction	Fitted
Number of replicates	3 10 for detection limit measurements

The on-line addition of reductant results in an unusually large quantity of liquid being nebulized. It is recommended that the fast pump option be disabled during the sample uptake delay, to minimize droplet condensation in the injector tube of the torch.

Materials and Reagents

All chemicals and reagents used were of high-purity grade.

- HCl, Tracepur, 36%, Merck.
- HNO_3, Ultrapure, 60%, Merck.
- 1000 mg/L Hg certified standard solution, EM Science.
- $SnCl_2$, Analar, BDH.
- Milli-Q water.

Sample Preparation

Preparation of $SnCl_2$ Solutions

Stannous chloride ($SnCl_2$) can dissolve in less than its own mass of water without apparent decomposition, but as the solution is diluted, hydrolysis occurs to form an insoluble basic salt (Sn(OH)Cl) which is readily oxidized by air. Therefore HCl must be added to suppress hydrolysis and to help prevent the oxidation of $SnCl_2$ by air. An insufficient amount of HCl will result in a yellow-colored solution. In this work, all $SnCl_2$ solutions were prepared in 20% HCl. It is recommended to dissolve $SnCl_2$ in concentrated HCl prior to mixing with water.

Sample Preparation for NIST 1641D Mercury in Water [3]

The certified reference material NIST 1641D mercury in water is supplied in an ampoule. After the ampoule was opened, a 0.2 mL aliquot was transferred to a 100 mL volumetric flask to make a 1 in 500 dilution with 2% v/v HNO_3.

Calibration solutions and blank were prepared in 2% v/v HNO_3.

Cleaning of the Torch After Analysis

After the analysis, it was observed that the end of the outer tube of the torch was covered by a layer of white powder, which is presumably SnO_2. This can be removed by simply soaking the torch directly in concentrated HCl to dissolve the white deposit. After soaking, rinse the torch with de-ionized water and allow to dry.

Results and Discussion

Effect of $SnCl_2$ on Hg Signal Intensity

The effect of different $SnCl_2$ concentrations on the signal intensity of a 50 µg/L mercury solution was studied. As illustrated in Figure 1, on-line addition of $SnCl_2$ has enhanced the Hg signal over 30 times.

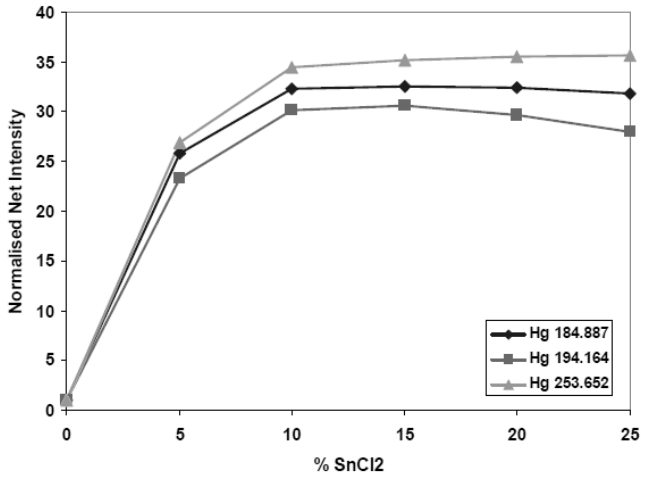

Figure 1. Effect of different $SnCl_2$ concentrations on the signal intensity of 50 µg/L Hg.

As the concentration of $SnCl_2$ increases, the Hg signal intensity increases, and then plateaus out at about 10–15% $SnCl_2$. Therefore, the concentration of $SnCl_2$ used in this work was 15%.

It is noted that for both the Hg 184 nm and Hg 194 nm lines, the Hg signal increases up to 15% $SnCl_2$, then slowly decreases as the concentration of $SnCl_2$ increases. This is caused by the spectral interference of Sn on both the Hg 184 nm and Hg 194 nm lines, respectively. The potential spectral interferences on various Hg lines are listed in Table 2.

Table 2. Potential Spectral Interferences on Hg Emission Lines

Wavelength (nm)	Potential interferences
184.887	Sn 184.821 nm
194.164	Sn 194.205 nm
253.652	Cr 253.634 nm Cr 257.692 nm

Figures 2 to 4 show the signal graphics for Hg at the 184, 194 and 253 nm lines with various $SnCl_2$ concentrations. The sloping background at the Hg 184 nm and Hg 194 nm lines is caused by the presence of high levels of Sn. The Hg 253 line is free of interference from Sn, but there is the potential for interference from Cr if the sample has been prepared in $K_2Cr_2O_7$.

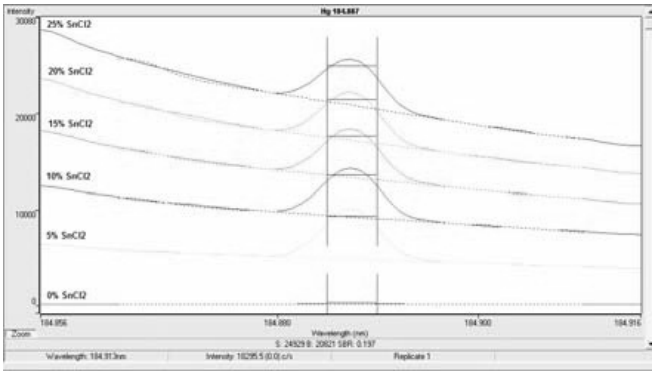

Figure 2. Signal traces of 50 µg/L Hg 184 nm at various $SnCl_2$ concentrations.

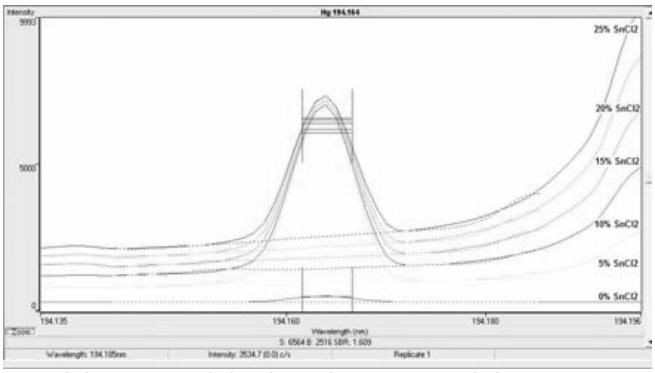

Figure 3. Signal traces of 50 µg/L Hg 194 nm at various $SnCl_2$ concentrations

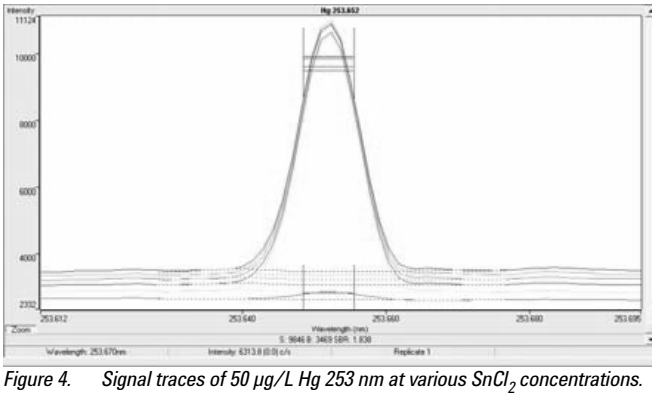

Figure 4. Signal traces of 50 µg/L Hg 253 nm at various $SnCl_2$ concentrations.

Detection Limit

Detection limit (DL) is expressed as three times the standard deviation of the blank intensity in concentration units. The detection limits for Hg with and without the addition of $SnCl_2$ are listed in Table 2. Generally speaking, an order of magnitude improvement in detection limit is achieved with the addition of $SnCl_2$ as a reductant.

However it is also noted that these detection limits are around a factor of 10 higher than those that can be achieved

with the use of the cold vapor technique using the VGA-77 (0.02 µg/L[2]).

Table 2. Detection Limits for Hg Achieved with On-line Addition of $SnCl_2$

Wavelength (nm)	DL (µg/L) with 15% $SnCl_2$	Without $SnCl_2$
184.887	0.4	2.0
194.164	0.2	2.0
253.652	0.2	3.0

Analysis of NIST 1641D Mercury in Water

Conventional aqueous Hg standards of 1, 5 and 10 µg/L were used to calibrate the instrument at each of the respective Hg wavelengths. The calibration graphs obtained for each Hg emission line are shown in Figure 5. The NIST 1641d mercury in water certified reference material was measured against these calibrations. The results are listed in Table. 3. The measured values are in good agreement with the certified values.

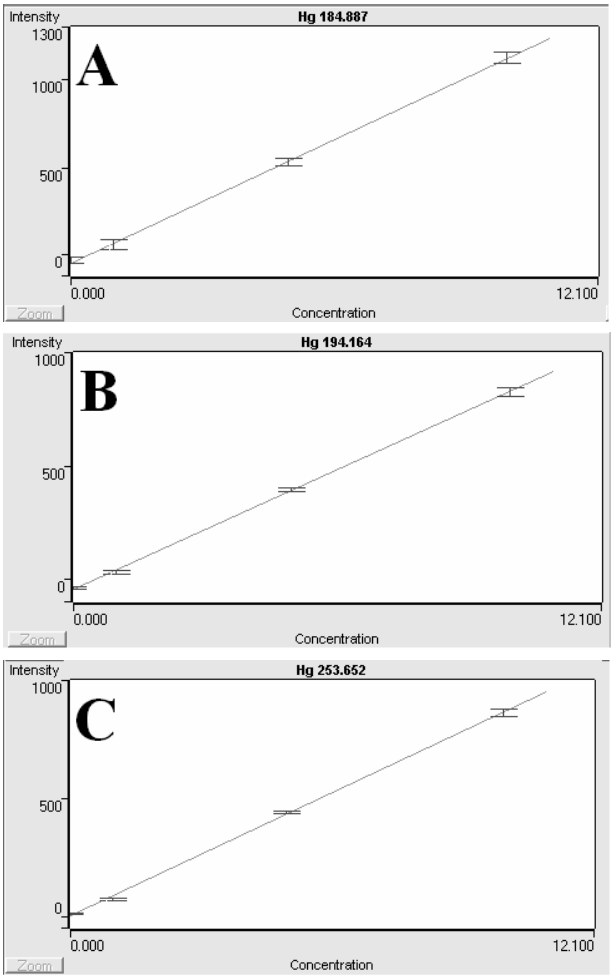

Figure 5. Calibration curves for Hg at (A) the 184 nm emission line, (B) the 194 nm emission line and (C) the 253 nm emission line.

Table 3. Results for Analysis of the NIST 1641D Mercury in Water Certified Reference Material

Wavelength (nm)	Concentration (mg/kg) measured	Certified
184.887	1.592 ± 0.010	1.590 ± 0.018
194.164	1.585 ± 0.010	1.590 ± 0.018
253.652	1.596 ± 0.012	1.590 ± 0.018

Conclusion

The determination of Hg by on-line addition of $SnCl_2$ using the Agilent 720-ES axial ICP-OES instrument has been described. The on-line addition of $SnCl_2$ reductant for Hg determination can provide a tenfold improvement in detection limit compared with routine determination. This on-line reduction method allows Hg determinations at µg/L levels without the use of a cold vapor generation technique. It is also simple and easy to implement.

References

1. US EPA Publication No. EPA-600/4-79-020, "Method for chemical analysis of water & wastes", (1979), Method 245.1.
2. P. Doidge, "Determination of mercury in a certified reference sludge material using the Varian 710-ES",

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Printed in the USA
November 1, 2010
SI-01319

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Determination of Rhodium in Catalyst Oil and Aqueous Samples Using the Agilent 710 ICP-OES

Application Note

Energy and Fuels

Author

Jin-Na Chang

Introduction

A series of oil catalyst samples and an unknown aqueous sample were supplied for analysis. The rhodium content in these samples was determined using the Agilent 710 ICP-OES axially viewing ICP-OES. The oil catalyst samples were prepared using microwave digestion.

The axially viewing ICP-OES was selected for measurement as the expected rhodium content was low, and this system provides better sensitivity than that achieved by the radially viewed configuration, independent of the complexity of the sample medium [1]. The improvement in sensitivity with the axially viewed configuration is typically up to a 20 fold improvement in detection limits [1,2].

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Instrumentation

All sample measurements were performed on a Agilent 710 ICP-OES axially viewing ICP-OES with simultaneous CCD detection.

The Agilent 710 ICP-OES is a simultaneous ICP-OES featuring an Echelle polychromator and a megapixel CCD detector, which provides the benefit of simultaneous measurement and continuous wavelength coverage over the range from 177 to 785 nm. The polychromator can be purged with a low flow of either argon or nitrogen for improved detection capability when measuring emission lines at low UV wavelengths.

The system is supplied as standard with a 3-channel peristaltic pump and manual pressure control of the nebulizer gas flow. The standard sample introduction system consists of a glass concentric nebulizer (Conikal) and a glass cyclonic spray chamber. Agilent ICP Expert II software was used for instrument operation. The operating parameters of the system are listed in Table 1.

Table 1. Instrument Operating Parameters

Condition	Setting
Power	1.15 kW
Plasma gas flow	16.5 L/min
Auxiliary gas flow	1.5 L/min
Spray chamber type	Glass cyclonic (single-pass)
Torch	Standard one piece axial torch
Nebulizer pressure	200 kPa
Nebulizer type	Conikal
Replicate read time	5 s
Auto-integration	On
Number of replicates	3
Stabilization time	15 s
Pump tubing	Sample: white-white (1.05 mm ID) Waste: blue-blue (1.65 mm ID) Buffer/Reference element: black-black (0.76 mm ID)
Sample uptake delay time	15 s
Pump speed	15 rpm
Rinse time	10 s
Fast pump	On
Background correction	Fitted

Sample Preparation and Instrument Conditions

All chemicals and reagents used were of high purity grade.

- HNO_3, Ultrapure, 60%, Merck.
- 40 and 100 mg/L Rh standard solutions as supplied by the client.
- Milli-Q water with resistivity less than 18 Mohm–cm^{-1}.

The oil catalyst samples were prepared using a microwave digestion system with temperature and pressure control (Shanghai EU Microwave Chemistry Technology Co. Ltd. model WX-4000).

0.3 g of sample was accurately weighed and placed into a digestion vessel. The digestion vessel was heated on a conventional hot plate at 80 °C for approximately 20 minutes. The aim of this sample pre-treatment was to remove the volatile organic components from the sample. The digestion vessel was removed from the hot plate and allowed to cool.

This solution was quantitatively transferred into a microwave digestion vessel and 4 mL of nitric acid (HNO_3) was added. The digestion vessel was sealed and placed into the microwave digestion system. The microwave digestion method used is summarized in Table 2.

Table 2. Microwave Digestion Settings

Step	Temperature (°C)	Pressure (atm.)	Time (min.)
1	130	10	5
2	160	16	5
3	180	20	5
4	200	25	5

The digestion vessels were removed from the microwave digestion system and left to cool to room temperature. The digest was transferred to a volumetric flask and diluted on a mass basis. A summary of the initial sample weights, the final weights after dilution and observations on the digest obtained are summarized in Table 3.

As the expected concentration of rhodium in the aqueous sample was around 60 mg/L, no preparation was required for analysis. The aqueous sample was measured directly.

All the prepared samples were analyzed directly for rhodium.

Table 3. Actual Sample Weights Used for Digestion Together with Observations on the Samples Before and After Microwave Digestion

No.	Sample ID	Sample type	Expected Rh concentration	Observations	Sample weight (g)	Weight of predetermined volume (g)	Digest obtained
1	Q22321 2007-08-04	Oil catalyst	Very low	Light yellow, transparent oil sample with pungent odor	0.3033	23.8461	Clear
2	Q22321 2007-08-05	Oil catalyst	Very low	Light yellow, transparent oil sample with pungent odor	0.3260	27.5567	Clear
3	Q22321 2007-08-06	Oil catalyst	Very low	Light yellow, transparent oil sample with pungent odor	0.3442	23.2292	Clear
4	Q22321 2007-08-07	Oil catalyst	Very low	Light yellow, transparent oil sample with pungent odor	0.3386	22.7784	Clear
5	Q22321 2007-08-08	Oil catalyst	Very low	Light yellow, transparent oil sample with pungent odor	0.3043	24.2429	Clear
6	Q22321 2007-08-09	Oil catalyst	Very low	Light yellow, transparent oil sample with pungent odor	0.3380	24.9658	Clear
7	Q22321 2007-08-10	Oil catalyst	Very low	Light yellow, transparent oil sample with pungent odor	0.3025	22.2151	Clear
8	Q21011 2007-08-06	Oil catalyst	Est. 100-200 mg/L	Yellow, transparent oil sample with pungent odor	0.3096	52.3151	Light yellow color, but basically clear
9	Rhodium acetate solution	Aqueous solution	Est. 60 mg/L	Light yellow, transparent sample	N/A	N/A	N/A

Calibration Solutions

Conventional aqueous Rh standard solutions of 40 and 100 mg/L were provided by the client together with the samples.

For the determination of the aqueous sample, which was expected to be at a concentration of 60 mg/L, these standard solutions were used directly to calibrate the instrument.

For the determination of the digested oil samples, for which the Rh content was expected to be low, these standard solutions were diluted by a factor of 10 to concentrations of 4 and 10 mg/L respectively.

Results and Discussion

The calibration graphs obtained are shown in Figures 1 and 2.

The weight/dilution corrected sample results are listed in Table 4. These results have been converted back to report the actual rhodium content contained in the original samples. This takes into account the sample weight used for digestion and the applied dilution ratio during preparation.

The measured concentrations for rhodium in a number of the samples were close to the calibration blank solution. Accordingly, these results have been reported as "Not Detected" (ND).

Signal traces for the measured solutions where the Rh content could be quantified are included in Figures 3 to 5.

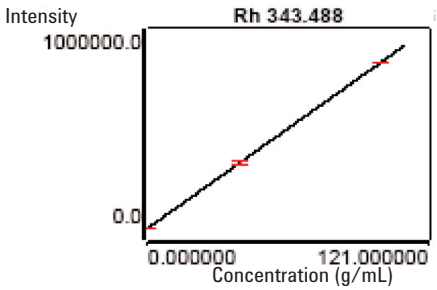

Figure 1. Calibration graph used for the determination of the aqueous sample at the Rh 343.488 nm emission line.

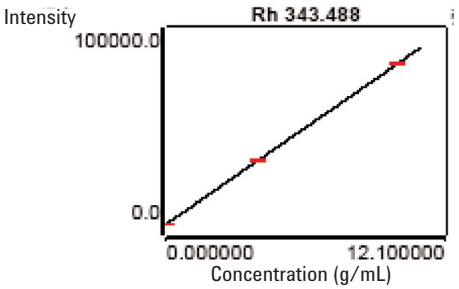

Figure 2. Calibration graph used for determination of the digested oil catalyst samples at the Rh 343.488 nm emission line.

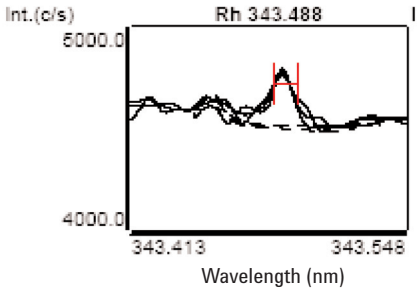

Figure 3. Signal trace for oil sample Q22321 2007-08-07.

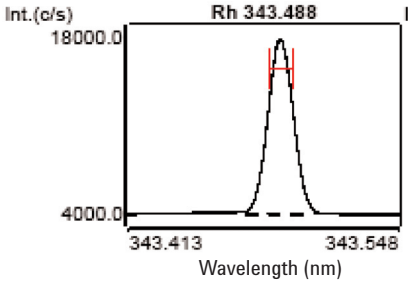

Figure 4. Signal trace for oil sample Q21011 2007-08-06.

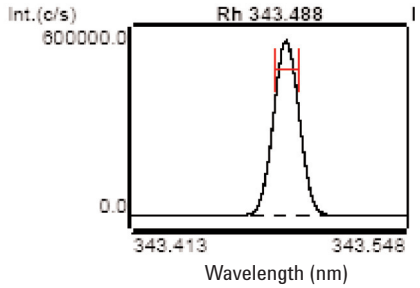

Figure 5. Signal trace for the rhodium acetate aqueous solution.

