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Application Compendium

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Application Note

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Analysis of PFAS and Other Environmental Contaminants in Soil and Oat Plants Using High-Resolution GC/Q-TOF

Authors

Luann Wong, Gabrielle Black, and Thomas Young
Department of Civil and Environmental Engineering, University of California, Davis

Sofia Nieto, Matthew Giardina, Matthew Curtis, and Tarun Anumol
Agilent Technologies, Inc.

Abstract

Soil is one of the major environmental repositories of per- and polyfluoroalkyl substances (PFAS)[1], and the presence of PFAS in soil can potentially lead to ground water and food contamination. Current PFAS methods typically only cover 40 to 80 PFAS and vastly underestimate their presence in many environmental samples based on mass balance studies.[2,3] Further, liquid chromatography/mass spectrometry (LC/MS) has significant limitations with respect to the analysis of some of the volatile classes of PFAS, which is where gas chromatography/mass spectrometry (GC/MS) should be considered as an important complementary technique.

This study describes different approaches for the extraction and analysis of PFAS in soil and plants using an Agilent 7250 gas chromatography/quadrupole time-of-flight mass spectrometer (GC/Q-TOF). PFAS and other environmental contaminants were detected using a target screening methodology based on an accurate mass personal compound database and library (PCDL) of these pollutants. A broader range of contaminants was also identified in the soil and plant samples, including polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and flame retardants, using a nontargeted screening and an extensive unit mass NIST23 library.

Introduction

PFAS are persistent synthetic organic pollutants with a potential to bioaccumulate.[4] The list of PFAS substances curated by the Environmental Protection Agency (EPA) currently includes nearly 8,000 PFAS chemicals based on structure[5] ranging from volatile PFAS, such as ubiquitous fluorotelomer alcohols (FTOHs), to long-chain PFAS, including the most commonly detected perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS). While long-chain PFAS are being phased out of production, the manufacturing of shorter chain length PFAS is increasing due to the assumption that more volatile PFAS are less toxic. These shorter chain length PFAS such as 6:2 FTOH are more difficult to detect with LC/MS using established methods, and recent studies have indicated that they are equally toxic.[6,7]

Soil is a significant reservoir of PFAS as well as of many other persistent environmental contaminants, and thus can contribute to contamination of ground water, atmosphere, and biota. Therefore, to better understand the source and transport of these contaminants, both soil and plant extracts have been analyzed using the Agilent 7250 GC/Q-TOF.

To maximize the sensitivity of PFAS detection, a target screening approach based on a PFAS accurate mass library was used. The PFAS PCDL used in this study included over 150 electron ionization (EI) PFAS spectra along with retention times (RTs) and retention indices (RIs), and is described elsewhere.[8]

In addition to PFAS, many persistent pollutants were identified in both soil and plants, where both target and nontarget screening workflows were used. These pollutants included pesticides, polyaromatic hydrocarbons (PAHs), PCBs, PBDEs, and flame retardants.

Experimental

Sample collection

Soil and oat plants were sampled from two fields in California (F1 and F2) that have historically received biosolids. The soil samples were collected prior to the application of biosolids (labeled PreA for preapplication). A certified USDA organic (Org) field was also sampled prior to treating the subplots with compost (Comp) and compost and lime (C&L). The compost was collected as well. The soil was also sampled at harvest time (Hvst). Plants were collected in the same regions as the soil samples.

Sample preparation

The soil and plant samples were either extracted with methylene chloride (DCM) for liquid injections or subjected to headspace solid-phase microextraction (HS-SPME). For DCM extraction, 2 g of soil was weighed into a 50 mL glass centrifuge tube and 5 mL of DCM was added. The 50 mL centrifuge tubes containing the samples were vortexed using a Heidolph Multi Reax Vibrating Test Tube Shaker at speed 5 for 5 minutes and centrifuged at 3,000 rpm for 5 minutes. Approximately 0.5 mL of supernatant extract was transferred into 2 mL autosampler vials. Whole plant samples that included stems, leaves, seeds, and seed pods were cut into 2 to 5 mm sections. Then, 2 g of plant samples were extracted with methylene chloride the same way as the soil. Method blank samples for each set were also generated.

For HS-SPME, the soil (2 g) and finely chopped plant material (1 g) were transferred into a 20 mL headspace vial, and either 2 or 3 mL of DI water were added, respectively.

SPME conditions

The HS-SPME was performed using an Agilent PAL 3 CTC autosampler. Four different fibers were tested (Agilent 100 µm PDMS, 95 µm CWR/PDMS, 65 µm DVB/PDMS, and 80 µm DVB/CWR/PDMS, part number 5191-5878), and the SPME conditions were optimized. The fiber conditioning was carried out at 300 °C for 5 minutes. The samples were equilibrated for 10 minutes, and the SPME fiber was inserted into the vial headspace. Extraction was carried out at 50 °C for 35 minutes at 300 rpm (programmed for 10 seconds on, 2 seconds off cycle), with the desorption into the GC inlet at 250 °C for 7 minutes. The GC injection port was equipped with the 0.75 mm id liner for SPME analysis and the resistant to wear Merlin Microseal septa.

Data acquisition

The GC/MS analysis was performed using an Agilent 7250 GC/Q-TOF system. All the data were acquired in full spectrum acquisition mode. Two different GC columns were used to acquire the data. The DB-624 is a midpolar GC column and provided the best retention and separation for GC-amenable PFAS compounds. This column was used for PFAS screening using the PFAS PCDL. The nonpolar DB-5ms column was also used to take advantage of RI information available for all the compounds with EI spectra in the NIST23 library. The data acquisition parameters are described in Table 1.

Table 1. Data acquisition parameters.

	Agilent DB-5ms	Agilent DB-624
MS	Agilent 7250 GC/Q-TOF	
GC	Agilent 8890 GC	
Inlet	Multimode inlet, Agilent Ultra Inert 4-mm liner single taper with wool	
Inlet Temperature	70 °C for 0.01 min, 300 °C/min to 250 °C	
Injection Volume	1 µL	
Column	Agilent J&W DB-5ms Ultra Inert (UI), 30 m × 0.25 mm, 0.25 µm	Agilent DB-624 Ultra Inert, 30 m × 0.25 mm, 1.4 µm
Oven Temperature Program	35 °C for 2 min, 7 °C/min to 210 °C, 20 °C/min to 300 °C, 4 min hold	30 °C for 2 min, 3 °C/min to 75 °C, 2 °C/min to 110 °C, 10 °C/min to 210 °C, 20 °C/min to 240 °C, 2 min hold
Column Flow	1.2 mL/min constant flow	1 mL/min constant flow
Carrier Gas	Helium	
Transfer Line Temperature	250 °C	
Quadrupole Temperature	150 °C	
Source Temperature	200 °C	
Electron Energy	70 eV	
Emission Current	Variable by time segment, 0.01 to 5 µA	
Spectral Acquisition Rate	5 Hz	
Mass Range (Tune)	50 to 1,200 *m/z*	

Data processing

The nontargeted workflow was performed in Agilent MassHunter Unknowns Analysis software (version 12.1) and involved the SureMass chromatographic deconvolution and the NIST23 EI library search. RIs and accurate mass information were used to confirm the compound identification. The suspect screening was performed using the GC/Q-TOF Screener tool of MassHunter Quantitative Analysis software (version 12.1) and accurate mass libraries for pesticides and PFAS.