Table 4. Measured Rhodium Concentrations in Each of the Samples

Sample Type	Sample ID	Expected Rh Concentration (mg/L)	Measured Rh Concentration (mg/L)	Precision (% RSD)
Oil samples	Q22321 2007-08-04	Very low	ND	N/A
	Q22321 2007-08-05	Very low	ND	N/A
	Q22321 2007-08-06	Very low	ND	N/A
	Q22321 2007-08-07	Very low	3.08	4.15
	Q22321 2007-08-08	Very low	ND	N/A
	Q22321 2007-08-09	Very low	ND	N/A
	Q22321 2007-08-10	Very low	ND	N/A
	Q21011 2007-08-06	Est. 100–200 mg/L	216.20	0.197
Aqueous samples	Rhodium acetate	Est. 60 mg/L	55.26	0.842

Conclusion

Using the Agilent 710 ICP-OES axially viewing ICP-OES, it was possible to determine the rhodium content in both the oil catalyst and the aqueous samples without interferences. The oil catalyst samples were prepared using a microwave digestion system with temperature and pressure control. The digests obtained were clear, confirming complete digestion. The method demonstrated good results, although the Rh concentration in most samples was not detectable.

The results demonstrate this method can be readily applied to the routine determination of rhodium in oil catalyst and aqueous samples.

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Printed in the USA
November 1, 2010
SI-1342

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Using the Agilent Cary 670 FTIR Spectrometer to observe rotational and isotopic bands in CO through high resolution FTIR Spectroscopy

Application Note

Author

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Agilent Technologies, Inc.

Introduction

The advent of Fourier transform infrared (FTIR) spectroscopy has permitted the observation of spectra at higher spectral resolutions previously unattainable with traditional scanning spectrometers. Resolution of rotational-vibrational bands within gas-phase samples using modern FTIR spectrometers is now commonplace.

The observation of rotational-vibrational bands at high resolution requires the use of small apertures to limit the divergence of the IR radiation through the interferometer, leading to a reduced throughput in power at the sample focus. This translates to a degradation of signal-to-noise (S/N) performance, thereby necessitating longer sampling times to resolve weak bands.

To overcome this, an instrument designed specifically for high optical performance is required. The Agilent Cary 670 FTIR spectrometer incorporates several design enhancements, including large collection optics and a retro-reflected source, which result in increased infrared energy at the sample focus. The results discussed in this application note demonstrate that weakly-abundant isotopic peaks can be resolved in a relatively short time using the Agilent Cary 670 FTIR spectrometer.

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Instrumentation

An Agilent Cary 670 FTIR spectrometer was used for all measurements with the settings listed in Table 1. The instrument was purged with nitrogen prior to spectral collection.

Table 1. Instrument parameters in Resolutions Pro software used in all collections

	Instrument Parameters	Settings
Detector	MCT narrow band	
	Speed (kHz)	25.0
	Filter (kHz)	17.4
Source	MIR	Normal
Collection	Sample scans	4
	Background scans	4
	Resolution (cm^{-1})	0.09
	Aperture (cm^{-1})	0.10
	Symmetry	asym
Computation	Apodisation type	boxcar
	Zero filling factor	16
	UDR	2

Materials and reagents

A carbon monoxide cell (KBr windows, 10 cm path length) was obtained from Pike Technologies, Madison WI, USA. The cell contains carbon monoxide at a reduced pressure of 4 Torr to ensure that the resolution of the obtained spectra are instrument-limited and not physically limited by the sample. The cell was mounted on the slide-on sample holder and placed in the left hand side position of the sample compartment to account for the extra length of the gas cell.

Results and discussion

Isotopomers of carbon monoxide

Sample spectra were recorded (Figure 1) and the region between 2250–2000 cm^{-1} analyzed to reveal the rotational-vibrational transitions of the C≡O stretching band centered at 2143.3 cm^{-1}. These transitions are grouped into two branches reflecting a simultaneous rotational transition: the lower energy P-branch where the change in rotational state (ΔJ) = -1, and the higher energy R-branch where ΔJ=1.

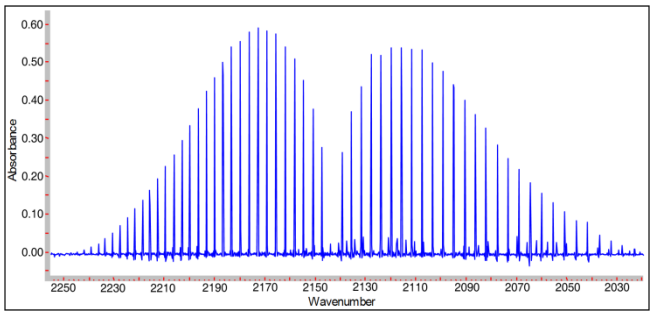

Figure 1. Sample spectrum displayed between 2250–2000 cm^{-1} revealing the P (right) and R (left) branching of the C≡O stretching band centered at 2143.3 cm^{-1}

These bands are due to transitions of the $C^{12}O^{16}$ isotopomer. Atmospheric carbon monoxide is known to contain the isotopomers $C^{13}O^{16}$ and $C^{12}O^{18}$ at abundances of 1.1% and 0.2% respectively. The frequency of the fundamental transition, $v_0 \rightarrow v_1$, ΔJ=0 of these isotopomers is given by the ratio of the bond strength for the C≡O bond, k, and the reduced mass, μ, of the molecule (Equation 1 below).

$$\nu = \frac{1}{2\pi}\sqrt{\frac{k}{\mu}} \qquad (1)$$

The reduced mass of the various isotopomers of this molecule are given by:

$$\mu = \frac{m_1 m_2}{m_1 m_2} \qquad (2)$$

where m_1 and m_2 are the atomic masses of each atom. The fundamental frequency of the $v_0 \rightarrow v_1$ transition in the $C^{12}O^{16}$ molecule[1] is 2143.3 cm^{-1}, while those of the other isotopomers can be estimated by considering the ratio of their reduced masses with $C^{12}O^{16}$ (as given by Equation 3 below).

$$\frac{\nu_a}{\nu_b} = \sqrt{\frac{u_b}{u_a}} \qquad (3)$$

Using the above equations, the fundamental frequency of the $v_0 \rightarrow v_1$ transition can be calculated for each isotopomer.

Table 2. Calculated fundamental frequency for the isotopomers of C≡O

	Reduced Mass	⇔ (cm^{-1})
$C^{12}O^{16}$	6.8571	2143.3
$C^{13}O^{16}$	7.1724	2095.7
$C^{12}O^{18}$	7.2000	2092.1

By focusing on the region between 2170 and 2095 cm^{-1} (as highlighted in Figure 2), the R branch of $C^{13}O^{16}$ is clearly visible extending right to left from the forbidden ΔJ=0 transition centered at 2095.7 cm^{-1}.

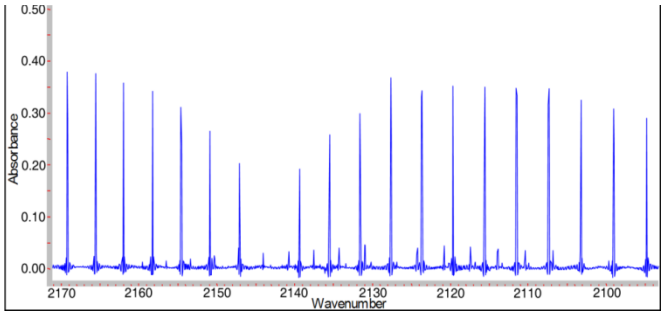

Figure 2. Spectrum of C≡O between 2170–2095 cm^{-1} highlighting the fine detail of the R branches of $C^{13}O^{16}$

Figure 3 exhibits some visually discernable differences between the transitions of $C^{12}O^{18}$ and the other isotopomers of carbon monoxide. The most intense of these is $C^{13}O^{16}$ (at 2114.0 cm^{-1}) followed by $C^{12}O^{18}$ (at 2113.4 cm^{-1}) reflecting the natural abundances of these isotopomers within atmospheric carbon monoxide.

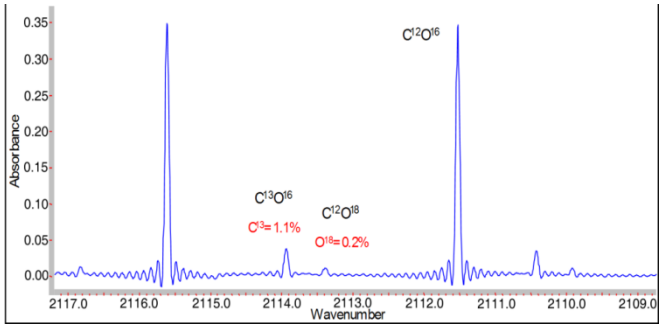

Figure 3. Expanded view of the same spectrum of carbon monoxide between 2117 and 2109 cm^{-1}, illustrating transitions of the $C^{12}O^{16}$, $C^{13}O^{16}$ and $C^{12}O^{18}$ isotopomers

The observation of the peaks from the weakly abundant species requires good S/N performance at high resolution. Typically, resolution of these peaks would require a large number of co-additions in order to average out noise within the spectrum.

Due to its superior optical design, the Agilent Cary 670 FTIR delivers excellent signal-to-noise performance even at very high spectral resolutions and facilitates fast analysis of weak bands with a high degree of precision and accuracy.

Spectra recorded in this study were collected with just 4 scans which correlate to a 40 second collection time. The high throughput and performance are attributable to a number of design features including:

- 57 mm optics that ensure a larger collection area
- A novel source with retro-reflector to ensure the collection of a greater portion of the source's output
- An AC-powered source to prevent the wandering of the hot spot within the source and to ensure that perfect alignment of the source is maintained.

All of these factors combine to deliver more power to the sample focus translating to better S/N performance. In addition, the frictionless air-bearing interferometer and dynamic alignment system of the Agilent Cary 670 FTIR spectrometer deliver superb velocity control of the moving mirror. This leads to superior wavelength precision and accuracy and permits faster collection times, especially at higher spectral resolutions.

Wavelength resolution, accuracy and precision

The wavelength resolution, accuracy and precision values reported in Table 3 were determined. From three replicate collections of carbon monoxide using the collection parameters outlined in Table 1. The wavelength resolution, accuracy and precision of the spectrometer was calculated based on the R-5, R-6 and R-7 transitions of $C^{12}O^{16}$ in the C≡O spectra.

Table 3. Wavenumber accuracy, precision and spectral resolution results for the Agilent Cary 670 FTIR spectrometer using the R-5, R-6 and R-7 transitions of $C^{12}O^{16}$

	wavenumber reference [2] (cm^{-1})	wavenumber actual (cm^{-1})	precision (st dev) (cm^{-1})	resolution (cm^{-1})
R-5	2165.6010	2165.6010	0.0016	0.0572
R-6	2169.1979	2169.1986	0.0010	0.0598
R-7	2172.7588	2172.7586	0.0008	0.0552
R-5	2165.6010	2165.6010	0.0016	0.0572

The wavelength of maximum absorption for each of the R-5, R-6 and R-7 transitions was calculated using the mean of the three replicate scans of carbon monoxide. These were compared with reference values from the HITRAN database[2] to assess the wavelength accuracy of the spectrometer. All three transitions were found to lie within 0.001 cm^{-1} of reference values, highlighting the superior performance of the Agilent Cary 670 FTIR spectrometer.

Also calculated was the wavelength precision of the spectrometer, defined as the standard deviation of the peak wavelength from the mean of the three replicate scans. This was determined for each of the R-5, R-6 and R-7 transitions. In each case the precision was found to be better than 0.001 cm^{-1} again demonstrating the stability and performance of the Agilent Cary 670 FTIR spectrometer.

The instrument-limited spectral resolution was determined by calculating the full width at half maximum (FWHM) for each of the three transitions as illustrated in Figure 4. The resolution of the absorbance bands was determined to be better than 0.06 cm^{-1} for each transition, hence enabling well-resolved observation of rotational structure within gaseous samples.

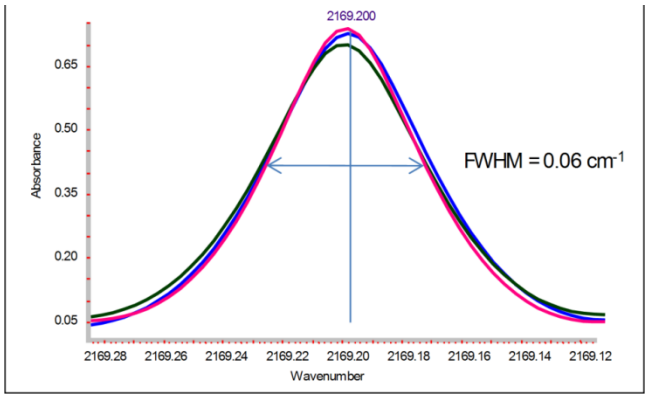

Figure 4. Spectra of three replicates of the R-6 transition of $C^{12}O^{16}$. Such spectra were used to calculate the wavelength precision, accuracy and resolution of the Agilent Cary 670 FTIR spectrometer

Conclusion

The rotational structure of the C≡O stretching band centered at 2143.3 cm^{-1} was observed with <0.06 cm^{-1} spectral resolution for the R-5, R-6 and R-7 transitions. Isotopic splitting of the stretching band was also observed, with the isotopomers $C^{13}O^{16}$ and $C^{12}O^{18}$ being resolved with just a 40 second collect reflecting the high optical performance of the Agilent Cary 670 FTIR spectrometer. The spectrometer's wavelength accuracy was determined to be better than 0.001 cm^{-1} against reference values, while the precision was found to be better than 0.001 cm^{-1}. These performance attributes are the result of a number of design features which have been incorporated into the Agilent Cary 670 FTIR spectrometer and demonstrate the suitability of the instrument for high resolution gas phase applications.

References

1. Rao, K.N. and Mathews, C.W., editors, Molecular Spectroscopy: Modern Research, Academic Press, New York, 1972.

2. Rothman L.S. et al. 2004. The HITRAN 2004 molecular spectroscopic database. *J. Quantitative Spectroscopy & Radiative Transfer*, 96(2): 241-250.

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Published March, 2011
Publication Number si-01378

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Agilent Technologies

Analysis of Polylactide and Poly(lactide-*co*-glycolide) by GPC with Viscometry

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Polylactide (PLA) and copolymers with glycolic acid (PLGA) are polyesters derived from corn starch and sugar cane. Synthesized by the tin-mediated ring-opening polymerization of the dimeric acid formed from bacterial fermentation, these materials can be manufactured to reasonably high molecular weights. PLA and PLGA have received considerable attention due to their non-toxic, biocompatible and biodegradable properties. The polymers are used in a number of biomedical applications, including sutures, stents and as dialysis media. Fibers of PLA are also employed in the manufacture of upholstery and diapers.

The accurate molecular weight distributions of a sample of polylactide and of poly(lactide-*co*-glycolide) were compared by gel permeation chromatography with viscometry. Light scattering could not be used with these samples as one was a copolymer and therefore not of uniform chemistry across the molecular weight distribution. This could lead to errors in the light scattering calculations. However, the Universal Calibration technique employing a viscometer can be used with copolymers, to provide molecular weights that are independent of the standards used in the column calibration. To investigate the molecular structure of the materials they were analyzed on an integrated GPC system.

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Instrumentation

The copolymer was assessed on an Agilent PL-GPC 50 Plus with differential refractive index detector, Agilent PL-BV 400RT viscometer and Agilent PLgel 5 µm MIXED-D columns, which provide high resolution of resin and condensation polymers.

Columns:	2 x PLgel 5 µm MIXED-D, 300 x 7.5 mm (part number PL1110-6504)

Materials and Reagents

Samples:	Polylactide and poly(lactide-*co*-glycolide)
Eluent:	Tetrahydrofuran

Conditions

Flow Rate:	1 mL/min
Temperature:	40 °C

Results and Discussion

Figure 1 shows chromatograms for the polylactide. Figure 2 reveals that the samples were clearly quite different in molecular weight, the copolymer having a lower molecular weight than the homopolymer. The Mark-Houwink plot indicated that there might also be some structural differences between the homopolymer and the copolymer, with the copolymer showing a deviation to lower intrinsic viscosities as a function of molecular weight, indicating a smaller and more compact structure in solution (Figure 3).

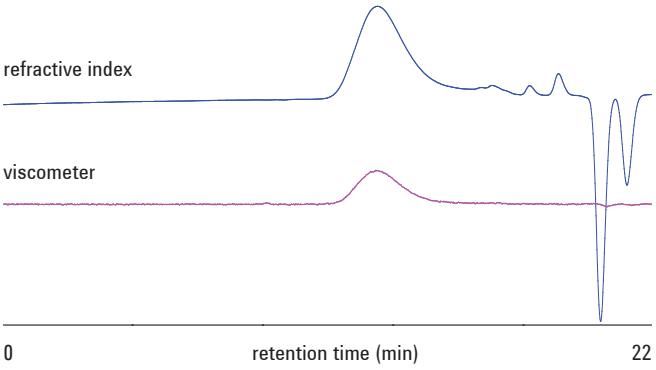

Figure 1. Chromatograms for the polylactide sample

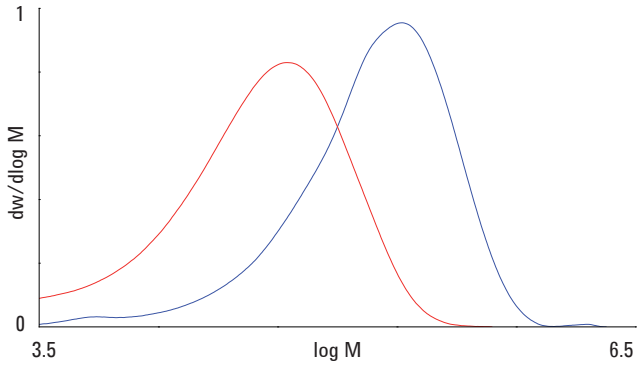

Figure 2. Overlaid molecular weight distributions for polylactide (blue) and poly(lactide-co-glycolide) (red) samples

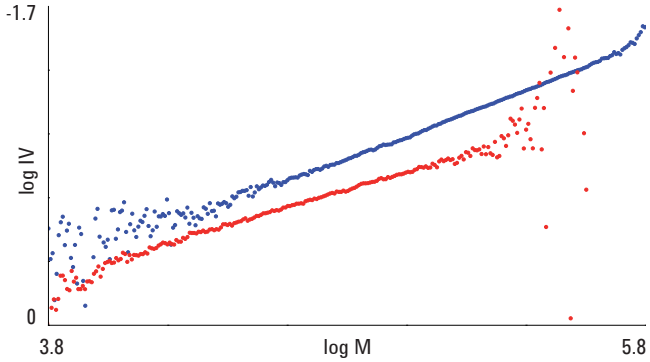

Figure 3. Overlaid Mark-Houwink plots for polylactide (blue) and poly(lactide-co-glycolide) (red) samples

Conclusion

The PL-GPC 50 Plus is a high resolution, cost effective integrated GPC system designed for operation from ambient to 50 °C. The standard system comprises precision solvent delivery, sample injection, high performance differential refractive index detection and a column oven, with fully integrated software control. When coupled with PLgel 5um MIXED-D columns and a PL-BV 400RT viscometry detector, the PL-GPC 50 Plus provides accurate molecular weight determination for all polymer types based on the Universal Calibration principle, such as polylactide and its copolymers.

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Published in UK, September 2, 2010
SI-01520

Agilent Technologies

Analysis of Surfactants Using the Agilent 500 Ion Trap LC/MS

Application Note

Authors

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Abstract

Four surfactants were identified in rolling oil used to roll out large sheets of aluminum using the Agilent 500 Ion Trap LC/MS. The full scan sensitivity of the 500 Ion Trap makes it ideal for identification of unknown components in a mixture at an unknown concentration.

Introduction

In this analysis, surfactants were identified in rolling oil used to roll out large sheets of aluminum. It was suspected that surfactant contaminants were causing the oil to foam up over time, and in order to identify the surfactants in the oil, samples were analyzed by LC/MS using the Agilent 500 Ion Trap LC/MS. In addition to using the 500 Ion Trap for initial confirmation of oil contaminants, continuous monitoring of the oil for presence of surfactants maintains optimal operation and efficiency of these machines over time. The full scan sensitivity of the 500 Ion Trap makes it ideal for this type of analysis, where the analysis must identify unknown components in a mixture at an unknown concentration. In addition, the MS/MS capabilities of the instrument make it a powerful tool for structural elucidation and identification of unknown compounds.

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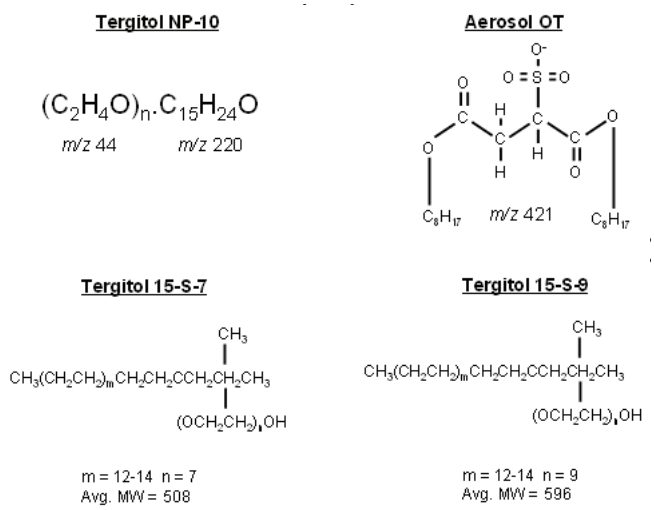

Figure 1. Chemical structures of Tergitol NP-10, Tergitol 15-S-7, Tergitol 15-S-9, and Aerosol OT surfactants.

Instrumentation

The following instruments were used in this study:

- Agilent 500 Ion Trap LC/MS with ESI source
- Agilent 212-LC Binary Solvent Delivery Modules
- Agilent ProStar 430 AutoSampler

Materials and Reagents

A clean sample of Hygold 100 petroleum distillate rolling oil was supplied for analysis, along with neat standards of Tergitol NP-10, Tergitol 15-S-7, Tergitol 15-S-9, and Aerosol OT.

Sample Preparation

ESI positive compounds

- **Neat rolling oil** (blank) was Hygold 100 petroleum distillate, Ergon Refining MSDS, CAS #64742-52-5. The oil was agitated in water (acidified) to release any water soluble components into the aqueous phase. The aqueous layers were injected and analyzed by LC/MS.
- **Neat surfactant materials** included Tergitol NP-10 in Hygold 100, Dow MSDS 1985; Tergitol 15-S-7 surfactant, Union Carbide MSDS 1917; Tergitol 15-S-9 surfactant, Union Carbide MSDS 1912. The neat surfactant materials dissolved in rolling oil were added to acidified aqueous (0.2% acetic acid) and agitated to release surfactants into the aqueous phase. The aqueous layer was analyzed by LC/MS.

ESI negative compounds

- **Neat rolling oil** (blank) was Hygold 100 petroleum distillate, Ergon Refining MSDS, CAS #64742-52-5 and was agitated in water (alkaline) to release surfactants into the aqueous phase. The aqueous layer was injected and analyzed by LC/MS.
- **Surfactant material** was Aerosol OT surfactant in Hygold 100, Alcoa MSDS #120778. The surfactant dissolved in rolling oil was added to alkaline aqueous (0.2% ammonium hydroxide) and agitated to release surfactants into the aqueous phase. The aqueous layer was injected and analyzed by LC/MS.

HPLC Conditions

Column:	Inertsil ODS-3,100 × 4.0 mm, 3 µm (Pursuit C18 Recommended Agilent Alternative p/n A3001100X020)			
Solvent A:	0.2% acetic acid in water (ESI+) 0.2% ammonium hydroxide in water (ESI-)			
Solvent B:	Methanol			
LC program:	Time (min:sec)	%A	%B	Flow (µL/min)
	00:00	95	5	200*
	01:00	95	5	200*
	11:00	0	100	200
	31:00	0	100	200
	31:01	95	5	200*
	36:00	95	5	200*
	* diverted			
Injection volume:	10 µL			

MS parameters

Ionization mode:	ESI (positive) and ESI (negative)
API drying gas:	15 psi at 300 °C
API nebulizing gas:	20 psi
Needle:	4500 V –5500 V
Shield:	600 V –600 V

Table 1. MS Segment Parameters

Analyte	Scan range	Retention time (min)	Capillary voltage (V)	RF load
Tergitol NP-10	200–700	13.48	100	85
Tergitol 15-S-7	329 > 280–320	11.12	75	82
Tergitol 15-S-9	315 > 280–320	11.68	75	82
Aerosol OT	313 > 280–320	12.30	75	82

Results and Discussion

The surfactants analyzed in this method were spiked into blank Hygold rolling oil. Figure 2 shows a chromatogram that compares a full scan blank injection with the subsequent spiked injections containing the surfactants of interest. The blank chromatogram is the extracted ion chromatogram for all of the ions of interest for all the surfactants tested. As the chromatogram shows, no background signal for the surfactants was observed in the blank rolling oil.

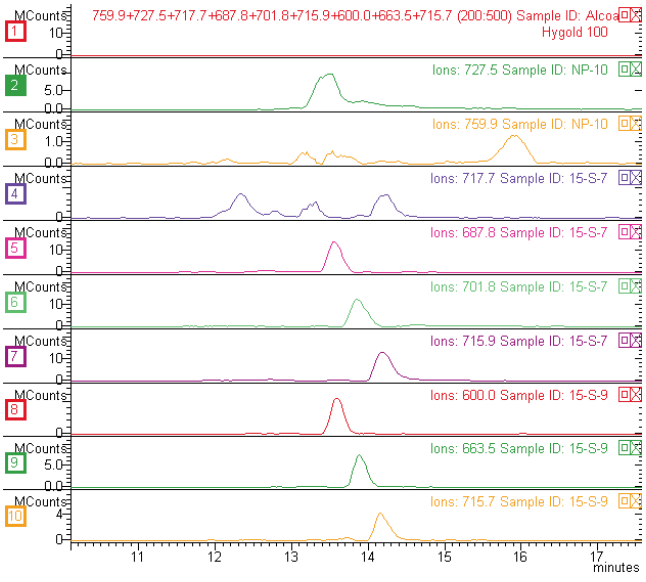

Figure 2. Chromatogram of a blank injection (top) followed by the same blank spiked with Tergitol NP-10, Tergitol 15-S-7, and Tergitol 15-S-9.

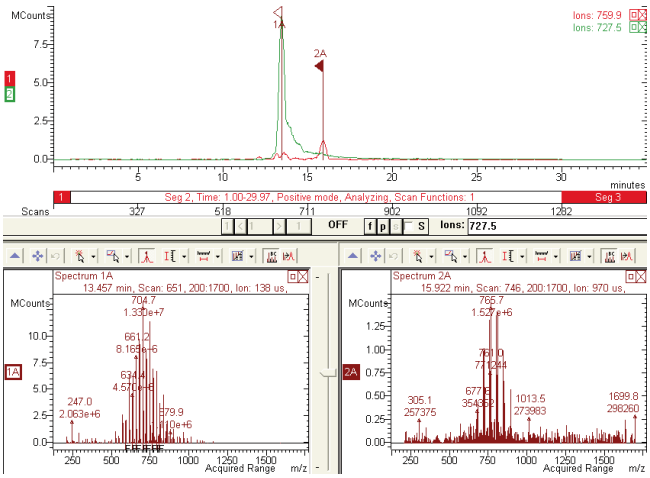

Figure 3. Extracted ion chromatogram and mass spectra for Tergitol NP-10 surfactant in Hygold 100 rolling oil, full scan analysis.

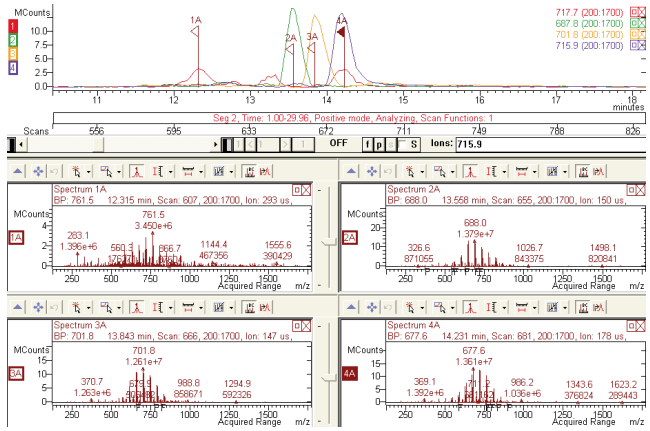

Figure 4. Extracted ion chromatogram and mass spectra for Tergitol 15-S-7 surfactant full scan analysis.

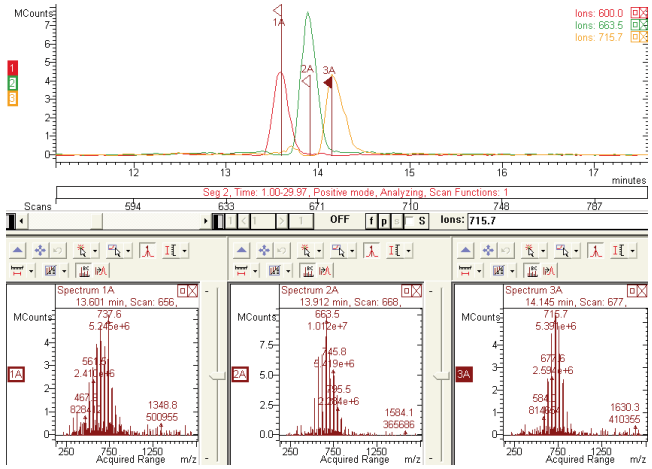

Figure 5. Extracted ion chromatogram mass spectra for Tergitol 15-S-9 surfactant full scan analysis.

The surfactant samples have interesting mass spectra with repeated losses of 44 mass units, representing a loss of C_2H_4O groups from the polymeric chain. Figure 6 shows the mass spectrum of Tergitol NP-10. The fragmentation of NP-10 is observed and a characteristic cluster is seen.

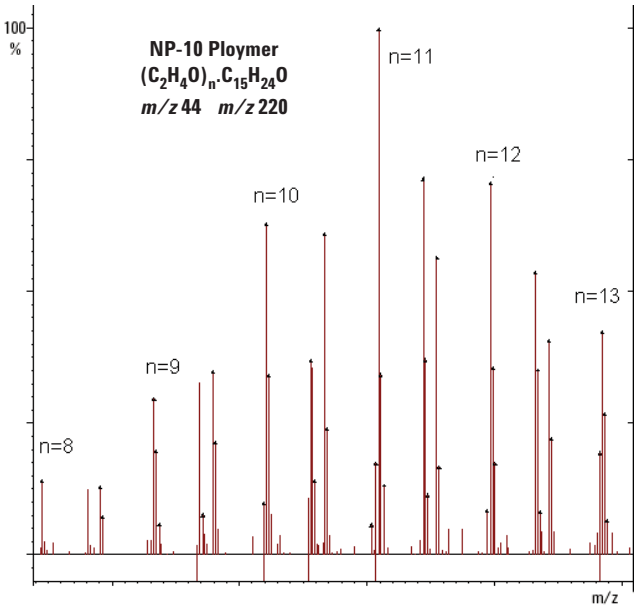

Figure 6. Mass spectrum of Tergitol NP-10.

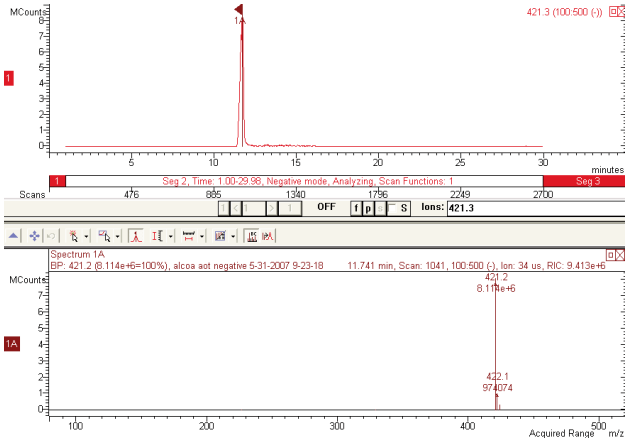

Figure 7. Extracted ion chromatogram of full scan analysis of Aerosol AOT surfactant.

Conclusion

Four surfactants were extracted from rolling oil for machines that roll out large aluminum sheets. The components of the oil were unknown, which made the full scan sensitivity of the Agilent 500 Ion Trap LC/MS ideal for this analysis. By extracting the ions of interest, clear chromatographic peaks are observed and the unique mass spectra are identified.

For More Information

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Printed in the USA
February 24, 2011
SI-1558

Agilent Technologies

SEC Analysis of Sodium Polystyrene Sulfonate

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Sodium polystyrene sulfonate is an ion-exchange resin used to treat high levels of blood potassium by substituting potassium in the diet and body for sodium in the resin. The potassium is then excreted and levels in the body are reduced. A sample of sodium polystyrene sulfonate was analyzed by aqueous SEC using Agilent PL aquagel-OH columns. These columns combine high pore volume and high column efficiency (>35,000 plates/meter) for maximum resolution. As the polymers are both ionic and relatively hydrophobic, eluent conditions are chosen to minimize sample to column interaction, which would otherwise result in late elution times.

Agilent Technologies

Conditions

Samples: Sodium polystyrene sulfonates
Columns: 2 x PL aquagel-OH 40 8 µm, 300 x 7.5 mm (p/n PL1149-6840)
Eluent: 80 % 0.3 M $NaNO_3$ + 0.01 M NaH_2PO_4 at pH 9 + 20 % Methanol
Flow Rate: 1.0 mL/min
Detection: RI

Conclusion

SEC using PL aquagel-OH columns successfully analyzed samples of sodium polystyrene sulfonate. Aqueous SEC not only provides molecular weight data but also provides information on the polydispersity and the shape of the molecular weight distribution. The excellent chemical and mechanical stability of these columns offer high performance with good repeatability and column lifetime.

This product is not cleared or approved by the United States FDA or other international regulatory agency for use in clinical diagnostic procedures. No use of this product as a diagnostic medical device is implied or should be inferred.

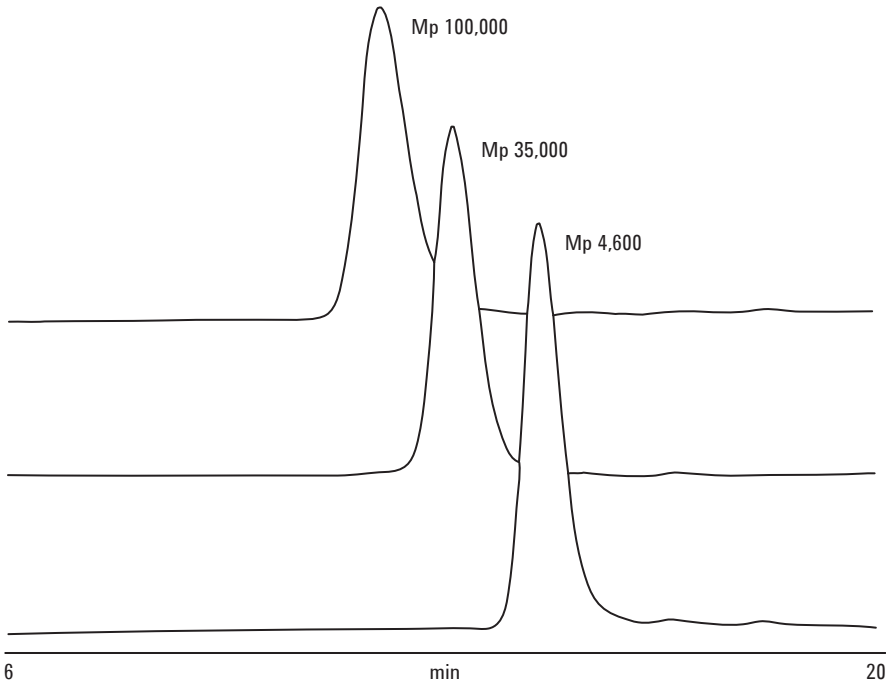

Figure 1. Raw data chromatograms of three samples of sodium polystyrene sulfonate

www.agilent.com/chem

Published in UK, September 2, 2010
SI-01574

Agilent Technologies

SEC Analysis of Poly 2-Vinyl Pyridine

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Poly 2-vinyl pyridines are non-toxic, water soluble, cationic polymers with the ability to chelate heavy metals. This makes them valuable in removing metals found in poisoning and contaminated environments. They also have useful electrical conductivity properties when reacted with halogens such as iodine. This property is made use of in heart pacemakers. A sample of poly 2-vinyl pyridine was analyzed by aqueous SEC using Agilent PL aquagel-OH columns. These columns combine high pore volume and high column efficiency (>35,000 plates/meter) for maximum resolution. The pH of the salt buffer eluent was reduced to 3 in order to minimize ionic interaction between the sample and column. In order to dissolve the sample, the solvent pH had to be reduced further to pH 1.5 but the polymer remained in solution at pH 3 for analysis.

Agilent Technologies

Conditions

Sample: Poly 2-vinyl pyridine
Columns: 2 x PL aquagel-OH 50 8 µm, 300 x 7.5 mm (p/n PL1149-6850)
Eluent: 0.8 M $NaNO_3$ + 0.01 M NaH_2PO_4 at pH 3
Flow Rate: 1.0 mL/min
Detection: RI

Conclusion

SEC using PL aquagel-OH columns successfully analyzed a sample of poly 2-vinyl pyridine. Aqueous SEC not only provides molecular weight data but also provides information on the polydispersity and the shape of the molecular weight distribution. The excellent chemical and mechanical stability of these columns offer high performance with good repeatability and column lifetime.

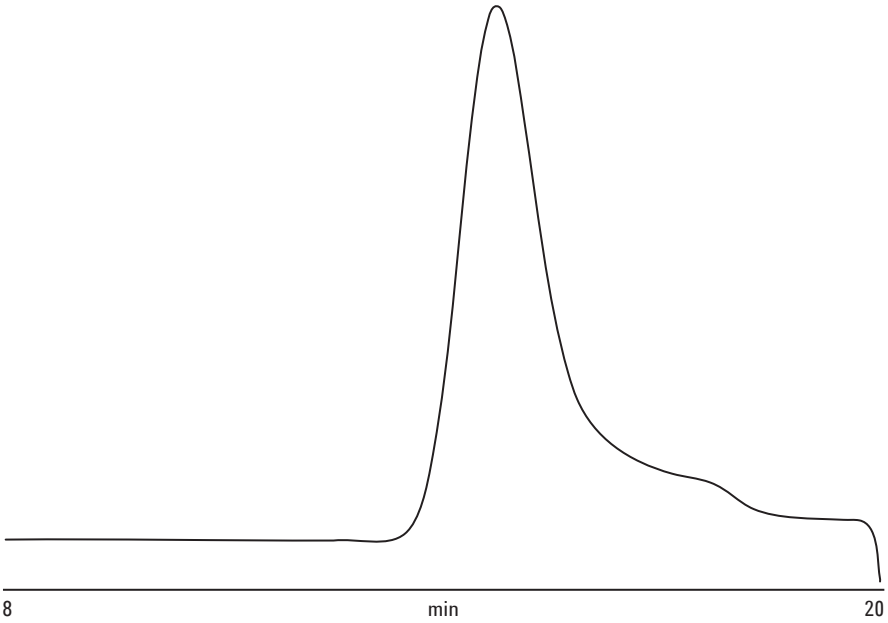

Figure 1. Raw data chromatogram of poly 2-vinyl pyridine

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Published in UK, September 2, 2010
SI-01575

Agilent Technologies

SEC Analysis of Sodium Polyacrylate

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Sodium polyacrylate ($-CH_2-CH(COONa)_n$) is an acrylic acid polymer sodium salt. The value of this polymer lies in its ability to absorb up to 1,000 times its weight in distilled water, giving it a high gel capacity. Such polymers are referred to as 'super absorbents' or 'water crystals'. Unsurprisingly, sodium polyacrylate finds applications that make use of its superabsorbency, such as diapers and incontinence pads, and in agriculture for spray drift control, seed germination, soil conditioning, hydroponics and as a water storage agent for soils, where it soaks up soil moisture and releases it when the soil dries out. It is also used by many industries as a thickener and stabilizer. SEC using Agilent PL aquagel-OH columns is an excellent system for characterizing this polymer. The columns combine high pore volume and high column efficiency (>35,000 plates/meter) for maximum resolution.