Results and discussion

Characteristic EI fragmentation of PFAS

One of the approaches that could be beneficial for PFAS screening in complex matrices is a suspect screening approach since it allows for high sensitivity and specificity of detection. When using a high-resolution accurate mass GC/MS, this approach can be greatly facilitated by using accurate mass libraries to screen for a large number of target compounds that could, in theory, be unlimited. Thus, the accurate mass GC/MS PCDL for over 100 volatile and semivolatile PFAS compounds that was previously created[6] was used in this work for PFAS screening in soil and plant samples. The PFAS compound classes in the PCDL included perfluoroalkyl iodides (PFAIs), fluorotelomer iodides (FTIs), fluorotelomer alcohols (FTOHs), fluorotelomer olefins (FTOs), fluorotelomer acrylates (FTACs), fluorotelomer methacrylates (FTMACs), fluorotelomer carboxylic acids (FTCA), fluorotelomer unsaturated carboxylic acids (FTUCA), perfluoroalkane sulfonamides (FASA), among others, many of which are uniquely amenable to GC/MS analysis. The electron ionization (EI) mode was chosen for the PFAS PCDL as a more universal technique compared to chemical ionization. EI covers a broader range of PFAS compound classes and allows users to easily screen for other contaminants in the same data file. While many PFAS compounds can highly fragment in EI, most of them nevertheless have specific fragment ions that could be selected by the GC/Q-TOF suspect screening algorithm or manually, as target or qualifier ions. Some of the most typical and specific fragments for the different PFAS compound classes are shown in Table 2.

Table 2. Characteristic fragments of volatile PFAS in EI.

Characteristic Fragments	Neutral Loss (*m/z*)	PFAS Class; % of Base Ion, as Maximum Observed for a Given PFAS Class							
		FTOH	PFAI	FTI	FTAC	FTMAC	FTO	PFAL	FASA
[M]+	0	–	40	100	30	90	–	–	–
[M-I]+	126.9045	–	100	–	–	–	–	–	–
[M-H_2O-HF]+	38.0168	100	–	–	–	–	–	–	–
[M-CHO-F]+	48.011	–	–	–	–	–	–	90	–
[M-H_2O-F-HF-C_2H_2]+	83.0308	80	–	–	–	–	–	–	–
[M-H_2O-2F]+	56.0074	70	–	–	–	–	–	–	–
[M-C_2F_5]+	118.992	–	–	50	–	–	–	–	–
[M-HF-I]+	146.9107	–	–	50	–	–	–	–	–
[M-H_2O-CF_3]+	87.0058	30	–	–	–	–	–	–	–
[M-H_2O-2F-CF_3]+	126.0026	30	–	–	–	–	–	–	–
[M-F]+	18.9984	6	–	–	10	5	5	–	1
[M-CHO-2F]+	66.9995	–	–	–	–	–	–	25	
[M-SO_2-CH_3]+	78.9854	–	–	–	–	–	–	–	25
[M-H_2O-CF_2]+	68.0074	25	–	–	–	–	–	–	–
[M-HF]+	20.0062	20	–	–	–	–	–	–	–
[M-2F-CF_3]+	106.992	–	–	–	–	–	20	–	–
[M-H]+	1.0078	15	–	1	–	–	–	–	–
[M-CH_3]+	15.0235	–	–	–	–	10	–	–	5
[M-H-HF]+	21.0141	15	–	–	–	–	–	–	–
[M-F-2HF]+	59.0109	15	–	–	–	–	–	–	–
[M-H_2O-F]+	37.009	15	–	–	–	–	–	–	–
[M-CF_3-HF]+	89.0014	–	–	10	–	–	5	–	–
[M-F-HF]+	39.0046	–	–	–	–	–	10	–	–
[M-NH_2SO_2]+	79.9806	–	–	–	–	–	–	–	10
[M-C_2H_3-2F]+	65.0203	–	–	–	–	–	10	–	–
[M-CHO]+	29.0027	–	–	–	–	–	–	5	–
[M-SO_2-H]+	64.9697	–	–	–	–	–	–	–	5
[M-SO_2-F]+	82.9603	–	–	–	–	–	–	–	5
[M-SO_2-CF_3-HF]+	152.9633	–	–	–	–	–	–	–	5

Selection of SPME fiber for soil analysis

Four different SPME fibers were evaluated for the ability to extract volatile compounds (including PFAS) from soil: PDMS, CWR/PDMS, DVB/PDMS, and DVB/CWR/PDMS. The test was performed using soil (2 g) sampled from the same location, mixed with 2 mL of water, and run under the same SPME conditions. Total ion chromatograms (TIC) generated by each fiber tested are shown in Figure 1. Both DVB/PDMS and DVB/CWR/PDMS fibers produced a significant number of peaks and showed the ability to extract a wide range of compounds.

The number of the identifiable peaks was also evaluated for each of the fibers (Table 3). DVB/PDMS and DVB/CWR/PDMS had a comparable number of library hits that was slightly higher for DVB/CWR/PDMS. Therefore, it was selected for further analysis.

Table 3. SPME fibers performance. The number of components generated by the SureMass deconvolution algorithm as well as number of NIST23 library hits (Library Match Score cutoff 75) are shown.

Fiber Type	Number of Components	Number of Hits
PDMS	422	228
CWR-PDMS	514	419
DVB-PDMS	687	560
DVB-CWR-PDMS	683	570