Agilent Technologies

Conditions

Samples: Sodium polyacrylate
Columns: 2 x PL aquagel-OH 40 8 µm, 300 x 7.5 mm (p/n PL1149-6840)
Eluent: 0.2 M $NaNO_3$ + 0.01 M NaH_2PO_4 at pH 7
Flow Rate: 1.0 mL/min
Detection: RI

Results and Discussion

The chromatogram shows a relatively low molecular weight sample with Mw approximately 13,000. The sample was readily soluble and the salt/buffer eluent was selected to minimize ionic effects.

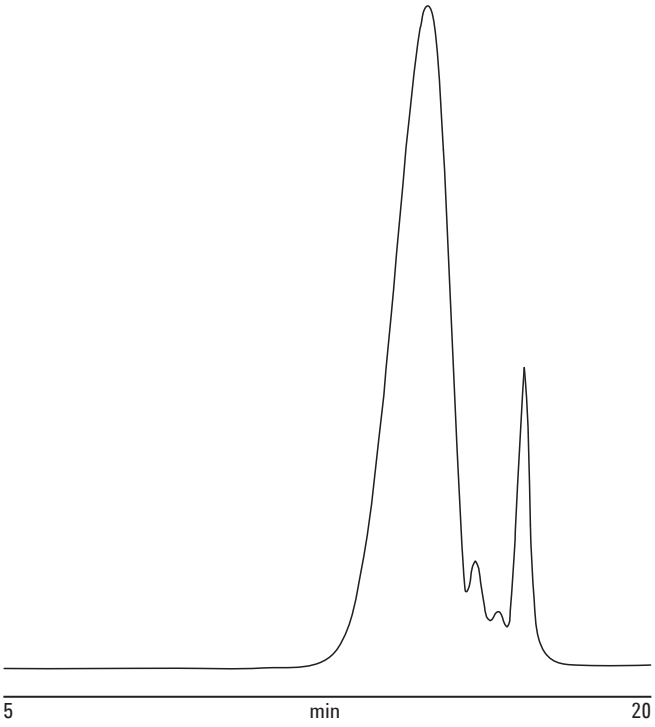


Figure 1. Raw data chromatogram of sodium polyacrylate

Conclusion

SEC using PL aquagel-OH columns successfully analyzed a sample of sodium polyacrylate. Aqueous SEC with PL aquagel-OH columns provides information not only on the molecular weight of the polymer but also on the polydispersity and the shape of the molecular weight distribution. The excellent chemical and mechanical stability of these columns offer high performance with good repeatability and column lifetime.

www.agilent.com/chem

Published in UK, September 2, 2010
SI-01579

Agilent Technologies

SEC for the Analysis of Non-ionic Surfactants

Application Note

Authors

Greg Saunders, Ben MacCreath
Agilent Technologies, Inc.

Introduction

Non-ionic surfactants are used in a variety of applications ranging from cleaning and personal care products to industrial processing. The hydrophobic and the hydrophilic portions of the surfactant molecule can be incrementally varied and manipulated to any molecular weight, in order to adjust its properties to suit a particular application. The determination of the surfactant molecular weight distribution is therefore extremely important in terms of performance as well as batch to batch reproducibility. This type of sample is normally relatively low in terms of molecular weight. Agilent PL aquagel-OH 30 8 µm columns are ideal for these analyses, because they combine low exclusion limit, high pore volume and high column efficiency (>35,000 plates/meter) for maximum resolution. Column calibration was achieved using polyethylene oxide Agilent EasiVial standards (Figure 1). EasiVials provide a rapid and convenient means of constructing an aqueous SEC column calibration curve over a wide molecular weight range (typically 100 to 1,200,000 g/mol). Each vial contains a mixture of four individual, highly characterized, narrow dispersity standards. The amount of each individual standard is carefully controlled during manufacture, allowing their use in SEC-viscometry, which requires accurate concentrations.

Agilent Technologies

Conditions

Non-ionic surfactants contain hydrophobic species so the addition of 30% of a weak organic solvent (methanol) is required to inhibit hydrophobic interactions between the sample and the column packing. The PL aquagel-OH packing material allows such eluent modifications while retaining the high column efficiency.

Samples: Surfactants; EasiVial PEO standards
Columns: 2 x PL aquagel-OH 30 8 µm, 300 x 7.5 mm (p/n PL1120-6830)
Eluent: Water + 0.2 M $NaNO_3$ + 0.01 M NaH_2PO_4 at pH 7 + 30 % methanol
Flow Rate: 1.0 mL/min
Detection: RI

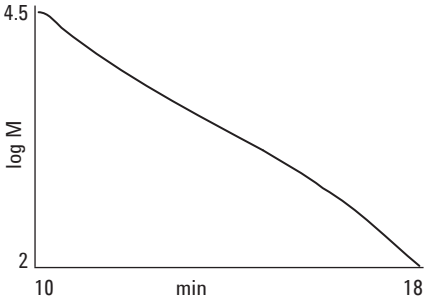


Figure 1. EasiVial calibration of PL aquagel-OH columns

Results and Discussion

A typical raw data chromatogram for the sample is illustrated in Figure 2. The sample displays a low molecular weight shoulder.

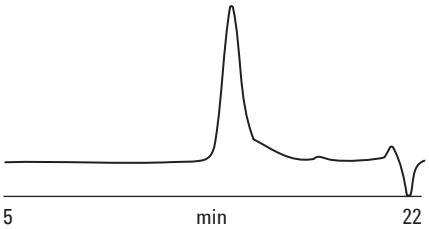


Figure 2. Chromatogram of a non-ionic surfactant

Conclusion

The presence of significant hydrophobicity in a surfactant is no barrier to its resolution by SEC with PL aquagel-OH columns. The column's ability to handle eluents containing up to 50% methanol means that such compounds can be resolved without interactions.

www.agilent.com/chem

Published in UK, September 2, 2010
SI-01642

Agilent Technologies

Analysis of Aliphatic Alcohols by Ligand-Exchange Chromatography

Application Note

Chemical

Author

Stephen Ball

Agilent Technologies, Inc.

Introduction

This application note demonstrates how an Agilent Hi-Plex H column can be used to separate aliphatic alcohols.

Agilent Technologies

Materials and Reagents

Column	Agilent Hi-Plex H (8% crosslinked), 7.7 × 300 mm, 8 µm (p/n PL1170-6830)
Mobile phase	100% DI H_2O
Flow rate	0.6 mL/min
Temperature	40 °C
Detector	RI

Conclusion

Using only pure HPLC-grade water as eluent, the Agilent Hi-Plex H column is capable of separating a range of aliphatic alcohols. In addition to those shown in Figure 1, it may also be possible to separate a much wider range of this type of compound. Molecular weight and degree of branching are critical factors in determining the amount of retention on a Hi-Plex H column.

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

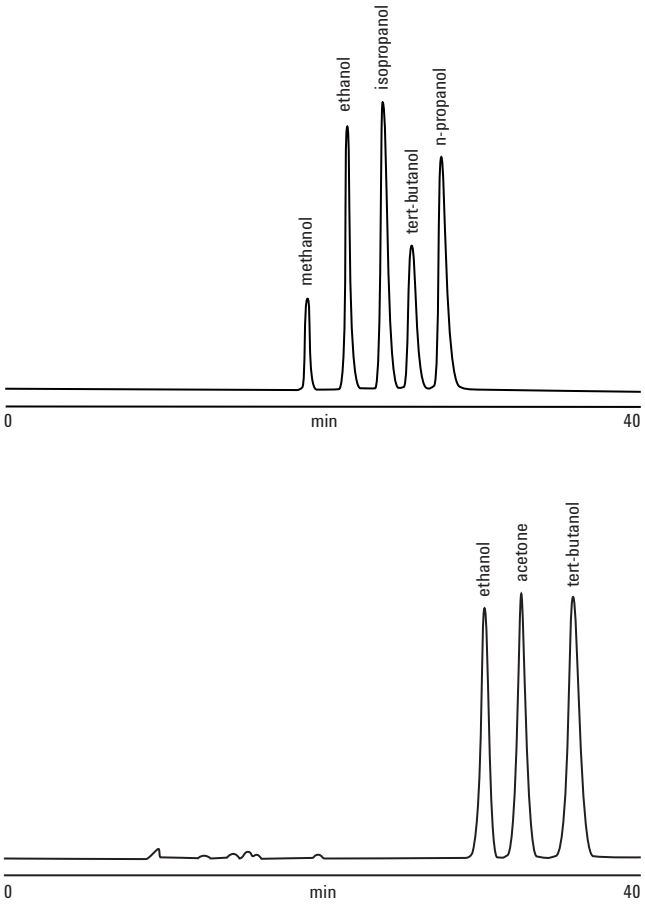


Figure 1. Separation of different aliphatic compounds on an Agilent Hi-Plex H column.

www.agilent.com/chem

Published in USA, June 30, 2011
SI-02030

Agilent Technologies

Separation of Permanent Gases on a Liquid Phase

Separation of 5 permanent gases on a WCOT column with a liquid phase with high retention

Application Note

Authors

Rick Hamerlinck and Norbert Reuter
Agilent Technologies, Inc.

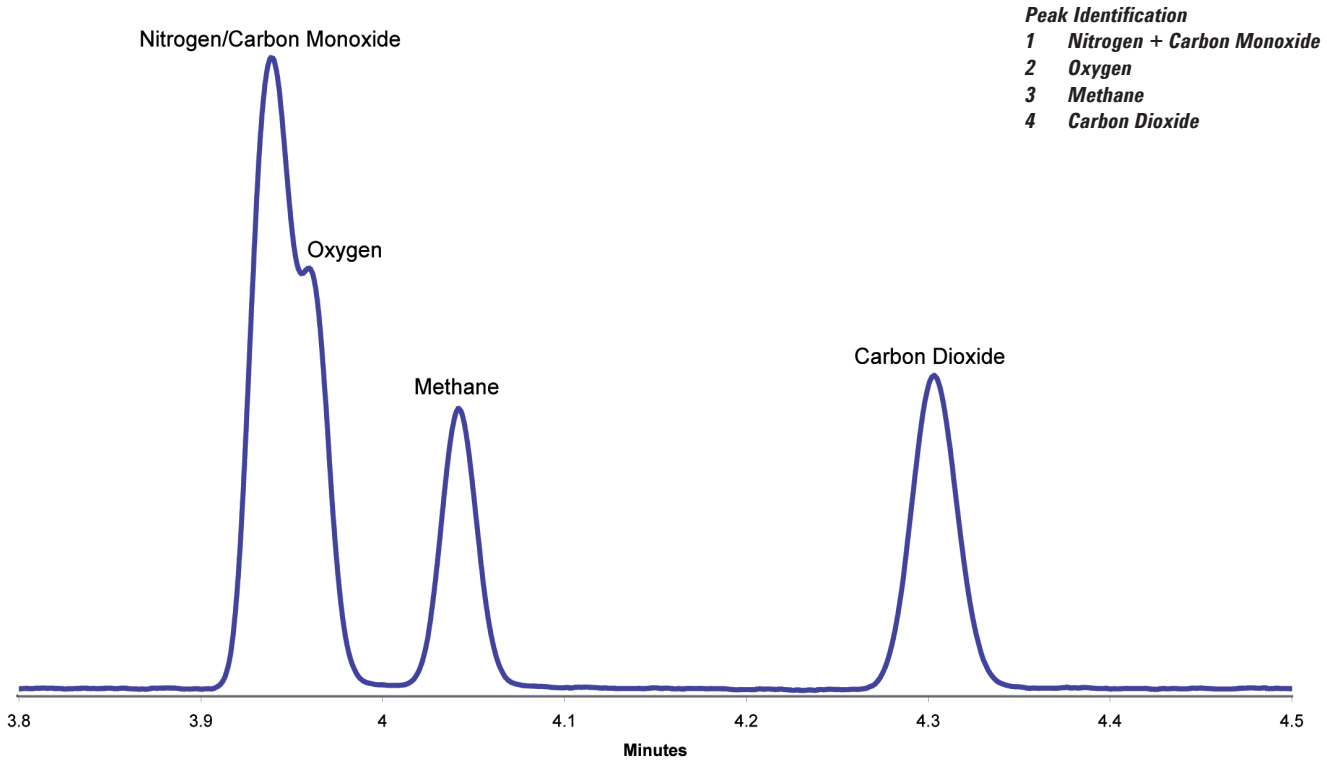
Introduction

Normally permanent gases are separated by PLOT (porous layer open tubular) columns with their high retentive phases. With WCOT (wall coated open tubular) columns sub-ambient temperatures are normally necessary. Thick films, like the 8 µm film thickness of the Agilent J&W Select CP-Sil 5CB for Formaldehyde, allow the use of high-inert liquid phases for the (pre-) separation of the standard permanent gases from carbon dioxide for possible column switching at normal ambient temperatures.

Agilent Technologies

Materials and Methods

Technique:	GC-Capillary Medium Bore	Carrier Gas:	Helium at 25 psi (170 kPa)	Detector:	Thermal Conductivity Detector at 220 °C (Filament Temp. 280 °C)
Instrument:	GC Gas Chromatograph	Temp Program:	35 °C isothermal	Sample:	All Gases 1% in Helium
Column:	CP-Sil 5 CB for Formaldehyde, 0.32 mm x 60 m, df=8 µm (part number CP7475)	Injector:	Split/Splitless-Injector (1177) at 250 °C		
		Inj Volume:	500 µL (split ratio 1:20)		



Peak Identification

1. ***Nitrogen + Carbon Monoxide***
2. ***Oxygen***
3. ***Methane***
4. ***Carbon Dioxide***

Analysis of permanent gases at 35°C

www.agilent.com/chem

Published in UK, October 08, 2010
SI-02166

Agilent Technologies

Material analysis by infrared mapping: A case study using a multi-layer paint sample

Application Note

Author

Dr. Jonah Kirkwood, Dr. John Wilson and Dr. Mustafa Kansiz

Agilent Technologies, Inc.

Introduction

Agilent's 610 FTIR fourier transform infrared (FTIR) microscopes are routinely used for the analysis of heterogeneous materials. They provide an ability to characterize the spatial distribution of components as well as the ability to identify the specific chemical nature of a sample. Agilent's infrared microscopes can be used on both the microscopic and macroscopic scale using multiple measurement modes including:

- transmission
- reflection
- attenuated total reflectance (ATR)
- grazing angle reflection analysis
- 'large sample' mode using Agilent's large sampling side-port accessory

They are ideal for advanced materials characterization as they are simple to use, provide the best sensitivity and versatility, and can be customized to suit a desired area of analysis. By adding a motorized sample stage to an Agilent Cary 610 FTIR single-element detector microscope system, the capabilities can be extended to include automated infrared mapping analysis.

Agilent Technologies

Infrared mapping allows for multiple infrared spectra to be sequentially acquired from different spatially-resolved points on the same sample and provides both spectral and spatial information, thereby facilitating the study of within-sample chemical heterogeneity. Common infrared mapping applications in material sciences include simple material characterization, the analysis of the homogeneity of coating materials, the investigation of multi-layer sample interfaces such as polymer laminates and paint cross-sections, the automated screening of samples for defects or contamination, the characterization of the total reflectance of optical surfaces and other process control applications.

This paper highlights the simplicity and power of Agilent's Agilent Cary 610 infrared mapping microscope for the rapid and automated analysis of a multi-component paint sample.

Instrumentation

The infrared mapping experiment was conducted using a Cary 610 FTIR spectrometer, equipped with a 610 FTIR infrared microscope (containing a 250 micron single-element, narrow-band Mercury Cadmium Telluride detector and a motorized sample stage) operating under Resolutions Pro 5.0 software. A constant flow of dry air was used to purge the system, limiting the contributions from carbon dioxide and atmospheric water vapor.

The infrared map was collected in reflection-mode using a pre-loaded grid mapping template that was customized to collect a 19 × 19 grid (totaling 361 spectra) using a 20 µm step size from an area measuring 380 × 380 microns. The infrared spectra were sequentially recorded over the range of 4000–700 cm^{-1} at a spectral resolution of 8 cm^{-1} by co-adding 16 scans per point (~40 mins for the entire infrared map).

Sample preparation

The paint chip cross sections were prepared from vehicle paint fragments provided by a police forensic laboratory. Samples were mounted in a clear casting polyester resin, and then polished using a 12,000-mesh Micromesh polishing cloth. The embedded paint fragments were microtomed to a thickness of ~10 µm, and the samples transferred to a standard glass microscope slide that was covered with aluminum foil to allow for reflection/absorption analysis.

Results and discussion

Infrared mapping using Agilent's Cary 610 FTIR Microscope allows for the automated sequential acquisition of hundreds of high-quality infrared spectra from analytical samples. Using Resolutions Pro software, mapping experiments are extremely flexible. Users can either select individual spectral collection locations themselves or use one of several grid mapping templates that can be customized to a sample, saved and re-applied later. In this experiment, a paint fragment found at an automobile crime scene was embedded in a polymer resin, then microtomed to obtain an appropriate sample thickness. This sample was deposited onto the surface of a reflective infrared support slide which was then placed on the motorized stage of the microscope. A visual image of the paint sample was acquired, followed by the sequential collection of the 361 spectra (19 × 19 grid map; 380 × 380 µm area) using automated infrared mapping. The visual image of the sample and the spectral acquisition locations are shown in Figure 1. Each spectrum in the infrared map results from a spatial resolution of 20 µm.

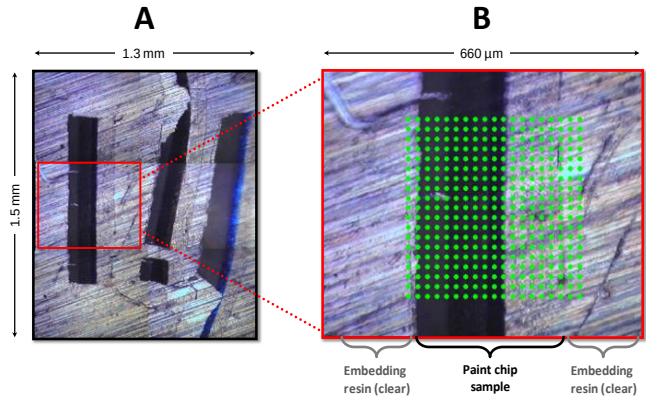


Figure 1. (A) Visual image of 3 sections of a paint chip sample (vertical bars), which were embedded into a polyester resin (clear). The reflective aluminum IR-slide upon which the samples are deposited can be seen through the resin. (B) Higher resolution view of a paint chip sample overlaid with the locations of spectral acquisition (represented by the grid of green circles). The overall area of analysis for the spectral map was 380 × 380 µm, yielding a total of 361 spectra.

The investigation and interpretation of the infrared data was simplified by several intuitive software features. For example, the grid of green circles that is overlaid on the surface of the visual image of the sample can be used to extract spatially resolved data. Simply clicking on a desired sample location (or multiple locations) will fill in the green circle(s) and will display the corresponding IR spectra in the software's 'spectrum' display panel. Spectral peaks of interest can then be compared or used for quantitative analysis, and the selected spectra can be overlaid or stacked to facilitate visual interpretation. Upon cursory visual examination of the forensic evidence in Figure 1, the vertical black strip appeared to be uniform in composition with only minimal variations. However, infrared investigation revealed that the sample is heterogeneous and composed of multiple spatially-resolved vertical layers. Exploratory investigation of the spectra in the map revealed the presence of four chemically distinct layers. In addition, the high spatial resolution of the infrared map allowed for the identification of localized areas with different chemical compositions within the stratified layers. Figure 2 illustrates selected absorbance spectra from the paint chip sample.