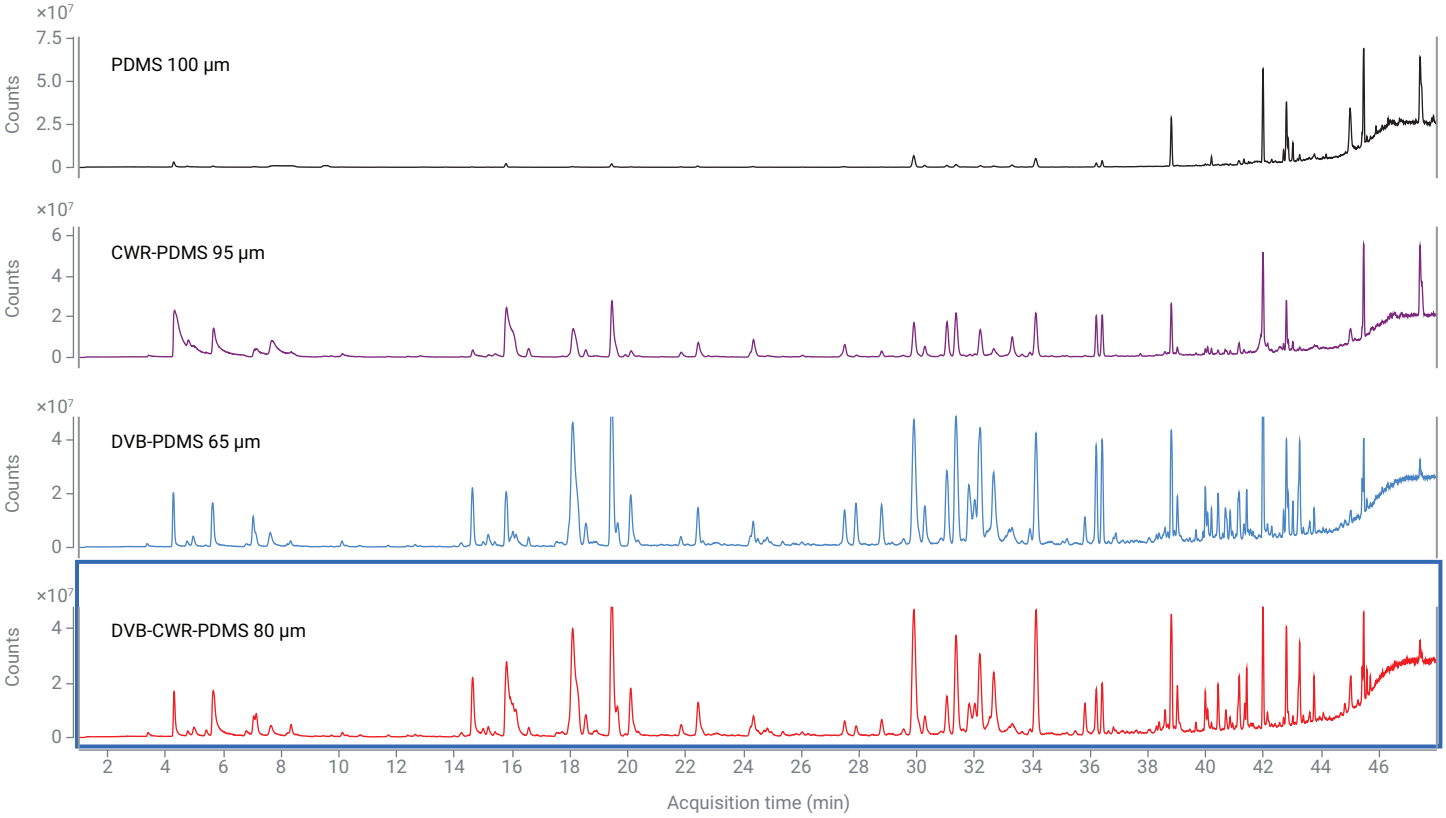


Figure 1. SPME fiber performance on soil samples.

Detection of volatile PFAS in soil and plant samples using accurate mass PFAS library

For PFAS detection using the accurate mass PFAS PCDL for GC/Q-TOF, the midpolar DB-624 GC column (for more detail, see Table 1) was used.

Both targeted and nontargeted methodologies that involved the GC/Q-TOF Screener and the Unknowns Analysis software, respectively, were used to identify PFAS in soil and plant samples. An advantage of using the nontargeted analysis is that both accurate mass libraries, as well as large comprehensive public libraries such as NIST, could be used to screen for the contaminants simultaneously without reinjection.

There are several benefits of the targeted, PCDL-based suspect screening approach, which is performed entirely in MassHunter Quantitative Analysis software and has been described in detail previously.[9,10] Some of the main advantages of this approach include high sensitivity and a high degree of flexibility and automation of the data analysis method setup and results validation. The validation requires minimal human involvement prior to the reporting. Together this provides a user with a highly effective and time-saving tool for targeted analysis.

A few PFAS compounds were detected when analyzing the data from SPME. An example of a compound identified in a few soil and plant samples using the GC/Q-TOF Screener is shown in Figure 2. This compound is a volatile 6:2 fluorotelomer alcohol, frequently detected in environmental matrices. Due to the trace amount of this compound, it was not found in a nontargeted approach.

Sample: soil, F1

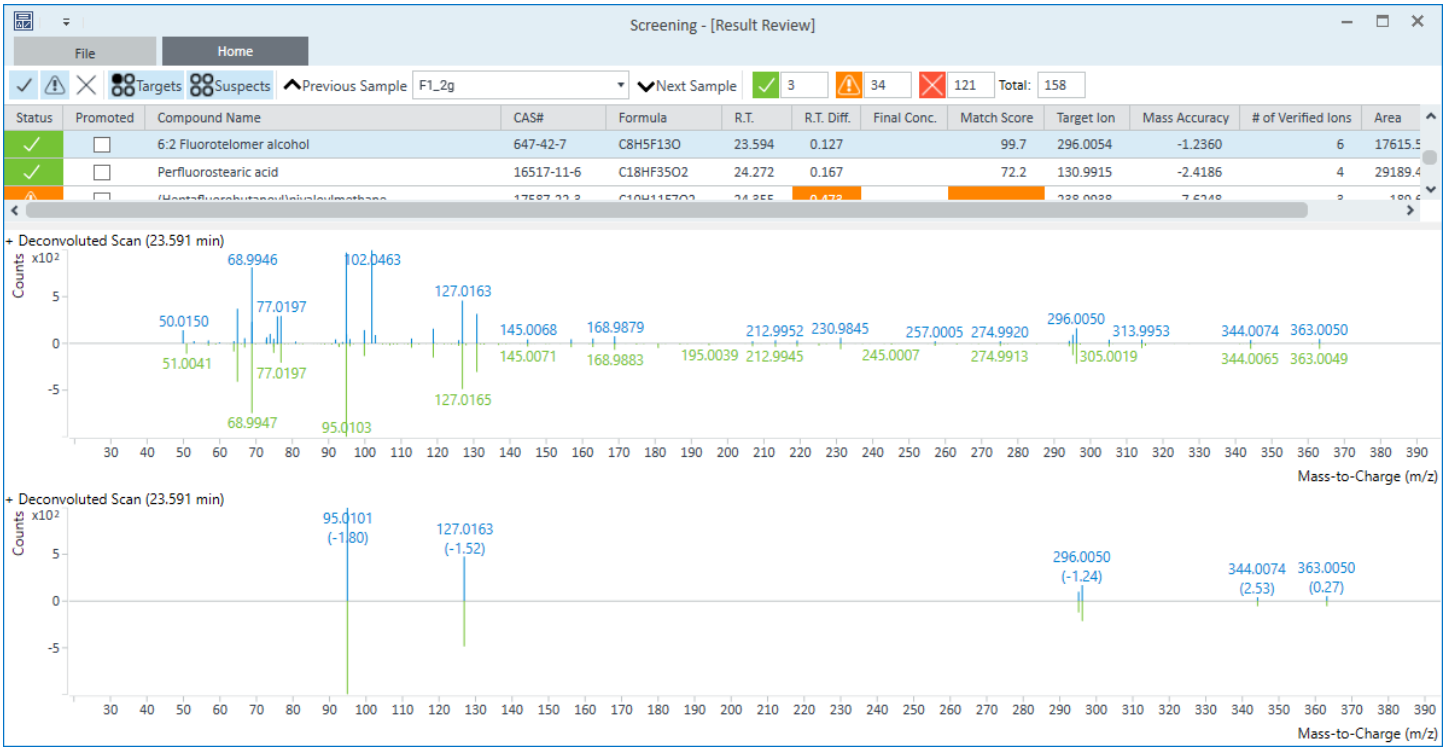
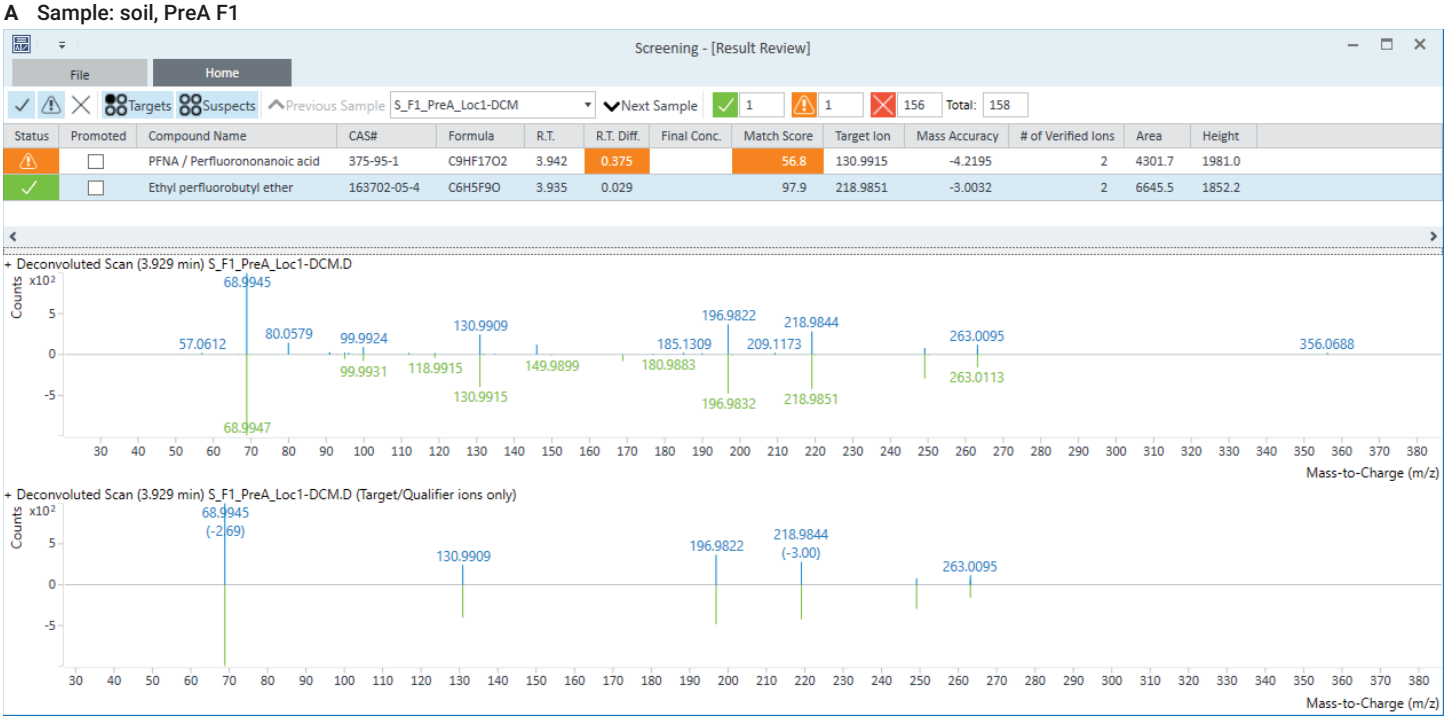


Figure 2. 6:2 FTOH detected in soil using SPME and PFAS PCDL-based screening approach. The mirror plot at the top shows the deconvoluted compound spectrum versus the spectrum from the PFAS PCDL. The mirror plot at bottom displays only target and qualifier ions.

When performing nontarget analysis, the chromatographic deconvolution uses the SureMass algorithm, which is specifically optimized for high-resolution EI data to ensure high speed, sensitivity, and integrity of spectral extraction. While the suspect screening approach provided the highest degree of sensitivity and was able to detect more PFAS compounds, one of the more abundant PFAS was detected in both approaches (Figures 3A and 3B).

All the PFAS compounds identified in soil and plant samples were detected by matching the PFAS PCDL.

A Sample: soil, PreA F1



B Sample: soil, PreA F1

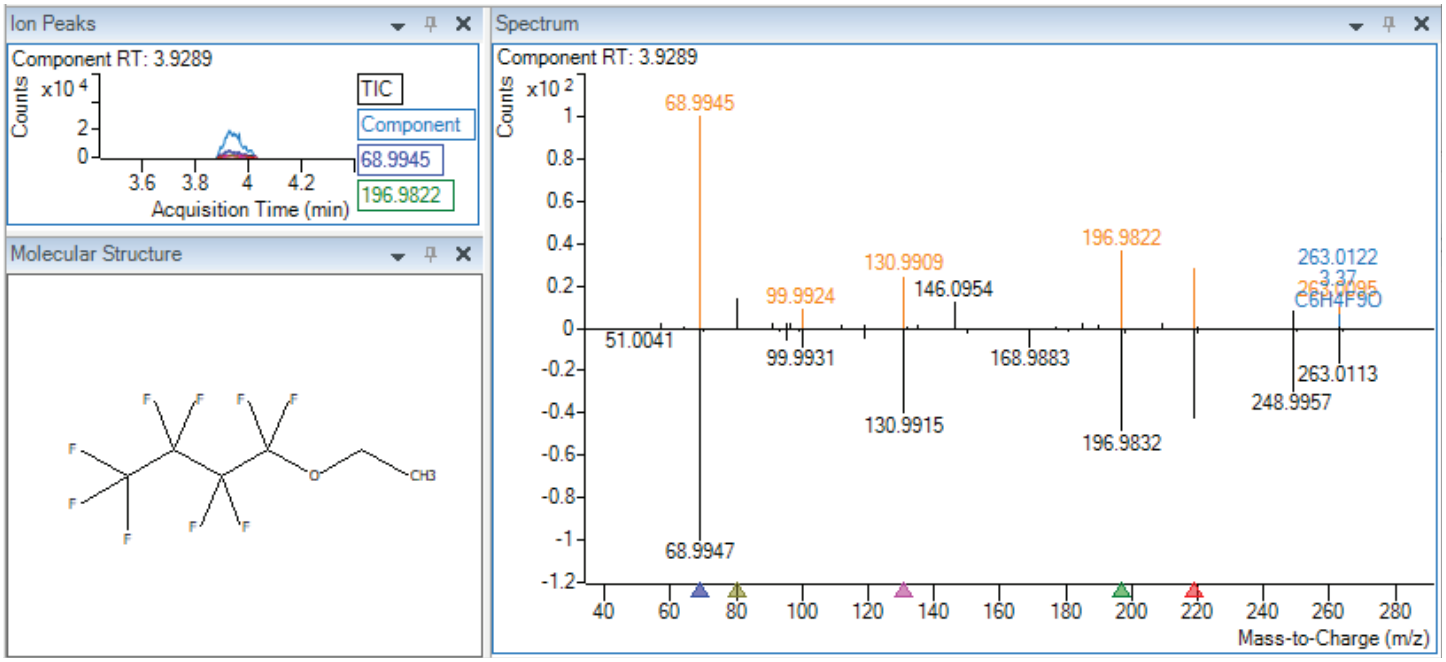


Figure 3. PFAS (ethyl perfluorobutyl ether) identified in DCM soil extracts using PFAS PCDL in both target screening (A) and an untargeted, deconvolution-based approach in the Unknowns Analysis software (B).

Overall, the HS-SPME approach for PFAS extraction worked best and provided a higher number of identified volatile PFAS compounds. The amounts of PFAS detected (summarized in Table 4) were estimated based on the standard injections where the actual concentrations in soil and plant samples have not been determined.

Identification of other contaminants in soil and oat plants

The identification of the additional contaminants in soil and plant samples was performed for both DCM extracts and SPME. However, while SPME allowed better detection of volatile compounds, a higher number of the environmental contaminants that included PCBs, PBDEs, pesticides, PAHs, and flame retardants, were detected in DCM extracts and will thus be the focus of the further discussion.