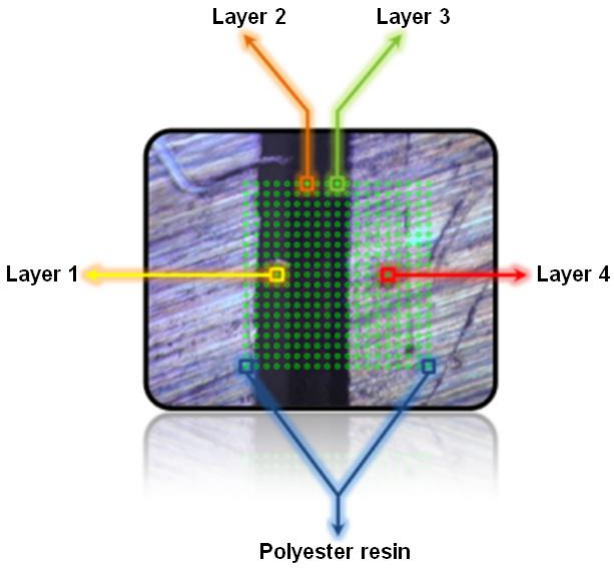
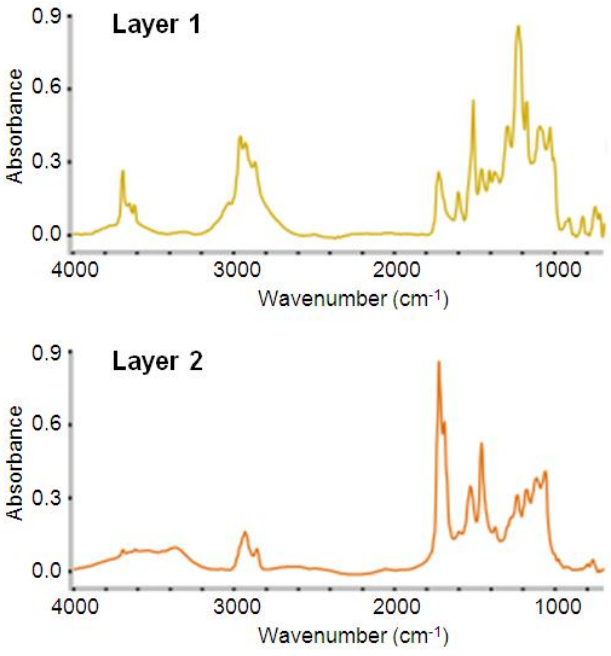
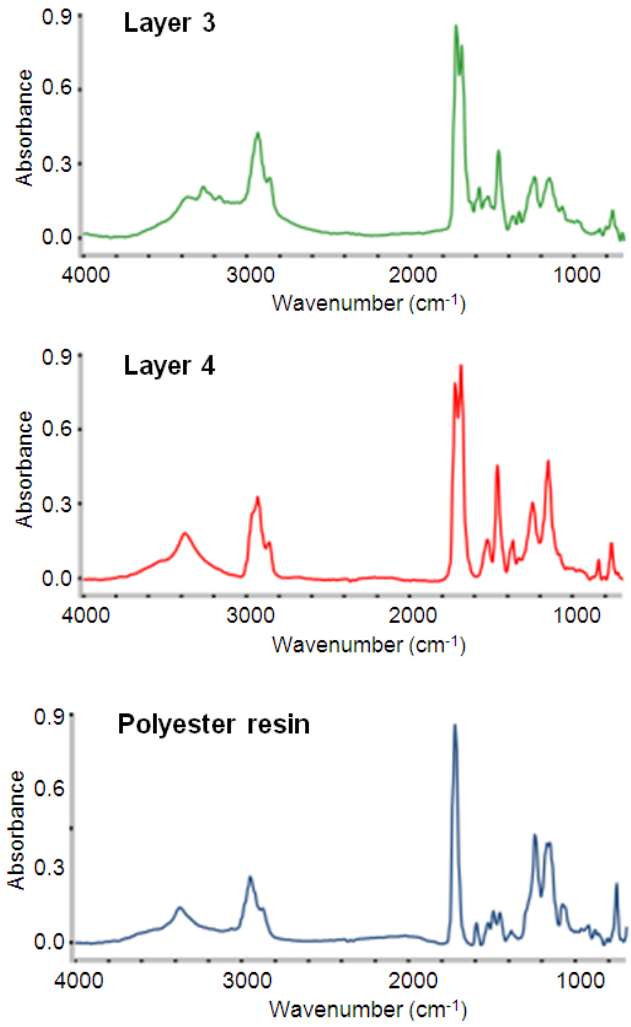


Figure 2. Representative FTIR spectra from the four layers of the paint chip sample as well as a spectrum of the embedding resin. Three of the spatially-resolved layers are in the black vertical bar, while one layer is transparent, as is the polyester resin. See layer spectra in the five images below.





The spectra in Figure 2 are visually distinct and contain sufficient information to allow for the characterization of each individual layer. Based on these spectra, forensic scientists are able to search spectral databases of paint and coating samples to identify the vehicle's make, model, year, and color. In this instance, the ability to detect trace materials in the evidence proved to be very useful in extending the knowledge of the sample's composition far beyond that which could have been obtained by in-bench FTIR experiments or by other analytical techniques.

Without a clear delineation of the layers, it is difficult to study the variations in sample chemistry across the infrared map by using the spectrum display alone. Resolutions Pro software makes it easy to view chemical differences across an entire infrared map of a sample. One means of probing a sample is to generate a feature image based on one or multiple spectral peaks (one or multiple functional groups of interest). A feature image assigns a color to the absorbance value of a selected peak (or spectral region) and plots the intensity across the infrared map to easily view spatially-resolved chemical differences on the visual image of a sample. The color red indicates a high absorbance value, while the color blue indicates a lower absorbance value. Figure 3 shows a feature image generated from a spectral peak that is unique to one layer of the paint chip. It is equally possible to view the feature image without displaying the locations of spectra acquisition, or to view it as a '3D' chemical image as shown in Figure 3.

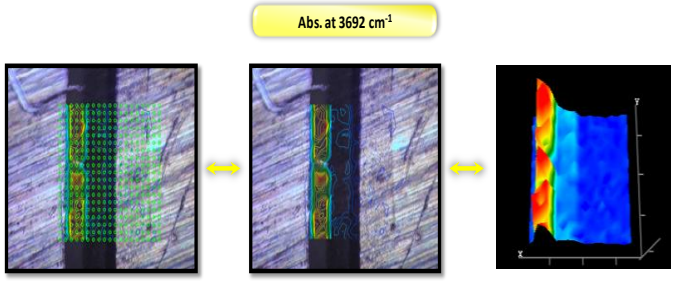


Figure 3. A feature image generated from a spectral peak that is unique to one layer of the paint chip (left), the same feature image shown without the spectral acquisition grid for clarity (center), and the 3-dimensional view of the feature image (right). These images were generated by plotting the intensity of the peak at 3692 cm^{-1} in the spectrum from each pixel across the entire infrared map.

Advantageously, feature images can be generated in real-time using any spectral range or absorbance peak to provide users with a better understanding of a sample's composition. Figure 4 illustrates the feature images generated from the four chemically distinct paint chip layers.

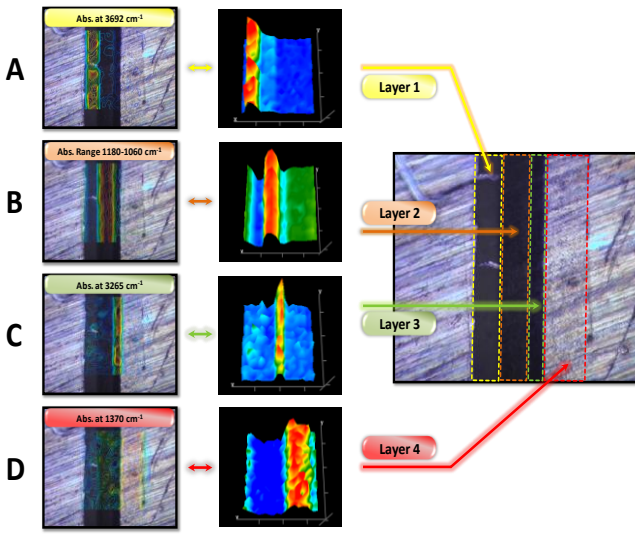


Figure 4. Feature images based on spectral peaks that are unique to each layer in the four-layer paint chip sample. The feature image in 'A' is based on the absorbance of the peak centered at 3692 cm^{-1}, which is primarily found in layer 1 of the paint chip; while the feature image in 'B' was generated from the absorbance peaks between 1180–1060 cm^{-1}, which are largely found in the second layer; 'C' shows the spatial distribution of the absorbance peak centered at 3265 cm^{-1}; while 'D' shows the feature image of the clear coating layer of the paint sample based on the absorbance at 1370 cm^{-1}. Legend for feature images: red = high intensity, green = medium intensity, blue = low intensity.

The chemical image display of the infrared mapping software was particularly useful to highlight the clear external coating of the paint sample, designated by layer 4 in Figure 4D. Depending on the visible contrast of a sample, it is occasionally easier to view the distribution of a selected spectral peak (or range) in different feature image views. From the feature images it is a simple task to estimate the approximate width of each stratified vertical layer; layer 1 is ~80 µm, layer 2 is ~80 µm, layer 3 is ~40 µm, while layer 4 is ~120 µm. It is equally possible to probe the heterogeneity within each layer for an improved characterization of the sample. For example, layer 1 in Figure 4A is not uniform in chemical composition and has a number of visible defects that can also be observed in the visible and feature images. With Resolutions Pro software, it is simple to investigate the chemical differences between adjacent spectra by displaying spectra simultaneously. However, for a more in-depth understanding of the samples' heterogeneity on the micro-scale, a higher spatial resolution infrared image would be required.

An alternate approach to acquiring IR spectra with a significantly higher spatial resolution involves the use of an infrared imaging system equipped with a focal plane array (FPA*) detector. An FPA-FTIR system would provide a superior means of investigating the subtle chemical differences found in each layer of the paint sample. Unlike infrared mapping using a single-element detector, an FPA* detector collects hundreds to thousands of spectra simultaneously within seconds, thereby providing dramatic savings in spectral acquisition time compared to infrared mapping techniques that perform sequential data collection. In practical terms, this infrared map required ~40 minutes acquisition time to collect 361 spectra for the area of 380 × 380 µm using a 20 µm spatial resolution; comparatively, Agilent's 128 × 128 FPA-FTIR system could acquire over 16,000 spectra with an identical signal-to-noise ratio from an area of 700 × 700 µm within a few seconds using an even higher spatial resolution of 5.5 µm per spectrum.

In addition, Agilent's FPA-FTIR imaging spectrometers have a number of easily user-changeable spatial resolution modes including: 1.1 µm (ATR Analysis), 5.5 µm, 11 µm, 22 µm and even larger sizes with pixel binning or macro imaging (for example, >40 µm). FPA-FTIR analysis would involve the same minimal sample preparation and could be used to reveal even the smallest features of the forensic evidence sample.

While this experiment focused on the characterization of a sample obtained from a crime scene, the application of FTIR microscopy and mapping in paint analysis extends far beyond forensic applications. They are commonly used for the characterization of historical art works, and for the development of conservation and preservation strategies for paintings and photographs. FTIR microscopy and mapping are equally important in the QC analysis of raw materials used in the manufacture of paints and inks, and are routinely applied to the analysis of resins, pigments, solvents and additives.

Conclusion

Agilent's Cary 610 FTIR Microscope provides the ability to collect high quality chemical information from multi-layer samples with a high spatial resolution. It provides an excellent means of probing a sample's chemistry as it can be used to visualize the relative distribution of specific components across a sample area of several centimeters. In this experiment, a 380 × 380 µm infrared map was automatically collected using a pre-defined acquisition grid to investigate the chemical heterogeneity of a paint chip sample. Four chemically distinct layers were resolved in the forensic evidence, including a miniscule layer measuring ~40 µm.

Feature images also were used to highlight each layer within the infrared map and to probe localized areas with varying chemical compositions within the stratified layers. The rapid nature and the simplicity of automated infrared mapping make it a key technique for the advanced characterization of material and polymer samples.

References

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Published March, 2011
Publication Number SI-02255

Agilent Technologies

Sensitivity Enhancement for Flame Atomic Absorption Spectrometry Using an Atom Concentrator Tube, the ACT 80

Application Note

Atomic Absorption

Author

Jonathan Moffett

Abstract

A simple attachment to enhance the sensitivity of flame atomic absorption spectrometry (FAAS) is described along with some performance results and practical applications. An historical review is also presented.

Introduction

In theory, atomic absorption spectrometry (AAS), is very simple: introduce ground state (metal) atoms into the appropriate instrument's optical path and measure the absorption of light at an appropriate wavelength [1]. The device that generates the atoms is called an atomizer and there are several types:

- Flame
- Vapor generation (cold and heated)
- Graphite furnace
- Cathodic discharge [2,3]

The flame atomization system offers several advantages:

- Relative freedom from interference
- Low capital cost
- Low running cost
- Rapid and simple operation

Agilent Technologies

Flame atomic absorption spectrometry (FAAS) is routinely used to measure solutions at the parts per million level–equivalent to one gram of element per 1000 kg of solution–which is suitable for a wide range of analyses. The other atomizers offer such benefits as greater sensitivity or minimal sample preparation. However the initial outlay and running expenses can be higher. Much closer attention to the chemistry of the samples is also required. Consequently various schemes have been devised to enhance the sensitivity of FAAS without incurring the expense associated with the other techniques. Some of the more commonly used methods as well as some speculative ideas will be outlined.

Enhancements in FAAS

All methods to improve the sensitivity of FAAS must involve at least one of the following stages:

- Sample preparation/preconcentration
- Nebulization
- Atomization

Each of these techniques is discussed in turn.

Sample

The simplest and cheapest methods for improving sensitivity rely on increasing the concentration of the sample solution. After sample dissolution, one of the following methods of sample preconcentration may be applied:

- Solvent evaporation
- Solvent extraction (for example, APDC/MIBK)
- Ion-exchange (for example, Chelex-100)
- Co-precipitation

While all are used [4], the method of solvent extraction (chelating the analyte and extracting with an organic solvent) is probably the most common. All of the methods are slow, increase the possibility of contamination and need a sample volume of at least 10 to 100 mL. The ion-exchange technique is the only one which could be developed into an automated online system and may overcome the speed and contamination problems.

Nebulization

Nebulization is the physical process of changing the bulk solution into a spray of fine droplets and mixing the droplets with the combustion gases.

The premix (laminar flow) burner assembly is invariably used in commercial FAAS instruments (Figure 1). A venturi is used to create a low pressure zone which draws up and causes nebulization of the solution. An impact bead breaks up the droplets even further. Mixing paddles or baffles may also be used to improve gas mixing and to remove larger droplets. The gas mixture is then passed into the burner and the combustion zone.

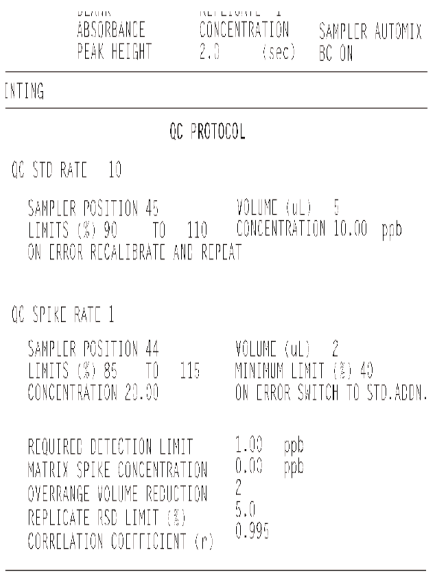

Figure 1. The Agilent Mark-VI spraychamber: (1) nebulizer, (2) ceramic faceplate, (3) adjustable glass bead, (4) drainage tube, (5) dual-head mixing paddle, (6) enhanced slope floor.

The main advantage of the premix burner assembly is its low noise and reproducibility. Agilent Technologies has introduced a new nebulizer [5], spraychamber [6], and a burner [7] to enhance further these benefits. However these improvements were not intended to improve the sensitivity significantly.

The difficulty of improving sensitivity can be demonstrated by using some typical numbers from this process. The nebulization process is only about 10% efficient so an uptake rate of 5 mL/min implies 0.5 mL/min passes through the burner. In most instruments 15–20 L/min of gas also flows through the burner. The effective dilution of the sample is therefore approximately 0.5/15000 or 1/30000.

The spraychamber would appear to be the obvious area to look for improvements in sensitivity. However even after decades of research and experimentation further significant improvements have yet to be made.

A heated spraychamber has been described which improves sensitivity for dilute, low solid solutions [8,9]. It appears likely that the premix spraychamber has been refined to its optimum

performance.

Logically the next potential area for improvement would be the nebulizer. Indeed it is possible to adjust the standard Agilent nebulizer to improve substantially the sensitivity for aqueous copper solutions. However the penalty of this mode of operation is an increased uptake rate and larger droplets in the flame. This would be perfectly acceptable if all samples behaved like aqueous copper solutions. In practice, under these conditions most solutions are known to cause unacceptable problems such as inter-element interferences, signal noise and blocking of the burner or nebulizer. Therefore obtaining sensitivity by increasing uptake rate is not recommended. Other nebulization schemes have been proposed. For example, it is quite feasible to use ultrasonic vibrations for improved nebulization. A different approach is to use electrostatic precipitation of the solid solutes in the aerosol [10-12]. However both techniques have yet to find wide acceptance in FAAS.

Atomization

The physical changes occurring to the solution aerosol in a flame are summarized in Reference 1. Work has been done on trying to understand the process better [8,13,14] but knowledge is still somewhat empirical, even without considering the chemical aspects or interferences. The number of analyte atoms present should in principle depend only on the volume of liquid reaching the combustion zone and the efficiency of atom formation. The flame sensitivity is determined by the number of ground state analyte atoms present in the optical path.

If the removal rate of the atoms from the optical path could be reduced, then an improvement in sensitivity should be observed. Such an approach was pioneered by Robinson [15] on a total combustion burner. Watling [16,17] experimented using a laminar flow burner with a slotted tube above the flame and Brown *et al* [18–20] have done additional work. (It should be mentioned that the Delves cup technique [21] also uses a tube.) This scheme is discussed in more detail in the following section.

A closely related approach pioneered by Lau [22] and investigated by several others [23–31] is to trap the atoms physically on the surface of a narrow diameter water-cooled silica tube placed just above the cone of the flame. After a suitable collecting period, the atom-trap tube is allowed to heat up (by stopping the flow and removing the water) and atoms are released to give an enhanced transient signal. Enhancements of 10 to 30 times have been reported. Practical difficulties have limited the application of this technique.

Atom Concentrator Tube, ACT 80

Watling, in 1977, described a slotted quartz tube which he placed over a conventional AA-6 air-acetylene burner and observed an improvement in analytical sensitivity [16,17].

The commercially available ACT 80 is a quartz tube 150 mm long with two lengthwise cuts. The longer slot is 100 mm × 2 mm, the shorter 80 mm × 2 mm. These cuts are angled at 120 degrees to each other relative to the tube's axis. The ACT 80 is installed in a standard Agilent Vapor Generation Accessory (VGA 76) cell holder and fits on a burner as does the VGA 76 cell. The longer slot is aligned over the burner slot; the shorter faces towards the rear of the instrument away from the holder. As with the VGA 76 cell, only the air-acetylene flame can be used as a hotter flame would destroy the tube. Figure 2 shows the tube in its holder.

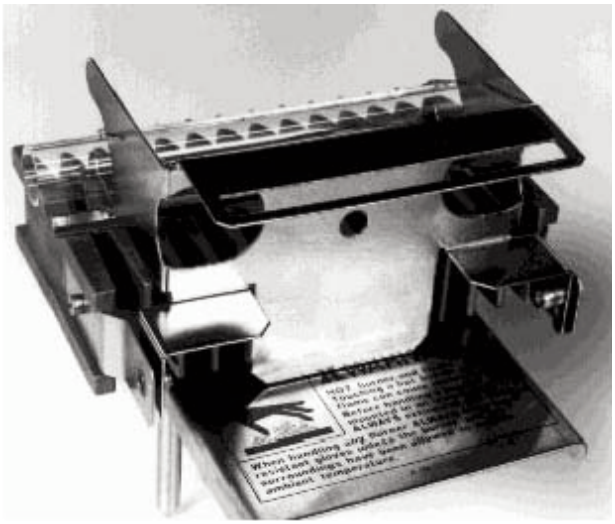

Figure 2. The ACT 80 Atom Concentrator Tube.

The ACT 80 tube must also be optically aligned so that the long axis of the tube coincides with the light beam. It was found in practice that the burner and ACT 80 needed to be lowered about 7 mm (equivalent to the radius of the tube).

Experimental

The performance of the ACT 80 was evaluated using SpectrAA-300/400 spectrometers fitted with a Mark VI spray-chamber and a Mark VA or a Mark VI air-acetylene burner. A VGA cell holder clamp was attached to the burner. Instrument default conditions were used for all measured elements. Where nitrous oxide-acetylene was the default flame, air-acetylene was used instead. Oxidant flow was 13.5 L/min and

acetylene flow 2.0 L/min. Delay time was 20 s and the read time period was 10 s integrated. All measurements were made after the system had been operated at least ten minutes to reach equilibrium.

Results and signal graphics were sent out to a printer. In addition, sample absorbances were sent to an ASCII file for further data manipulation.