The separation was carried out using the DB-5ms UI column to be able to use the RI values from the extensive NIST23 library, and thus increase confidence in compound identification by using the RI penalty function for library hits. This column phase is also compatible with the GC/Q-TOF Pesticide PCDL, which could be considered for screening GC/Q-TOF accurate mass EI data for pesticides and PAHs. After a quick prescreening, the identified pollutants were grouped by contaminant classes and approached separately.

PCBs and PBDEs were identified in the nontargeted approach using the Unknowns Analysis and NIST23 library. To eliminate false positives based on the accurate mass EI data while searching a unit mass library, the Unknowns Analysis ExactMass tool was used. This tool is described in further detail in Figure 4A, which shows an example of one of the BDEs detected in a soil extract.

There were 20 different PCBs and PBDEs detected in the soil extracts (Figure 4B). The only oat plant extract where this group of contaminants (BDE-47) was detected was grown in field F2.

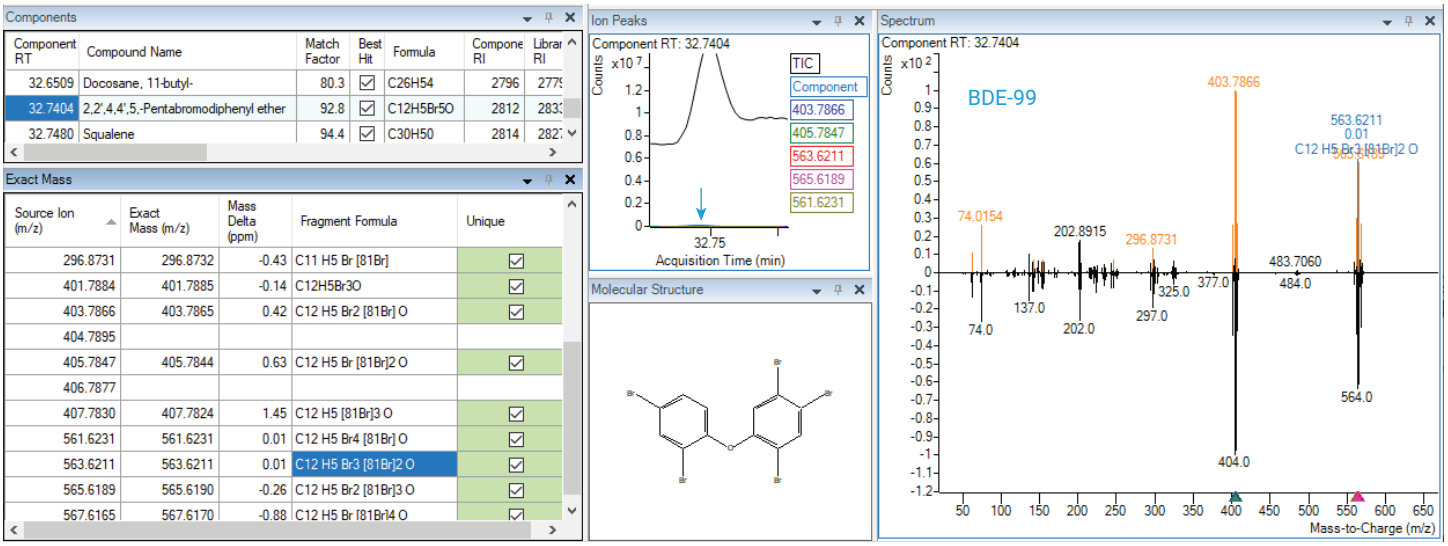
Note that PCBs and PBDEs were not detected by SPME due to their high boiling point.

Another prominent group of contaminants identified in soil extracts was pesticides, and the GC/Q-TOF Screener workflow with Pesticide PCDL was used for quick and streamlined detection of these pollutants. The original version of the Pesticide PCDL, which is RT-based, was supplemented with RIs to be able to use the PCDL in the screener workflow together with the data acquired using a different chromatographic method. The GC/Q-TOF Screener method was set up in agreement with the SANTE guidelines. However, RT windows were expanded to allow for an additional RT error introduced using a different chromatographic method.

Table 4. PFAS detected in soil and plants by HS-SPME using the accurate mass PFAS PCDL and suspect screening approach. The estimated amounts (in pg on column) are shown.

Compound	RT	Quantifier Ion	Soil Samples							Plant Samples				
			F1 PreA	F1 Hvst	F2 Hvst	C&L Hvst	Compost Hvst	Organic Hvst	Organic Compost	F1	F2	Compost	C&L	Org
Ethyl Perfluorobutyl Ether	4.4	218.9851	150.2	–	–	–	–	–	–	–	–	–	–	–
6:1 Fluorotelomer Alcohol	20.94	130.9915	–	2	–	–	–	–	–	2.2	–	–	–	–
6:2 Fluorotelomer Alcohol	23.59	296.0054	–	7.5	0.3	–	–	–	6.9	2.5	–	–	–	–
N-Methylperfluorooctanesulfonamide	43.1	93.9957	0.3	3.4	0.9	2.1	0.4	1.2	0.9	0.2	–	–	–	–