Standard solutions were made from BDH (Poole, England) Spectrosol 1000 mg/L standards. Solutions and blanks were acidified with Analar grade concentrated nitric acid to give 0.5% v/v in final volume. Water was distilled from a Pyrex still and deionized with a Waters Milli-Q system to 18 MOhms conductivity.

Practical Points

The ACT 80 must be tilted back out of the way when lighting the flame. Otherwise for tongue-of-flame igniters a significant amount of acetylene builds up inside the ACT 80 with subsequent noisy ignition. Mechanical igniters would physically damage the ACT 80.

Flame composition is also an important factor. It was found that a lean to stoichiometric flame was needed. A rich flame causes soot formation and the signal noise becomes unacceptably high. Elements requiring a rich flame such as arsenic, chromium or molybdenum are therefore not usefully measured using the ACT 80. It was noted with arsenic that each blank signal increased and the blank and solution absorbances tended to give the same value. While this observation is not strong evidence for a memory effect, it cannot yet be eliminated. Alkali and alkaline earth (Group I and II) metals which etch heated silica [22] are also not usefully measured with this technique.

Devitrification of the tube inevitably occurs and starts initially around the inlet slot. The presence of Group I and II metals tends to accelerate this process. However it is possible to aspirate strong solutions (1000 mg/L or greater) of aluminium or lanthanum which provide a protective coating [23] and so retard the devitrification process. This should be done each time the tube is used and must be repeated on a regular basis. Tube lifetimes for samples with simple acidified matrices for example, water or dilute solutions of solids should typically be several hours of continuous operation. At a rate of approximately 200 samples/hour many samples may be determined using one tube.

Lifetime is maximized by continuous operation because cooling and reheating stresses the quartz.

Results and Discussion

Performance

As a guide to performance, improvements in characteristic concentration and detection limit were measured for selected air-acetylene elements. For both values the absorbance of a dilute solution of the analyte must be measured. The absorbance must be determined on a linear portion of the calibration graph and so concentrations were selected to be approximately equal to the characteristic to determine the characteristic concentration (determined using values previously published by Agilent). In practice ten measurements of the solution were made interspersed by measurement of the blank solution. Measurements of each series were done without the ACT-80 and repeated with the ACT-80 fitted (the burner height was reoptimized as needed).

Each element required a large number of readings and to avoid transcription errors the measurements were also printed to an ASCII file. This file was subsequently read by a BASIC program written to extract the absorbance values and perform the necessary calculations. Each solution absorbance was corrected by subtracting the mean of the two adjacent blank readings. The mean and standard deviation of the ten corrected absorbances were used to determine the characteristic concentration and detection limit values. These values were then loaded into a a LOTUS1-2-3 spreadsheet to generate Table 1.

Table 1 also lists, for reference only, Agilent data on detection limit and characteristic concentration values. The values found from this study were obtained using fixed air-acetylene flows and should not be directly compared with values obtained by optimizing conditions for each element.

The following points are drawn from Table 1:

1. All the elements listed showed some improvement in sensitivity. These tended to be consistent as indicated by duplicate runs. Copper was repeated on different systems.
2. All improvements appear to be about 2X to 3X, which reflects the findings of Watling [16,17] and Brown [18–20].
3. Generally there was a corresponding improvement in detection limit. The statistical nature of detection limit means direct comparisons should be interpreted cautiously but since the improvement factor is almost always greater than unity it is inferred that the ACT-80 does improve detection limits. Gold, cadmium and lead appear to show the best improvements.
4. Iron and platinum showed no significant improvements in characteristic concentration or detection limit.

Table 1. Comparison of Detection Limits and Characteristic Concentrations for Selected Air-Acetylene Flame Elements

	Characteristic concentration				Detection limit			
Element	Literature FAAS	Standard FAAS (Ht=10)	Act-80 FAAS (Ht=3)	Act-80 improvement factor	Literature FAAS	Standard FAAS (Ht=10)	Act-80 FAAS (Ht=3)	Act-80 improvement factor
Ag	0.030	0.0134	0.0049	2.7	0.002	0.0019	0.0020	1.0
Au	0.100	0.1226	0.0451	2.7	0.010	0.0148	0.0036	4.1
Bi	0.200	0.2647	0.0919	2.9	0.050	0.0766	0.0177	4.3
Bi		0.2498	0.0903	2.8		0.0414	0.0211	2.0
Cd	0.010	0.0123	0.0054	2.3	0.002	0.0047	0.0011	4.3
Cu	0.030	0.0422	0.0214	2.0	0.003	0.0055	0.0056	1.0
Cu		0.0496	0.0212	2.3		0.0047	0.0034	1.4
Cu *		0.0448	0.0189	2.4		0.0066	0.0065	1.0
Fe	0.050	0.0538	0.0362	1.5	0.006	0.0110	0.0102	1.1
Hg	1.500	2.4278	0.8581	2.8	0.150	0.3094	0.1121	2.8
Mn	0.029	0.0291	0.0141	2.1	0.002	0.0025	0.0019	1.3
Pb	0.100	0.1182	0.0404	2.9	0.010	0.0301	0.0090	3.3
Pt	1.000	2.0064	1.9328	1.0	0.100	0.1220	0.0967	1.3
Sb	0.300	0.3866	0.1244	3.1	0.040	0.0678	0.0462	1.5
Se	1.000	0.3356	0.1010	3.3	0.500	0.1381	0.0927	1.5
Te	0.200	0.2476	0.0903	2.7	0.030	0.0760	0.0492	1.5
Tl	0.200	0.1509	0.0588	2.6	0.020	0.0112	0.0052	2.2

Notes:
-Ten readings were taken and the mean calculated for each value.
-Uptake rate was fixed at 6 mL/min.
-All conditions constant except for burner height ("Ht").
-"Ht" is burner position as shown on the instrument's burner vertical scale.
-Concentrations are about 10 times detection limit (except for Cu which was 100 times).*
-Quoted results for Se used nitrous oxide-acetylene flame. This study used an air-acetylene flame.
-Some elements show replicate results. With Cu, results were from different burners.

The following definitions apply:

$$\text{Detection limit} = \frac{2 \times \text{Standard Deviation} \times \text{Concentration}}{\text{Mean Absorbance}}$$

(IUPAC now recommend detection limit to be 3 times standard deviation, for comparison with literature values 2 times is used here.)

$$\text{Characteristic concentration} = \frac{0.0044 \times \text{Concentration}}{\text{Mean Absorbance}}$$

As an illustration, signal graphics for a standard lead solution measured with and without the ACT-80 tube in place are shown in Figure 3.

Variation in tube dimensions were not investigated, however Brown used a tube 8 mm id (Watling did not specify dimensions). The similarity between the results of this study and the published data indicates that the enhancement is not influenced greatly by the tube dimensions.

Watling suggested the flame characteristics are being affected in a way to encourage atom residence time in the optical path. Whether the flame has less entrained air or the reducing interconal zone is broadened or the diffusion of atoms is slowed down requires more work to elucidate. However, it appears that atoms are not trapped but merely delayed.

The sensitivity of the nitrous oxide-acetylene flame would perhaps also benefit from this technique but its higher temperature (2600 °C) means that the tube would need to be very refractory. The Delves cup method has been applied to the nitrous oxide-acetylene flame [32] so a refractory atom concentrator tube may be feasible.

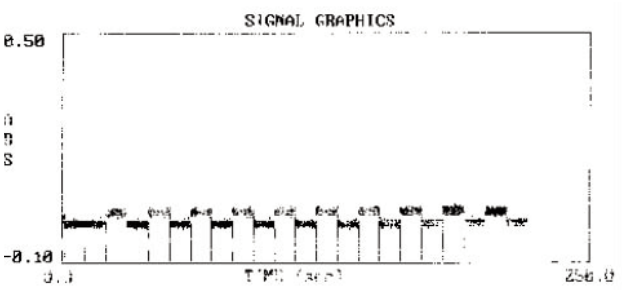

Figure 3(a). Pb signal compared to blank without ACT-80 tube.

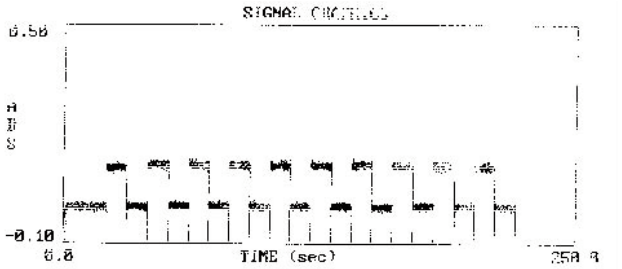

Figure 3(b). Pb signal compared to blank with ACT-80 tube.

Calibration Graphs

Calibration graphs were generated for four selected elements. The highest standard was selected to give about 0.3 Abs without the ACT-80 tube. As shown in Figure 4 the slope is clearly increased as would be anticipated from the improvements seen for the characteristic concentration. The graph for selenium shows that curvature is apparently more pronounced with the ACT-80 in place. However the same curvature is seen with higher solution concentrations without the tube in place. To corroborate this, the highest standard concentration used with the ACT-80 gave an absorbance equivalent to a standard three times the concentration without the tube.

Practical Applications

To illustrate the use of the tube in practical applications, quality control samples supplied by the United States Environmental Protection Agency (US EPA) were measured against aqueous standards. The levels of cadmium, copper and lead in EPA samples #4 and #5 are at or below the quoted detection limits for normal flame operation. A limited amount of National Bureau of Standards SRM 1643b water was also available and used for cadmium determinations.

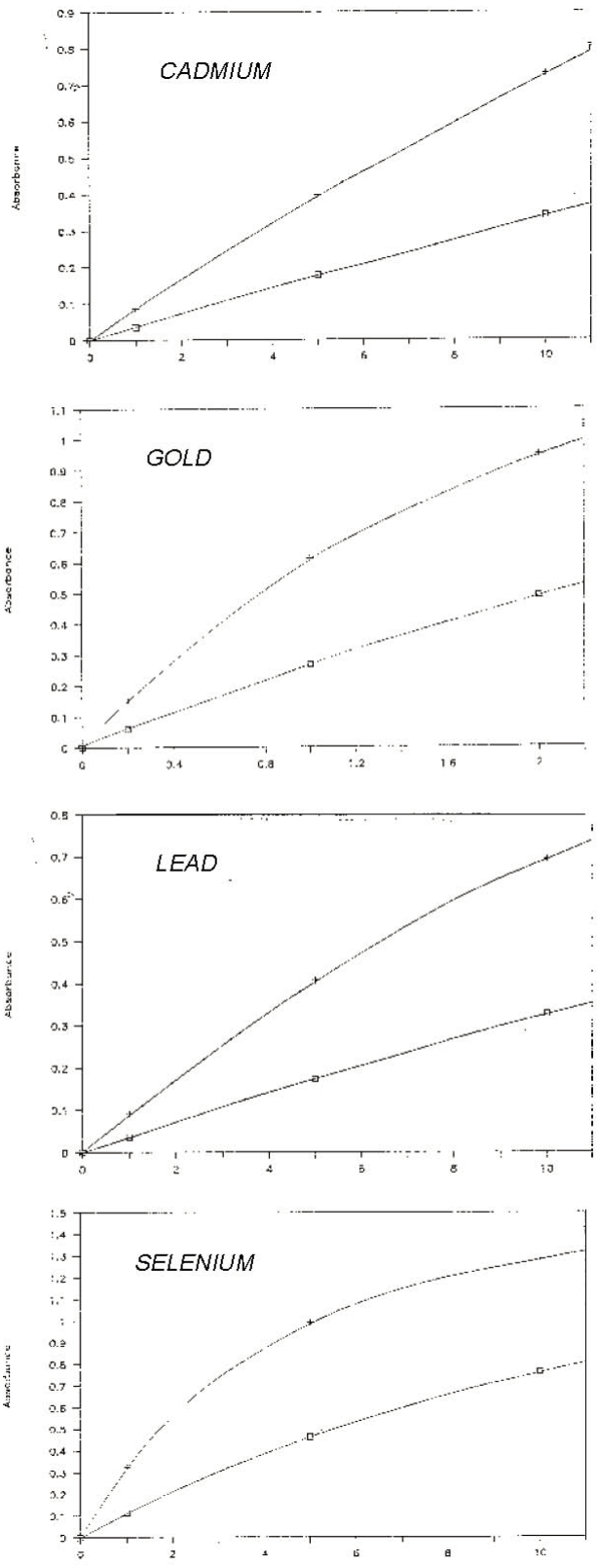

Figure 4. Calibration graphs of selected elements showing improvement in sensitivity. (+ = ACT-80, □ = normal FAAS)

The recommended instrument settings were used for each element. A delay time of five seconds and a read time of three seconds with three replicates were used. With these conditions about 200 solutions could be measured per hour. At least ten readings were taken for each sample to calculate standard deviations. The calibration graphs obtained are shown in Figure 5. A summary of the measured means and standard deviations are listed in Table 2. It can be seen that the measured results agree closely with the certified values even when working at the quoted detection limit for normal flame operation.

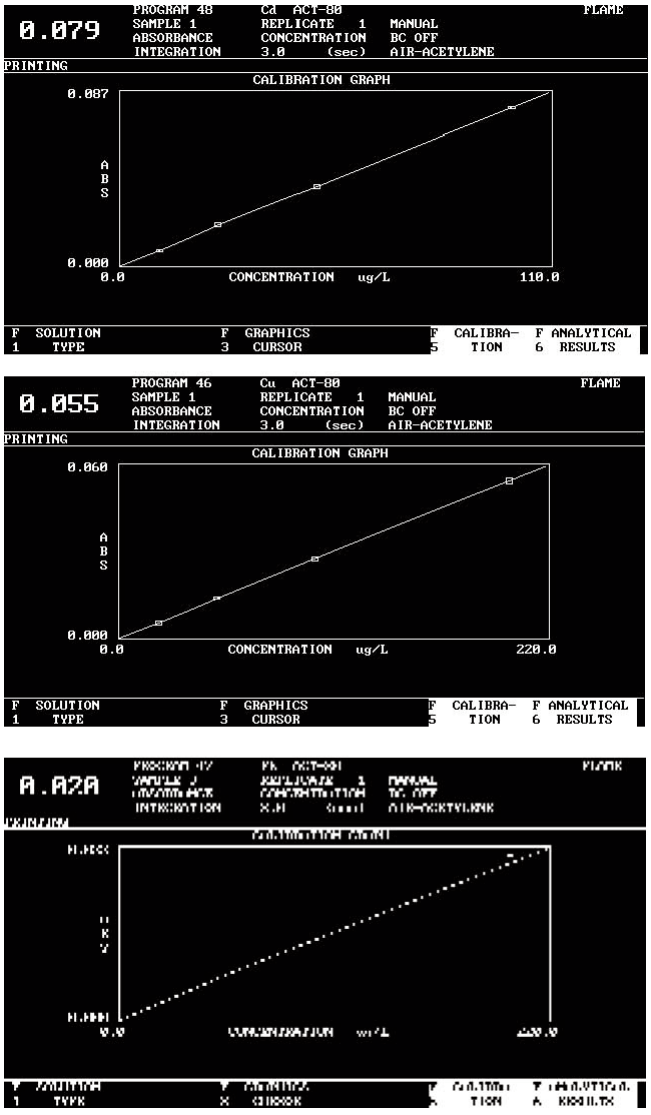

Figure 5. Calibration graphs used to measure quality control samples.

Table 2 Results for Quality Control Samples

Material	Mean ng/g	SD	Mean abs	Comments
Results for Cd using ACT-80				
US EPA sample 4	2.38	0.17		
Found	1.5	0.3	0.001	At quoted detection limit
US EPA sample 5	12.3	1.4		
Found	12.1	0.2	0.009	
NBS SRM 1643b	20	1		
Found	20.6	1.0	0.017	
Results for Cu using ACT-80				
US EPA sample 4	11.3	2.6		
Found	11.7	0.2	0.003	
US EPA sample 5	49.4	3.5		
Found	49.6	0.5	0.014	
Results for Pb using ACT-80				
US EPA sample 4	24.7	3.7		
Found	23.8	2.8	0.002	Twice quoted detection limit
US EPA sample 5	122	14.8		
Found	127.6	2.2	0.013	

Notes: Ten or more readings were taken for each solution.
SD is the standard deviation.

Conclusion

There is a measurable improvement in signal using the ACT-80. The improvements seen are comparable with those previously published. This study shows that there is an improvement in characteristic concentration between two and three times that of the normal FAAS. Detection limits generally show somewhat similar improvements. The ACT-80 is simple, cost effective and offers benefits in low level analyses.

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Printed in the USA
November 1, 2010
AA091

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Sensitivity Enhancement for Flame AAS Using an Atom Concentrator Tube for Elements Dissolved in Organic Solvents

Application Note

Atomic Absorption

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Introduction

The application of a slotted tube placed on an ordinary atomic absorption burner head in order to increase the sensitivity and detection limit for a number of elements in flame-atomic absorption spectrometry (FAAS) was first demonstrated by Watling [1,2]. A very similar technique had been used before in combination with either a nickel "cup" [3] or a tantalum "boat"[4] for the same purpose. The enhancement effect using the combination of a slotted tube and an ordinary acetylene/air flame was later confirmed by several authors who demonstrated that the sensitivity and the detection limit could typically be improved by a factor of 2–5 for easily atomized elements [5–11].

Extraction of aqueous samples into a small volume of an organic solvent after addition of a complexing agent in order to enhance the detection limit is a well established method [12–14]. A concentration factor of at least 20 times can easily be achieved.

Moreover, it is also well known that atomizing organic solutions (especially those rich in oxygen, for example, ketones) can result in 3–5 times better sensitivity for many elements [15] and references therein. Thus the improvement in sensitivity for flame-AAS after extraction should be about 20 x (3–5) = 60 – 100 times.

A combination of extraction into an organic solvent and the atom concentrator tube should thus theoretically result in a total improvement in sensitivity and detection limit of (60 to 100) × (2 to 3) = 120 to 300 times.

Surprisingly, the possibility of combining these techniques has not been investigated. The present paper therefore reports results from a number of experiments using the atom concentrator tube for organic solutions of some metals. For comparison the same solutions have been analyzed without the concentrator tube.

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Experimental

Apparatus

An Agilent SpectrAA-10BQ Atomic Absorption Spectrometer equipped with a Mark VI burner head was used together with an Agilent Atom Concentrator Tube (ACT 80) including a special metal holder constructed to fit the quartz tube to this particular burner–the holder being identical with that used for the quartz tube of the Agilent Vapor Generation Accessory (VGA-77). The quartz tube was 150 mm long with two lengthwise cuts 2 mm wide by 100 and 80 mm long respectively, angled at 120 degrees relative to each other. New tubes were conditioned in the flame by nebulizing a 1% lanthanum nitrate solution for 10–15 min before use in order to prolong the tube life.

The built-in instrument graphics together with an Epson RX-80 printer were used for the recording of the signals and for construction of the calibration graphs.

Gas flow-rates of acetylene for the organic and aqueous solutions were 1.2 and 1.8 L/min respectively. The air flow-rate was 12 L/min in both cases.

The instrument parameters were as follows:

Measurement time	4 sec
Delay time	4 sec
Replicates	3
Recommended SBW and wavelength for each element	Background correction was not used

Experiments

Test solutions containing mixtures of Ag, Cu, Fe Ni and Pb made by appropriate dilutions of a metallo-organic standard mixture of the elements (Conostan S-12 100 ppm (Wt)) with methyl isobutyl ketone (MIBK) were used. A corresponding series of aqueous metal standards were made by diluting a stock solution made from the appropriate amounts of the respective metal nitrates (of A.R. grade) dissolved in water.

The following concentrations were measured: 0, 2, 4, 6, 8 and 10 mg/L of each metal.

The instrument calculated and displayed the calibration graph for each element. From the four graphs: for example, water, MIBK, water + ACT and MIBK + ACT the relative enhancement factors were calculated for each element using the absorbance values for 6 mg/L. The factors are given in Table 1.

Results and Discussion

Both the aqueous and the MIBK-solutions were measured with and without the ACT tube. The No.1 value in the table should be compared with those obtained for No. 4. Both series demonstrated the enhancement factors that can be expected when the ACT is used and that the tube indeed has almost the same effect for organic solutions. Comparison of No. 2 and No. 6 confirms this.

Experiment No. 3 illustrates the total enhancement obtained using an organic solution combined with the concentrator tube relative to aqueous solutions without the tube.

No. 5 shows that atomizing MIBK-solutions without the tube is always more effective than atomizing aqueous solutions with the tube.

The results in Table 1 also confirm that the enhancement effect using the tube is best for the easily atomized elements.

Conclusion

The results show that using a quartz atom concentrator tube for metal compounds in methyl isobutyl ketone solutions will result in the same enhancement of the sensitivity as for aqueous solutions multiplied with a factor of 3–4 due to the beneficial (exothermal) atomizing conditions for organic solvents (see above). This can be utilized in the application of extraction methods for the determination of ions present in water samples thus achieving a much better detection limit relative to that obtained for aqueous samples without extraction.

It is evident that the enhancement effect is caused mostly by the prolonged residence time of the atoms in the light path and is most pronounced for the easily atomized elements. Thus for iron (and nickel) the tube does not seem to offer any advantage at all. This can be explained by the lower temperature inside the quartz tube, this being too low for an effective atomization of the more refractive elements. For such elements it is better to atomize an organic solution without tube.

In many cases, the combination of extraction of metal complexes into organic solvents using an atom concentrator tube for flame-AAS could be an alternative to the graphite furnace technique, for instance for sea-water samples. This approach can be even more attractive if using the extraction equipment recently described for a fast, non-manual extraction of large volumes which can solve the problems associated with the use of the conventional and inconvenient separatory funnels [15].

Alternatively, programmable probe height of the SPS-5 Flame Sampler may be used to advantage in the extraction procedure.

The SPS-5 probe operates through a range of 160 mm. When two immiscible liquids are in a test tube, the probe may be programmed to descend into the upper liquid layer. Thus, the extraction procedure could be as follows:

- Pipette a volume of sample into a stopped test tube, and add a known volume of extractant
- Then pipette a volume of organic solvent into the tube, stopper and shake it
- Remove the stopper, start the SPS-5 Flame Sampler
- The probe will then descend into the upper organic layer. This eliminates the use of separatory funnels.

Table 1. Enhancement Factors for Pb, Cu, Ag, Fe and Ni

	Pb	Cu	Ag	Fe	Ni
MIBK/ACT / MIBK	2.4	1.6	2.8	0.6	1.1
MIBK/ACT / AQ/ACT	3.3	4.0	3.8	2.1	n.d.
MIBK/ACT / aq	8.6	6.0	10.9	2.2	n.d.
AQ/ACT / aq	2.7	1.5	2.8	1.0	n.d.
MIBK / aq/ACT	1.3	2.5	1.3	3.5	n.d.
MIBK / aq	3.6	3.8	3.6	3.6	n.d.

n.d. = Not determined

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Printed in the USA
November 1, 2010
AA116

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Monitoring fast reactions using Stopped Flow Kinetics on the Cary 50/60 UV-Vis

Application Note

Chemical

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Introduction

There are many factors which control the rate of a chemical reaction. These include the type of metal center, the size and charge of the ligands, the concentration of the reactants, and environmental conditions such as pH and temperature. All these factors can produce systems with half lives in the region of milliseconds to hours to days.

One method of monitoring a reaction is through UV-Vis spectrophotometry. If the reactant or product exhibits a change in absorbance as a function of reaction time, this method can usually be employed. A conventional cell is adequate for reactions with half lives greater than a couple of minutes, however, reactions that are over within a second or milliseconds need specialized equipment. A Rapid Kinetics Accessory (or Stopped-Flow Apparatus) can be used to measure such fast reactions.

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Theory

Conventional spectrophotometric techniques cannot be used when investigating reactions that occur at a sub-second rate. If reactants are added manually, and then stirred for a few seconds to allow adequate mixing, the reaction is over and no changes in spectra are recorded. This is overcome by using a Stopped-Flow apparatus (Figure 1) that provides instantaneous mixing and recording of data on a tens-of-millisecond time scale. The technique rapidly mixes two solutions in a flow cell and starts recording data when the mixing ceases. The Cary 50 and Cary 60 spectrophotometer's can record a data point every 12.5 ms.

Figure 1. Rapid Mix Accessory - SFA20

The stopped flow apparatus is connected to the spectrophotometer through a remote-send-cable that is attached to an accessory controller port on the instrument. The reactants are placed in two syringes, labelled Solution A and Solution B, as shown in Figure 2. Upon pressing the plunger, the solutions travel separately to the cuvette and only mix upon entering the cell. Previously reacted solution is ejected into a waste syringe, which moves back until hitting a micro switch. Solution then stops flowing into the cell and data collection begins instantaneously, which eliminates any delay caused by a manual start.

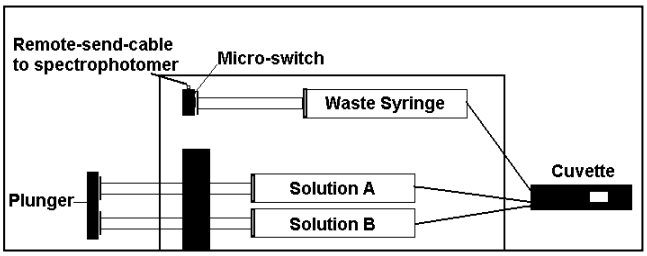

Figure 2. Schematic of Rapid Mix Accessory

The data are processed in much the same way as when using conventional techniques. The in-built algorithms, available in the Cary WinUV kinetics application, allows zero, first and second order fits to be applied to the experimental data. The fitted curve is displayed on the graph and the calculated parameters appear in the report. It is critical that there are a sufficient number of data points in the initial stage of the reaction as this is where most of the changes in the spectra occur. Enough data spanning 3-4 half lives should be collected. The Cary 50/60 UV-Vis spectrophotometers coupled to a Stopped-Flow apparatus can measure reactions that are over within a couple of hundred milliseconds!

Equipment

- Cary 50 UV-Vis spectrophotometer[1]
- Rapid Mix Accessory (SFA-20) [2]
- Green food dye
- Bleach in the form of White King[3]

Experimental

To demonstrate the extremely fast data collection capabilities of the Cary 50/60 UV-Vis, the rate of bleaching of green food dye was investigated. The rate of the reaction was controlled by varying the concentration of bleach, until the limits for measuring this reaction were achieved. First-order kinetic fits were then applied to the data.

A solution of green dye in de-ionized distilled water was prepared so as to give an absorbance of ca. 0.4 (Solution A).

Solution B was prepared by diluting White King (0.655 mL) with 30 mL of de-ionized distilled water.

The instrument parameters for the Cary 50/60 were set up as follows:

Wavelength (nm)	414
Ave Time (s)	0.0125
Y Min	0
Y Max	0.5
Cycle (min)	0
Stop (min)	0.2

Results

The color green is composed of the 2 primary colors yellow and blue, which is reflected in the UV-Vis spectrum of green food dye in water, Figure 3. The yellow component has an absorbance at 414 nm and the blue component at 629 nm. The addition of bleach causes a rapid change in the absorbance at 414 nm, following a first order decay path, the rate of which depends on the concentration of bleach. The reaction was monitored at 414 nm.

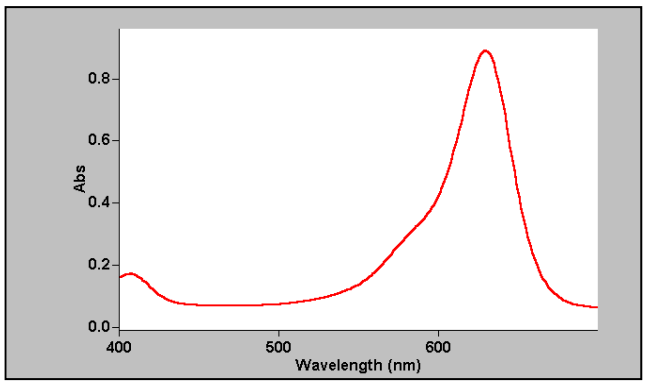

Figure 3. UV-Vis spectrum of green food dye in water

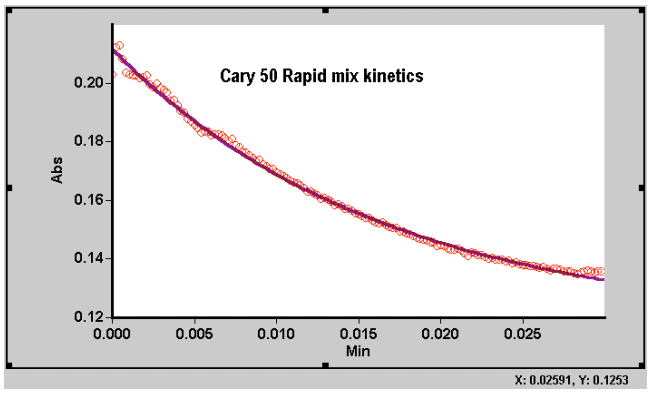

Figure 4. Change in absorbance at 414 nm of green dye and bleach on a Cary 50

Figure 4 shows the change in absorbance over time for the bleaching of green food dye collected on a Cary 50. This experiment could also be done on a Cary 60 UV-Vis. The reaction is over within 2 seconds, and the rate constant for a first order decay was calculated to be 60.320 min^{-1} with a SD of 0.0013.

Discussion

In order to accurately monitor reactions with sub-second half-lives, a spectrophotometer must have the following features:

1. Minimum delay time between mixing of reagents and recording the first data point.
2. The ability to acquire enough data points per second to allow accurate fitting of the data.
3. Remote read plug to accommodate a Rapid Mix Accessory.

The Cary 50/60 UV-Vis has minimized the waiting period between mixing and collecting the first data point by incorporating a 'Synch Start' function in its software. This essentially 'primes' the software, preparing it for its first reading. The overall delay is less than 50 ms on a Cary 50/60, which means that valuable data are not lost during the initial part of the reaction.

The WinUV kinetics software provides all the necessary tools to analyze and display the data. Curve fitting is completed within seconds and the fitted curve is overlaid on the experimental data. The option of displaying the experimental data as points and the fitted curve as a solid line results in a professional report, which can be customized by the user.
The instrument parameters used in the data collection and the results of the curve fitting are also presented in the report.

Conclusion

The Cary 50/60 is the fastest wavelength scanning UV-Vis spectrophotometer currently on the market. The minimum delay between mixing of the reagents and collecting the first data point allows for the collection of more data during the initial part of the reaction, where the greatest change in absorbance occurs. This enables accurate monitoring and analysis of reactions that occur on a sub-second level.

References

1. Part numbers for ordering:

Product	Part Number
Cary 60 UV-Vis with WinUV software and PC	G6860AA

2. Part numbers for ordering:

Product	Part Number
SFA-20	7910030400
Mounting bracket (Cary 300)	7910030500
Country Kit	depends on country

3. Household laundry bleach liquid with 40 g/L available chlorine present as sodium hypochlorite.

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Published March, 2011
Publication Number SI-A-1177

Agilent Technologies

Investigation of photochemical reactions using the Cary 50/60 UV-Vis

Application Note

Chemical

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Introduction

A photochemical reaction is a reaction that is initiated by the absorption of light. The reaction may then proceed with or without continuous irradiation. The reactivity arises from the absorption of light, which places the reactant molecules into an excited state. These molecules may then undergo a variety of subsequent reactions to form products. This reactivity is generally not observed in the ground state, i.e. when no light has been absorbed, however, very slow thermal reactions may occur[1].

In order to investigate a photochemical reaction *in situ*, using UV-Vis spectrophotometry, it is essential that the analyzing beam from the instrument does not degrade the sample, especially if the irradiating wavelength differs from that of the analyzing wavelength/s. This is a problem with commercial diode array spectrophotometers, where the sample is analyzed with white light. Significant photodegradation occurs over time.

Another requirement of the spectrophotometer is that it must accommodate a second external source used for irradiation. Light from this source may be introduced to the sample via a fiber optic bundle, which means that the instrument must be able to operate with the sample compartment open. A black cloth may be placed over the instrument and sample to prevent unwanted degradation from room light. A subsequent consideration then becomes stray light interference from the irradiating beam with the analyzing measurement from the spectrophotometer. If the instrument is not room light immune, then the intensity of the irradiating beam can cause major problems, particularly with deviations from photo-linearity instrument specifications. This can result in significant errors with the data collected.

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The Cary 50 and Cary 60 UV-Vis takes into consideration all of the aforementioned points. It is room light immune, so stray light is not a problem, and it does not degrade even the most photo-sensitive samples. Figure 1 shows the absorbance at 366 nm over time for the irradiation of a photoactive platinum compound in acetonitrile[2]. The experiment, performed on a Cary 50 and a commercial spectrophotometer, shows significant photo-degradation by the other instrument.

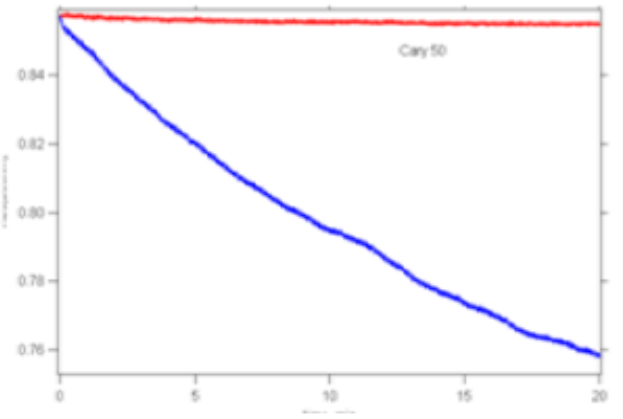

Figure 1. Absorbance(366 nm) vs time of platinum complex

This paper demonstrates the advantages of the Cary 50 and Cary 60 when used to monitor photochemical reactions *in situ*, by investigating the photochemical degradation of the compound potassium ferrioxalate ($K_3[Fe(C_2O_4)_3]$).

Background

The efficiency of a photochemical reaction is defined by its quantum yield of product formation, i.e. how much product is produced with respect to how much light is absorbed. For a reaction where A→ B via the absorption of light, the quantum yield of photo-product formation (Φ_{PR}) is defined by Equation 1, where I_{abs} is the amount of light absorbed by the reactant, A, and d[A]/dt is the change in concentration of A with time.

$$-\frac{d[A]}{dt} = \Phi_{PR} I_{abs} \qquad \textit{Equation 1}$$

The change in concentration is followed by measuring the absorbance at a specific wavelength and using the Beer-Lambert law to convert this to a concentration. The intensity of absorbed light is calculated using Equations 2-5, which require the measurement of the absolute intensity, I_0, of the irradiating source.

$$I_{abs} = \int_0^t I_{abs}(t)dt \qquad \textit{Equation 2}$$

$$I_{abs}(t) = I_{sol}(t)\alpha(t) \qquad \textit{Equation 3}$$

$$I_{sol}(t) = I_0\left(1 - 10^{-Abs_{\lambda,t}\,l}\right)\frac{S}{V} \qquad \textit{Equation 4}$$

$$\alpha(t) = \frac{\varepsilon_A[A]_t\,l}{Abs_{\lambda,t}} \qquad \textit{Equation 5}$$

Where :

I_{sol} = the amount of light absorbed by the solution

$\alpha(t)$ = the fraction of light absorbed by **1a**

S = the area exposed to irradiation (cm^2)

V = the volume of solution irradiated (dm^3)

The absolute light intensity of a source can be determined through actinometry. An actinometer is a substance or device that responds, in some measurable way, to the amount of light it absorbs. In the case of a chemical actinometer, the absorption of light leads to a photochemical transformation that is monitored by some analytical technique. The amount of reactant transformed is then used to calculate the intensity of irradiating light in units of Einsteins per unit time and area.

The sensitivity of the actinometer depends on the quantum yield for the photolysis reaction and the method of analysis. The latter is the most important as some analysis methods can cause significant degradation of the actinometer solution. Also, since most actinometers are extremely sensitive to room light, they must be protected and analyzed in a darkroom under red or yellow safelights.

Experimental

Reagents:

Potassium ferrioxalate (0.0114 M)

Buffer (CH_3COONa/H_2SO_4 pH 3.5)

1:10 phenanthroline (0.1% w/v)

Deionized distilled water

Apparatus:

Cary 50 (or Cary 60) UV-Vis

WinUV Software

Single cell Peltier temperature accessory

10 mm pathlength quartz cuvette

100 W Hg arc lamp/housing (Oriel)

366 nm bandpass filter

3' quartz fiber optic bundle (Dolan Jenner)

Potassium ferrioxalate (0.0114 M, 1.30 cm^3) was added to the quartz cuvette, immediately followed by CH_3COONa/H_2SO_4 buffer solution (pH 3.5, 0.70 cm^3) and 1:10 phenanthroline (0.1% w/v, 0.50 cm^3). The cuvette was placed in the thermostatted cell holder (25 °C) and allowed to equilibrate for 5-10 minutes in the dark. The solution was then irradiated with 366 nm Hg light and the absorbance at 510 nm monitored. The instrument parameters used are listed below and Figure 2 shows the setup used for irradiation.

Wavelength (nm)	366
Av. Time (s)	0.1
Y Min	0
Y Max	1.0
Cycle (min)	0
Stop (min)	30

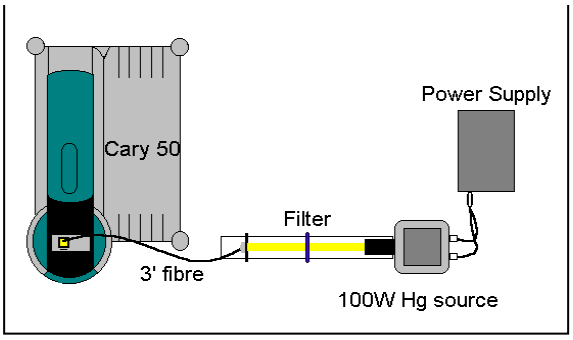

Figure 2. Setup of apparatus used for irradiating sample

Light from a 100 W Hg source passes through a bandpass filter isolating the intense 366 nm Hg line, and is directed via a 3 foot fiber optic bundle into the top of a quartz cuvette. The solution of $K_3[Fe(C_2O_4)_3]$ in the cuvette, which is continuously stirring, immediately undergoes photochemical reduction upon irradiation from the Hg source and the change in absorbance is measured at 510 nm The solution is analyzed using a Cary 50 UV-Vis with the Cary WInUV Kinetics software package. A Cary 60 UV-Vis can also be used for this experiment.

Discussion

Potassium ferrioxalate is an extremely sensitive actinometer. Investigated by Hatchard and Parker[3] in the mid 1950s, $K_3[Fe(C_2O_4)_3]$ undergoes photochemical reduction from Fe^{3+} to Fe^{2+} upon absorbing UV-Vis (λ<500nm) radiation, as shown in Equations 6-8. The quantum yield of Fe^{2+} formation is reported in Hatchard and Parker's paper, and the change in amount of Fe^{2+} produced during irradiation is followed by UV-Vis spectrophotometry at 510 nm. The addition of 1:10 phenanthroline to the irradiated solution results in the formation of $[Fe(phen)]^{2+}$, which has an absorption maximum at 510 nm.

$$[Fe^{III}(C_2O_4)_3]^{3-} \rightarrow [Fe^{II}(C_2O_4)_2]^{2-} + C_2O_4^{-} \qquad \textbf{Equation 6}$$

$$[Fe^{III}(C_2O_4)_3]^{3-} + C_2O_4^{-} \rightarrow [Fe^{III}(C_2O_4)_3]^{2-} + (C_2O_4)^{2-} \qquad \textbf{Equation 7}$$

$$[Fe^{III}(C_2O_4)_3]^{2-} \rightarrow [Fe^{II}(C_2O_4)_2]^{2-} + 2CO_2 \qquad \textbf{Equation 8}$$

In the past, the amount of Fe^{2+} produced has been determined by measuring the absorbance of $K_3[Fe(III)(C_2O_4)_3]$ at 510nm before irradiation, irradiating the sample for a known time, adding 1:10 phenanthroline in a dark room, and then measuring the absorbance of $[Fe(II)(phen)_3]^{2+}$ at 510 nm again.

The intensity of the irradiating source, I_0, is then calculated from the amount of Fe^{2+} produced during irradiation.

The Cary 50/60 allows the above procedure to be slightly modified. Due to the room light immunity of the instrument, the change in absorbance at 510 nm can be measured *in situ* during irradiation of the $K_3[Fe(C_2O_4)_3]$ solution. This reduces the handling of solution during the reaction which, in turn, eliminates unnecessary exposure to ambient light. In order to avoid degradation during the reaction due to room light, a thick black cloth was placed over the entire instrument.

Figure 3 shows the change in absorbance at 510 nm in the absence (0 - 1.8 min) and presence (1.8 - 4 min) of the irradiating beam.