A **Sample: soil, F1**



B

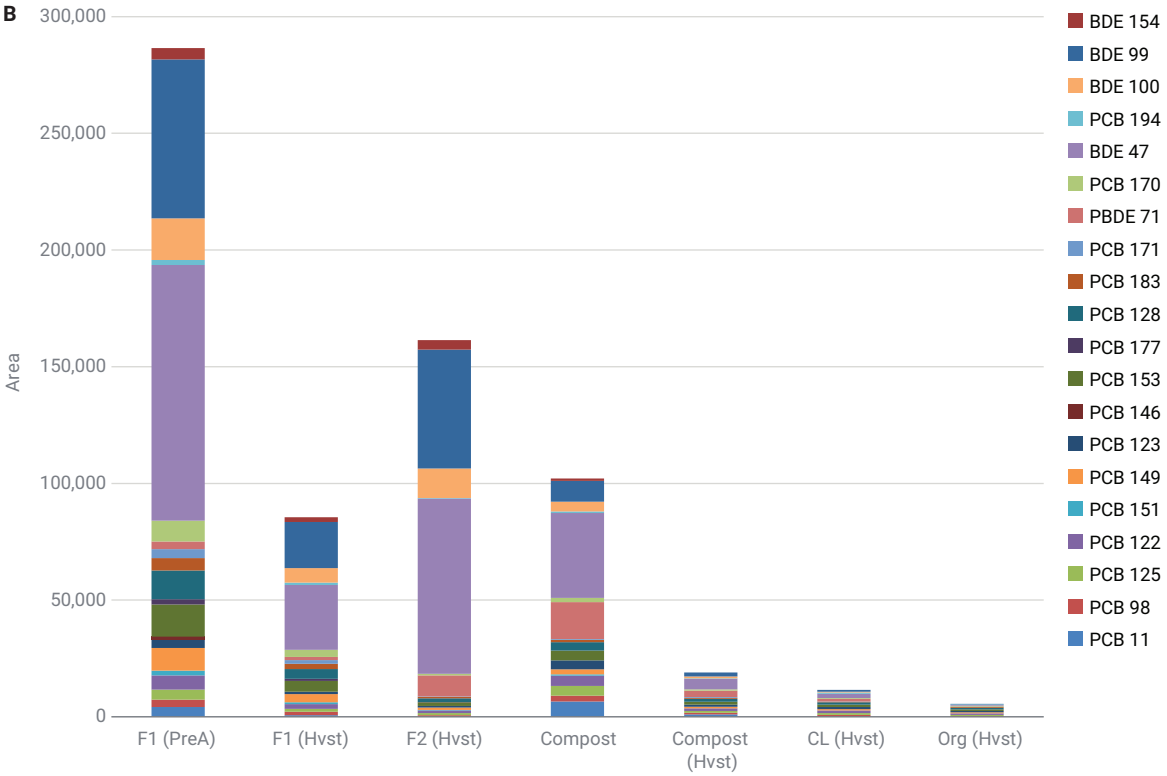


Figure 4. PCBs and PBDEs in soil DCM extracts using NIST23. (A) An example of BDE detected in soil sample collected from F1 at the time of harvesting. ExactMass table (bottom-left panel) shows how well the accurate mass fragment ions matched the unit mass library hit and thus provides the additional confirmation of compound ID. The most selective and abundant ions are highlighted in the mirror plot when *m/z* corresponds to the library hit formula. The arrow in the component chromatogram points to the identified component EICs. (B) Bar graph showing responses of PCB and PBDE for all the soil samples where they have been identified.

Since the DCM extraction method was not optimized specifically for pesticide recovery from plant matrices, only soil samples were processed. In total, over 50 pesticides were detected in soil extracts (Figure 5 and Table 5).

A large number of pesticides were detected in compost and compost-treated soils, and a few pesticides were also identified in organic soil extracts. Another interesting observation was that the insecticides fipronil sulfide and fipronil sulfone were consistently found in the same soil samples. Also, conazole fungicides such as propiconazoles, myclobutanil and difenconazole were mostly detected in compost and compost-treated soils.

Sample: soil, F1

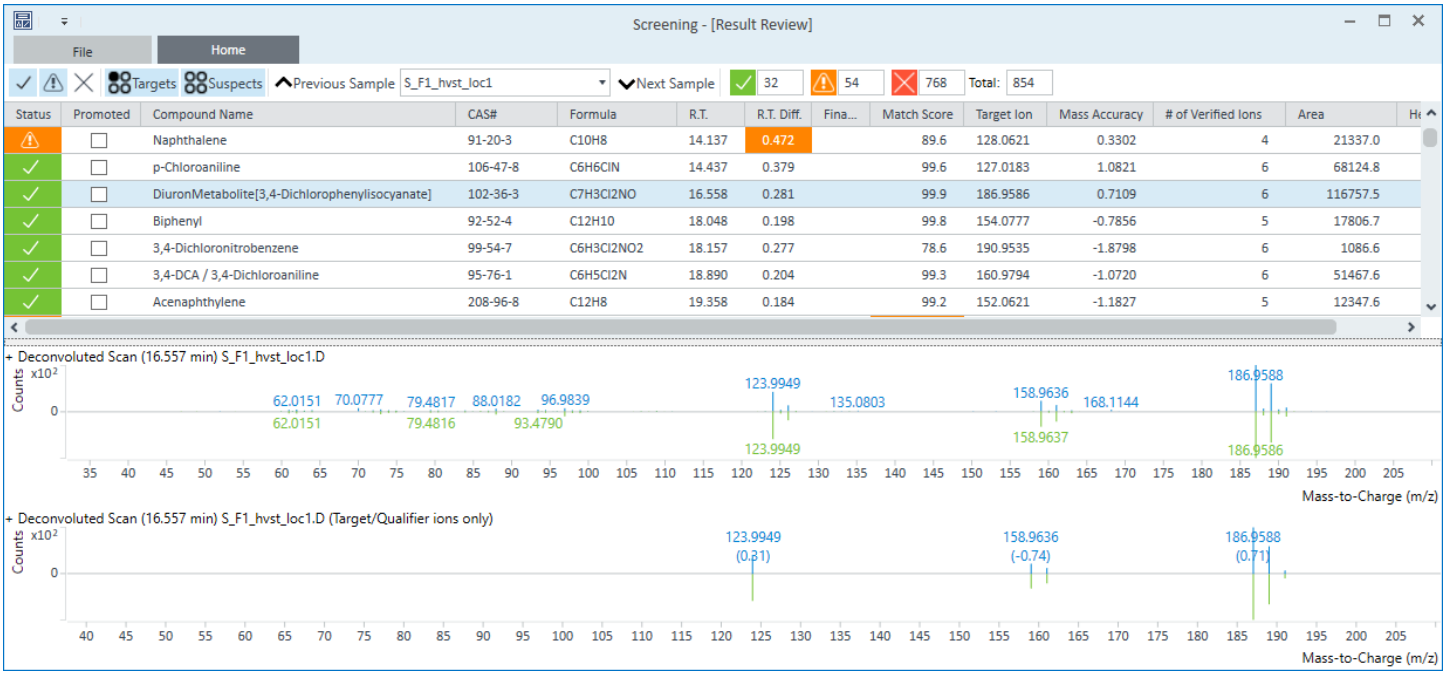


Figure 5. GC/Q-TOF Screener window of MassHunter Quantitative Analysis software when screening for pesticides in soil extracts using the Pesticides PCDL.

Table 5. Pesticides detected in soil extract using the accurate mass Pesticide PCDL and suspect screening approach.

Compound Name	RT	RT Delta*	Library Match Score	F1 PreA	F1 Hvst	F2 Hvst	C&L Hvst	Compost Hvst	Org Hvst	Org Compost
1,2,4-Trichlorobenzene	14.62	0.17	98.1	x						
Diuron Metabolite	16.56	0.28	99.9	x	x	x	x	x		x
1,2,3,5-Tetrachlorobenzene	16.98	0.32	90.7	x						
2,4,6-TCP/2,4,6-Trichlorophenol	17.44	0.25	99.3				x	x	x	Trace
Nicotine	17.56	0.05	97.9							x
Lufenuron	18.71	0.22	99.7			x				x
3,4-DCA/3,4-Dichloroaniline	18.88	0.21	99.9	x	x	x				
Pentachlorobenzene	20.42	0.35	99.4	x	x					x
DEET/Diethyltoluamide	21.46	0.22	82.1	Trace	Trace	Trace	x	x	Trace	x
2,3,4,5-Tetrachloroanisole	22.74	0.32	99.8	x						x
Bromoxynil	23.14	0.09	99.9	Trace			x	x		
HCB/Hexachlorobenzene	23.52	0.36	99.7	x	x	x	Trace	x	x	x
Dichloran (Dicloran)	23.84	0.16	97.8		x	x				x
Swep (MCC)	24.27	0.10	85.6	x	x	x				
PCP/Pentachlorophenol	24.27	0.24	99.7				Trace	x		x