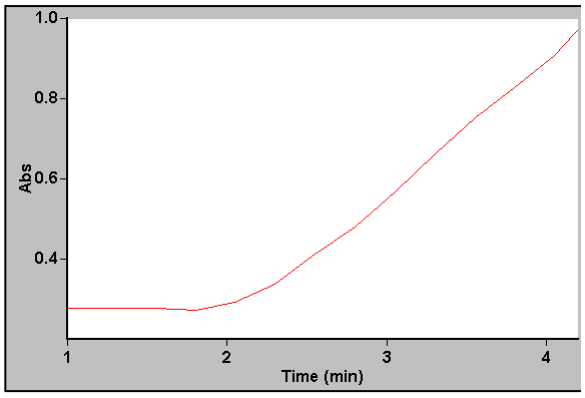

Figure 3. Absorbance(510 nm) vs time during irradiation with 366 nm Hg light

The change in absorbance at 510 nm only occurs during irradiation and is linear. This linearity and absence of noise in the data, shows that stray light has little effect on the measurements and also confirms that stirring was extremely efficient.

The intensity of the irradiating beam, in Einsteins s^{-1} dm^{-3}, was calculated from the change in absorbance using Equations 9 and 10[4].

$$\frac{n\left[Fe(phen)_3\right]^{II}}{dt} = \frac{dAbs_{510}}{dt}\frac{V}{\varepsilon_{510}\,l} \qquad \textit{Equation 9}$$

Where

V = volume of solution irradiated (dm^3)

ε_{510} = molar extinction coefficient at 510 nm of $[Fe(phen)_3]^{2+}$ ($M^{-1}cm^{-1}$)

l = pathlength (cm)

$$I_0 = \frac{\dfrac{n\left[Fe(phen)_3\right]^{II}}{dt}}{\phi_{Fe^{2+},\lambda}} \qquad \textit{Equation 10}$$

Equation 9 calculates the change in the number of moles of $[Fe(phen)_3]^{2+}$ with respect to irradiating time, which is used in Equation 10 to determine the intensity of the irradiating beam, I_0. Using the value of 1.21 for the quantum yield of Fe^{2+} formation when $K_3Fe(C_2O_4)_3$ is irradiated with 366 nm light[3], the intensity of the irradiating beam incident upon the cuvette is $(9.32\pm0.02)\times10^{-10}$ Einstein s^{-1} cm^{-2}.

Conclusion

The Cary 50 and Cary 60 UV-Vis allow measurements to be performed whilst using an external light source to irradiate the sample. The problems of noise and deviations from photo-linearity, associated with stray light, are not observed. The Cary 50/60 is able to study even the most light-sensitive of compounds *in situ* and does not cause photodegradation from the analyzing beam, which generally occurs when white light is used for analysis.

References

1. N. J. Turro, *Modern Molecular Photochemistry*, Benjamin/Cummings Pub. Co., California, **1978.**
2. J. Comerford and P. A. Tregloan, *Personal Communication*, The University of Melbourne, Australia, **1997.**
3. C. G. Hatchard and C. A. Parker, *Proc. Roy. Soc. A*, **278**, 518, **1956.**
4. J. F. Rabek, *Experimental methods in photochemistry and photophysics (Part 2)*, Wiley, New York, p944, **1982.**

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Published March, 2011
Publication Number SI-A-1251

Agilent Technologies

The investigation of the photo-kinetics of a platinum organoamine complex using the Cary 50/60

Application Note

Chemical

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Introduction

A photochemical reaction is a reaction that is initiated by the absorption of light. The reaction may then proceed with or without continuous irradiation. Reactivity arises from the absorption of light, which places the reactant molecules into an excited state. These molecules may then undergo a variety of subsequent reactions to form products. This reactivity is generally not observed in the ground state, i.e., when no light has been absorbed, however, very slow thermal reactions may occur[1].

In order to investigate a photochemical reaction *in situ*, using UV-Vis spectrophotometry, it is essential that the analyzing beam from the instrument does not degrade the sample, especially if the irradiating wavelength differs from that of the analyzing wavelength/s. This exemplifies the problem encountered with commercial diode array spectrophotometers, where the sample is analyzed with white light.

Another requirement of the spectrophotometer is that it must accommodate a second external source used for irradiation. Light from this source may be introduced to the sample via a fiber optic bundle, which means that the instrument must be able to operate with the sample compartment open. A black cloth is usually used to prevent unwanted degradation from room light. A subsequent consideration then becomes the stray light interference from the irradiating beam with the analyzing beam from the spectrophotometer. If the instrument is not room light immune, then the intensity of the irradiating beam can cause instrument problems, such as deviations from instrument photo-linearity specifications.

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Agilent Technologies

The Agilent Cary 50 and Cary 60 takes into consideration all of the aforementioned points. It is room light immune, so stray light is not a problem, and it does not degrade even the most photo-sensitive samples. Figure 1 shows the absorption at 366 nm vs time profile for the irradiation of a photoactive platinum compound, [N,N'-*Bis*(2,3,5,6-tetrafluorophenyl)ethane-1,2-diaminato(2-)] dipyridineplatinum(II) in acetonitrile (quantum yield of photosubstitution in acetonitrile of 0.96)[2], performed on a Cary 50 and a commercial instrument. As can be seen, significant photo-degradation is caused by the other spectrophotometer.

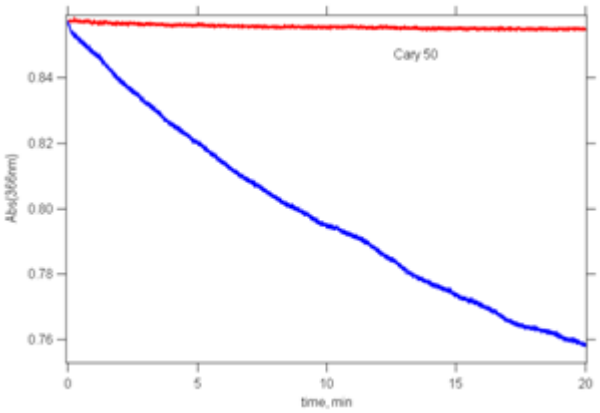

Figure 1. Absorbance(366 nm) vs time of platinum complex

This paper demonstrates the advantages of the Cary 50/60 UV-Vis when used to monitor photochemical reactions, by investigating the kinetics of [N,N'-*Bis*(2,3,5,6- tetrafluorophenyl)ethane-1,2-diaminato(2-)] dipyridineplatinum(II) in acetonitrile.

Background

The photochemistry of [N,N'-*Bis*(2,3,5,6-tetrafluorophenyl)ethane-1,2-diaminato(2-)] dipyridineplatinum(II)[i], referred from now on as **1a**, has been investigated in previous studies[2,4]. When irradiated with UV/blue light, solutions of **1a** undergo photochemical substitution in which a pyridine ligand is replaced by a solvent molecule (Figure 2). Continued irradiation sees the loss of a second pyridine and finally the formation of a tetrakis-platinum solvent species.

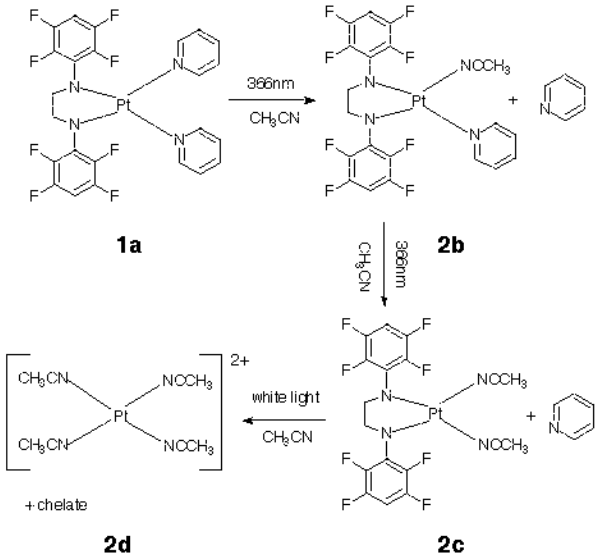

Figure 2. Schematic for the photosubstitution of 1a in acetonitrile

The quantum yield of photoproduct formation for the mono-solvento complex has been determined in acetonitrile, DMSO and THF[2]. In fact, the quantum yield measured in acetonitrile is the highest ever reported for a platinum photosubstitution reaction. The mechanism of photosubstitution is shown in Figure 3.

Irradiation with light of 366 nm populates a ligand-ligand charge transfer (LLCT) excited state, which is in thermal equilibrium with a ligand field (LF) excited state. High pressure photochemical experiments support a dissociative mechanism, in which the formation of the mono-solvento species occurs via a three coordinate transition state intermediate[2].

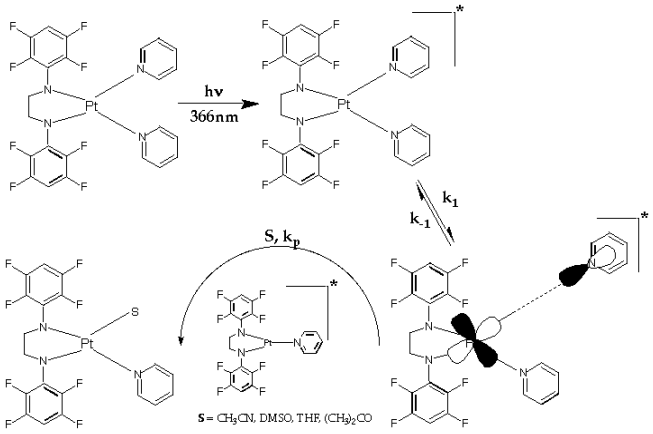

Figure 3. Mechanism of photosubstitution of 1a in solution

Experimental

Reagents:

- Acetonitrile (BDH, HPLC grade)
- [N,N'-*Bis*(2,3,5,6- tetrafluorophenyl)ethane-1,2-diaminato(2-)] dipyridineplatinum(II)
- Potassium ferrioxalate (0.0114 M)
- Buffer (CH_3COONa/H_2SO_4 pH 3.5)
- 1:10 phenanthroline (0.1% w/v)
- MilliQ water

Apparatus:

- Agilent Cary 50 (Cary 60) UV-Vis
- WinUV Software
- Single cell Peltier temperature accessory
- 10mm pathlength quartz cuvette
- 100W Hg arc lamp/housing
- 366 nm bandpass filter
- 3' quartz fibre optic bundle

Figure 4 shows the setup used for irradiation.

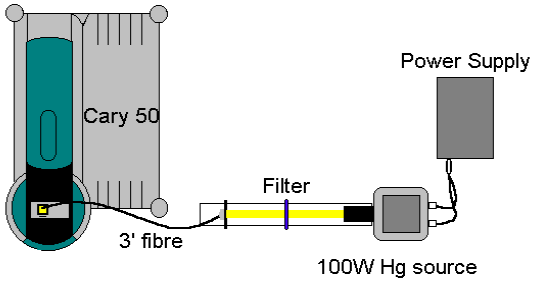

Figure 4. Setup of apparatus used for irradiating sample

White light from a 100W Hg source passes through a bandpass filter isolating the intense 366 nm Hg line, and is directed via a 3 foot fibre optic bundle into the top of a quartz cuvette. The solution in the cuvette, which is continuously stirred, immediately undergoes photochemical change upon irradiation from the Hg source. The solution is analysed using a Cary 50 UV-Vis with the WinUV Scanning and Kinetics software packages. A Cary 60 UV-Vis can also be used for this experiment.

Discussion

The intensity of the 366 nm irradiating beam from the Hg lamp was measured through chemical actinometry[5]. The method used is a modification from Hatchard and Parker's original procedure[5], the details of which are fully described in References 2 and 4 and summarised below.

Potassium ferrioxalate (0.0114M, 1.30 cm^3) was added to the quartz cuvette, immediately followed by CH_3COONa/H_2SO_4 buffer solution (pH 3.5, 0.70 cm^3) and 1:10 phenanthroline (0.1% w/v, 0.50 cm^3). The cuvette was placed in the thermostatted cell holder (25 °C) and allowed to equilibrate for 5-10 minutes in the dark. The solution was then irradiated with 366 nm Hg light and the absorbance at 510 nm monitored in the absence (no change in Abs, Figure 5) and presence (change in Abs, Figure 5) of the irradiating beam.

The instrument parameters used are outlined below.

Wavelength (nm)	510
Ave Time (s)	0.1
Y Min	0
Y Max	1.0
Cycle (min)	0
Stop (min) 30	30

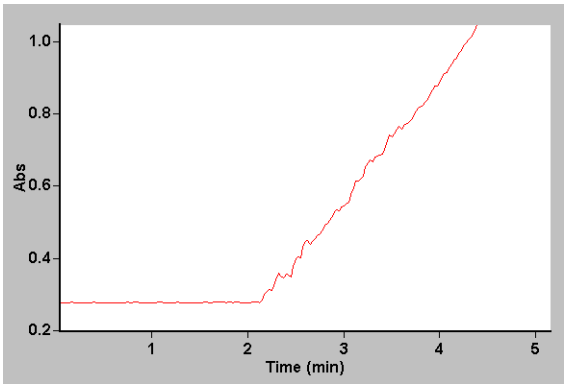

Figure 5. Absorbance(510 nm) vs time during irradiation with 366 nm Hg light

The change in absorbance only occurs in the presence of the irradiating beam. The intensity of the irradiating beam, in einsteins s^{-1} dm^{-3}, is calculated from this change in absorbance with time using Equations 1 and 2 below[6].

$$\frac{n\left[Fe(phen)_3\right]^{2+}}{dt} = \frac{dAbs_{510}}{dt}\frac{V}{\varepsilon_{510} l} \qquad \textbf{Equation 1}$$

Where :

V = volume of solution irradiated (dm^3)

ε_{510} = molar extinction coefficient at 510 nm of $[Fe(phen)_3]^{2+}$ ($M^{-1}cm^{-1}$)

l = pathlength (cm)

$$I_0 = \frac{\frac{n\left[Fe(phen)_3\right]^{2+}}{dt}}{\phi_{Fe^{2+},\lambda}} \qquad \textbf{Equation 2}$$

Equation 1 calculates the change in the number of moles of $[Fe(phen)_3]^{2+}$ with respect to irradiating time, which is used in Equation 2 to determine the intensity of the irradiating beam, I_0. The quantum yield for Fe^{2+} formation is 1.21, as determined by Hatchard and Parker[5]. The intensity of the irradiating source is determined before and after the photolysis of **1a**, and the result averaged to account for any intensity fluctuations in the Hg lamp over the course of the experiment. The lamp used is relatively stable over a period of days. The average intensity of the irradiating beam incident on the platinum solution is $9.32(2)x10^{-10}$ einstein sec^{-1} cm^{-2}.

Compound **1a** in acetonitrile (2.5 cm^3) was then irradiated with the Hg source. The change in spectra over a 250 nm range (Figure 6) and the absorbance at 366 nm (Figure 7) were recorded in two separate experiments using the WinUV Scan and Kinetics packages respectively.

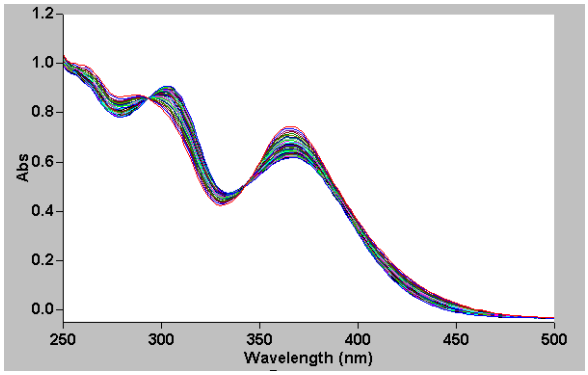

Figure 6. Change in absorption spectra of 1a in CH3CN during irradiation with Hg light

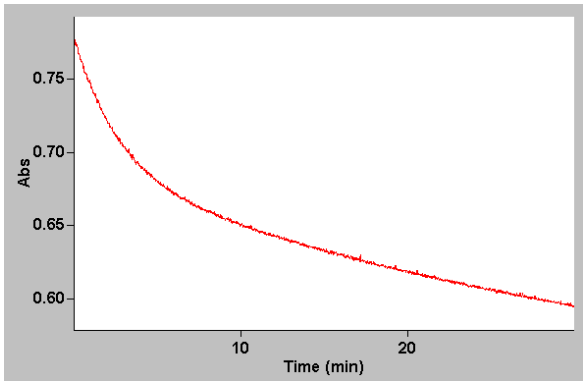

Figure 7. Change in absorbance(366 nm) as a function of time during irradiation

Figure 6 shows the formation of 2 isosbestic points, at 293 nm and 344 nm, during the initial stage of photolysis. During this time, only the mono-CH_3CN complex is being formed. The extremely fast scanning speed of the Cary 50/60 allows the fifty overlaid spectra in Figure 6 to be collected in less than 2 minutes.

The change in absorbance(366 nm) with time, $Abs_{\lambda,t}$, was used in Equation 3 to calculate the change in concentration of **1a**, $[A_t]$. The molar extinction coefficients at 366 nm of **1a** (ε_A) and **2b** (ε_B) have been previously determined[2], hence, d[**1a**]/dt can be calculated.

$$[A_t] = \frac{Abs_{\lambda,t} - (\varepsilon_B [A_0] l)}{l(\varepsilon_A - \varepsilon_B)} \qquad \textbf{Equation 3}$$

Where :

l = pathlength (cm)

[A0] = the initial concentration of 1a

The quantum yield of photoproduct formation (Φ_{PR}) of **2b** is defined as the amount of **2b** formed (or the amount of **1a** depleted), with respect to the amount of light absorbed by **1a**. The amount of light absorbed by **1a** over the course of the experiment (I_{abs}) is given by Equations 4-7[7].

$$I_{abs} = \int_0^t I_{abs}(t)dt \qquad \textbf{Equation 4}$$

$$I_{abs}(t) = I_{sol}(t)\alpha(t) \qquad \textbf{Equation 5}$$

$$I_{sol}(t) = I_0 \left(1 - 10^{-Abs_{\lambda,t} l}\right) \frac{S}{V} \qquad \textbf{Equation 6}$$

$$\alpha(t) = \frac{\varepsilon_A [A]_t l}{Abs_{\lambda,t}} \qquad \textbf{Equation 7}$$

Where:

I_{sol} = the amount of light absorbed by the solution

$\alpha(t)$ = the fraction of light absorbed by **1a**

S = the area exposed to irradiation (cm^2)

V = the volume of solution irradiated (dm^3)

The quantum yield of formation of **2b** is then determined by plotting the change in concentration of **1a** against I_{abs} (Figure 8), and fitting a linear regression over the first 10% of the reaction. The quantum yield is given by the gradient, as defined in Equation 8.

$$-\frac{d[A]}{dt} = \Phi_{PR} I_{abs} \qquad \textbf{Equation 8}$$

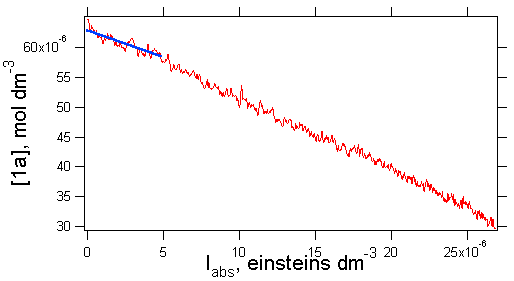

Figure 8. [1a] vs Iabs in acetonitrile

The WinUV software allows data to be exported as ASCII *.csv files. These files were imported into the mathematical analysis program IGOR PRO[8], where Equations 3-8 were calculated. Figure 8 was also generated in IGOR PRO. A calculated quantum yield of (0.92 ± 0.04) molecules per photon of light absorbed is in excellent agreement with the literature value of (0.96 ± 0.03)[2].

Conclusion

The Agilent Cary 50 and Cary 60 UV-Vis, with its room light immunity and fast scan speeds, has revolutionized the world of UV-Vis spectroscopy. Coupled with the powerful and versatile WinUV Kinetics and Scanning software, the Cary 50/60 UV-Vis is ideal for the study of photochemical kinetics. In analysing photochemical reactions, it allows the use of an irradiating light source without the problems associated with stray light. Also, studying even the most light-sensitive of compounds *in situ*, does not cause photodegradation from the analysing beam, which generally occurs when white light is used for analysis.

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Published March, 2011
Publication Number SI-A-1200

Agilent Technologies