Compound Name	RT	RT Delta*	Library Match Score	F1 PreA	F1 Hvst	F2 Hvst	C&L Hvst	Compost Hvst	Org Hvst	Org Compost
Pyrimethanil	25	0.10	82.5					Trace		x
Chlordene	25.1	0.03	98.5							x
Pentachloroaniline	25.74	0.24	99.7	x	x		x	x		x
Dithiopyr	26.85	0.40	93.7	x	x	x	x	x		x
Anthraquinone	27.59	0.05	99.7	x	x	x	x	x	x	x
4,4'-Dichlorobenzophenone	27.86	0.02	80.3	x						x
Fipronil Sulfide	28.13	0.43	99.7			x	Trace	x		x
Cyprodinil	28.27	0.05	99.1				Trace	x		x
Diuron	28.28	0.31	78.1				Trace	x		Trace
Fluopyram	28.49	0.18	93				x	x		x
Chlorbenside	28.99	0.21	92.2	x	x	x				
Chlordane-*trans* (γ-Chlordan)	28.82	0.03	99.7	x	x	x	x	x	x	x
Triclosan	28.85	0.03	99.3	x	x	x		x		x
Chlordane-*cis* (α-Chlordan)	29.06	0.04	99.9	x	x	x	x	x	x	x
Nonachlor-*trans*	29.11	0.07	99.9	x	x	x	x	x	x	x
Flutolanil	29.23	0.11	85.2					x		x
Fludioxonil	29.27	0.18	99.6							x
Dieldrin	29.5	0.05	84.6	x	Trace	Trace				
p,p'-DDE	29.42	0.04	99.2	x	x	x	x	x	x	x
Oxadiazon	29.43	0.14	97.7				x	x		x
o,p'-DDD (Mitotane)	29.52	0.07	99.9	x	x	x	Trace	x	x	
Fipronil Sulfone	29.34	0.33	98.6	Trace		x	Trace	x		x
Myclobutanil	29.48	0.11	98.8					x		x
p,p'-DDD	30.03	0.02	99.5	x	x	x	x	x	x	Trace
Nonachlor-*cis*	30.03	0.04	99.9	x	x	x	Trace	Trace		x
Carfentrazone-ethyl	30.32	0.13	98.8			x				
Bromoxynil octanoate	30.39	0.06	88.4	x			x	Trace		
Propiconazole I	30.43	0.11	96.4				x	x	Trace	x
Chloridazon (PAC)	30.44	0.06	91.5					x		
Propiconazole II	30.5	0.04	96				x	x	Trace	x
Tebuconazole	30.7	0.03	92.7				x	x		x
Chlorbenside Sulfone	30.7	0.39	89.6		Trace	Trace	x	x		x
Bifenthrin	31.08	0.09	98.6	Trace	x	Trace	x	x	x	x
cis-Permethrin	32.19	0.00	99.1	x	x	x	Trace	x	x	x
trans-Permethrin	32.28	0.01	81			x			Trace	Trace
Difenconazole II	34.09	0.01	88.9					Trace		x

* RT delta is recalculated from RI.
Trace indicates that the Library Match Factor is below 75.

PAHs are included into the Pesticide PCDL and were screened together with pesticides in a single workflow. PAHs were mostly detected in soil extracts. However, phenanthrene and fluoranthene were also identified in most plant samples. This was not unexpected considering that phenanthrene and fluoranthene were the most abundant PAHs detected in the soil.

A prominent group of contaminants identified in soil and oat plants was flame retardants. The accurate mass spectra for most of these compounds are already included in the Pesticide PCDL. For a more comprehensive coverage of this group of pollutants, a couple of flame retardants identified in the Unknowns Analysis with NIST23 library, missing in the Pesticide PCDL, were added to the Quant method directly from the Unknowns Analysis software, and thus were screened together with the rest of the targets. Remarkably similar responses were observed between soil and plant extracts for this group of pollutants (Figure 7).

Among the most abundant flame retardants identified in soil and plant samples were tributyl phosphate, tris(2-chloropropyl) phosphate, and tris(3-chloropropyl) phosphate, the phosphorus flame retardants of frequent use.

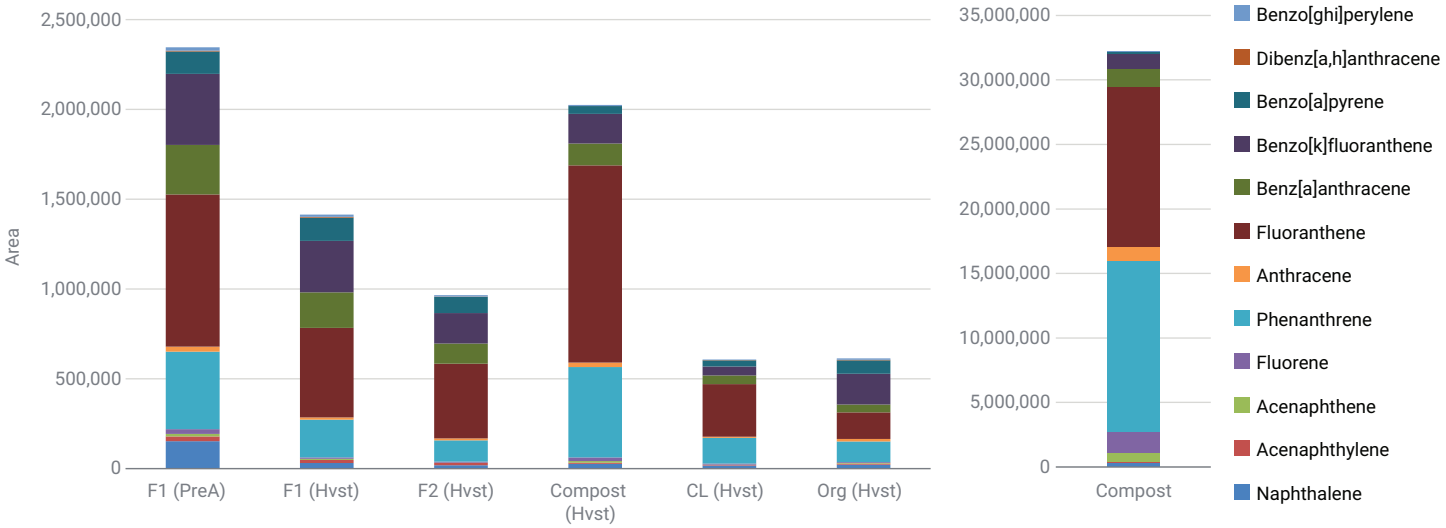


Figure 6. PAHs detected in soil DCM extracts using the accurate mass Pesticide PCDL and suspect screening approach. The bar graph shows the PAH peak area.

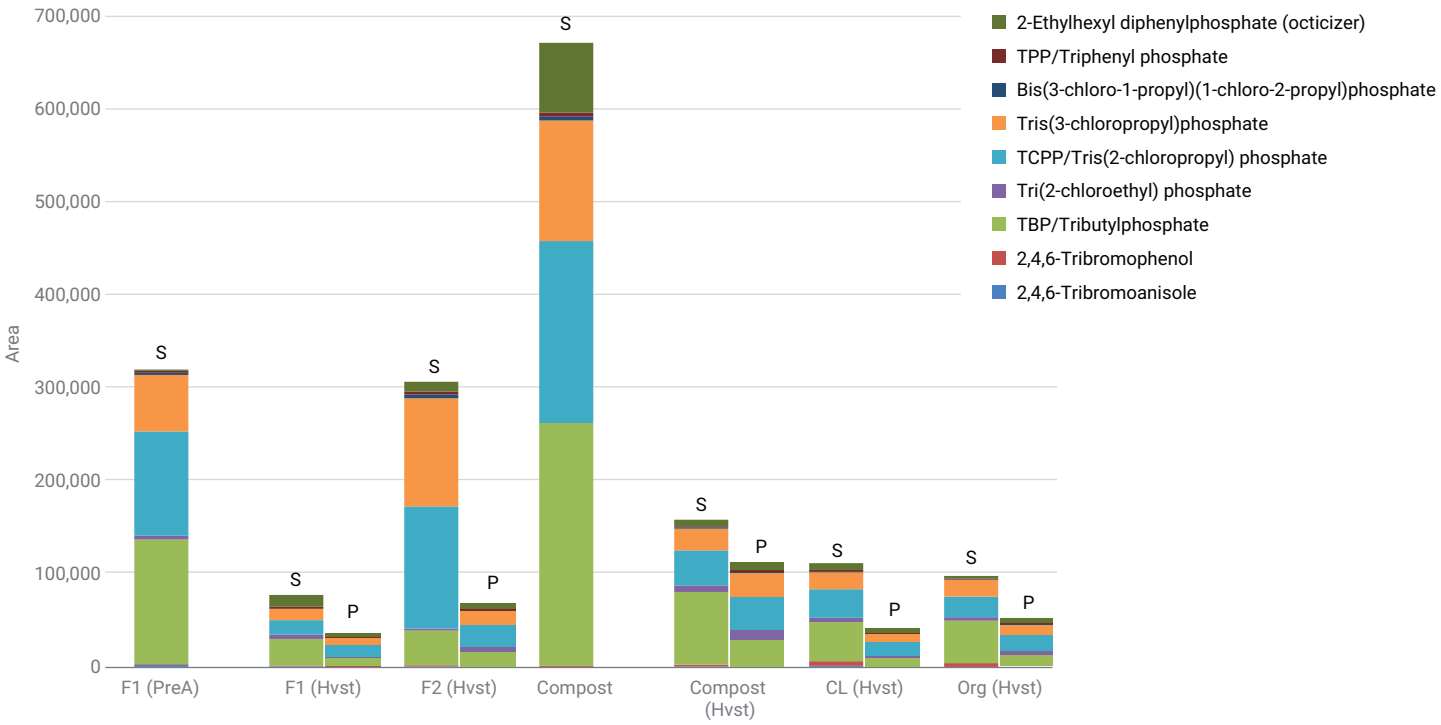


Figure 7. Flame retardants detected in soil and plant DCM extracts using a combined screening approach that included both accurate mass PCDL as well as NIST23 library. The bar graph shows flame retardant response in soil (S) and plant (P) extracts.

Conclusion

PFAS analysis in environmental matrices is a challenging undertaking. In this application note, different approaches for PFAS extraction from soil and plants as well as downstream workflows of data processing have been discussed. The most efficient and sensitive approach for volatile PFAS analysis in soil and plants suggested here is HS-SPME combined with the suspect screening based on the PFAS accurate mass library and high-resolution accurate mass GC/Q-TOF.

In addition, both soil and plant extracts were screened for other contaminants, and various pollutants including PCBs, PBDEs, PAHs, pesticides, and flame retardants were identified using targeted, nontargeted, and combined methodologies.

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Printed in the USA, May 6, 2024
5994-7351EN

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Analysis of Phthalate with Hydrogen Carrier Gas

Using the Agilent HydroInert source on a challenging cable sample in gas chromatography/mass spectrometry

Authors

Dinh Loc Dao,
Minh Trung Tran,
Xuan Dai Phan,
Quoc Trung Pham,
Thi Linh Nguyen, and
Tuan Dat Ho
IndoChina Center of Excellence and
Red Star Vietnam Company Limited – CMS.
Ho Chi Minh City, Vietnam

Boonraksa Srisawang
Agilent Technologies, Inc.
Singapore, Singapore

Abstract

Gas chromatography/mass spectrometry (GC/MS) plays a pivotal role in the analysis of phthalates in consumer products. In light of the recent helium supply challenges, the adoption of hydrogen (H_2) as a carrier gas has gained prominence. However, using hydrogen as carrier gas in many GC/MS analyses may cause hydrogenation or dechlorination problems in the ion sources that use helium gas as carrier gas. The Agilent GC 8890, coupled with the Agilent 5977C GC/MSD and using the Agilent HydroInert source with hydrogen as the carrier gas with the backflush technique, provides an effective solution to these challenges. It not only meets International Electrotechnical Commission (IEC) requirements but also successfully addresses the issue of instability when dealing with complex sample matrices.

Introduction

Phthalates are a group of chemical compounds that are synthesized from phthalic acid. They are widely used as plasticizers in many products, including children's toys and electrical and electronic equipment. Unfortunately, phthalates can leach out of plastic materials and enter the environment. This exposure can have adverse effects on human health.

The prevalence of phthalates in various products and the associated health risks have led to the implementation of regulations governing their use. Relevant policies include the European Union's updated directive under the Restriction of Hazardous Substances (RoHS) framework, designated as 2015/863/EU[1], and its 2007 Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH) framework.[2]

Traditionally, helium has been the preferred carrier gas for GC/MS analysis. However, due to recurring helium shortages and rising costs, hydrogen is emerging as a viable alternative. Hydrogen is more cost-effective and efficient than helium, making it a promising prospect for phthalate analyses.

This application note centers on the analysis of phthalates using a GC/MS system in Selected Ion Monitoring (SIM) mode with hydrogen serving as the GC carrier gas. When transitioning to hydrogen for GC/MS analysis, several critical factors must be considered.

Hydrogen, being a chemically reactive gas, can induce chemical reactions within various components of the GC/MS system, including the inlet, column, and sometimes the mass spectrometer Electron Ionization (MS EI) source. These reactions can alter the mass spectra of peaks in the total ion chromatogram (TIC), potentially leading to the misidentification of compounds in the analysis results.

The Agilent HydroInert source was developed to mitigate these concerns related to the MS source. The HydroInert source, when used with hydrogen as the carrier gas, preserves mass spectral accuracy and allows users to continue using existing helium-based mass spectral libraries and quantitative methods.

This application note uses the solvent extraction method, which is in accordance with the analytical methodology outlined in IEC standard 62321-8:2017.[3] The presence of residual sample matrix components in this method can contaminate essential column components, such as the inlet, column, and ionization source. This contamination can adversely affect the accuracy and reliability of the instrument during analysis.

The self-cleaning capability of the hydrogen gas ionization source, combined with the backflush technique, helps address issues related to sample background effects, reduces maintenance frequency, and ensures precision and accuracy throughout the analysis process.

Experimental

Reagents and chemicals

- Tetrahydrofuran (THF) 99.9% was purchased from Sigma-Aldrich.
- HPLC-grade acetonitrile (ACN) was obtained from Supelco.
- Benzyl benzoate (BB) 98%, benzyl butyl phthalate (BBP) 98%, dibutyl phthalate (DBP) 98%, di-isobutyl phthalate (DIBP) 98%, di-n-pentyl phthalate (DPENP) 2,500 mg/L, di-n-hexyl phthalate (DHEXP), dicyclohexyl phthalate (DCHP) > 98%, di(2-ethylhexyl) phthalate (DEHP) > 98%, dinoctyl phthalate (DNOP) > 98%, di-isononyl phthalate (DINP) > 98%, and di-isodecyl phthalate (DIDP) were obtained from Sigma-Aldrich.

Standards solution and standards preparation

Phthalate ester standard solutions were prepared by diluting a mixture of phthalate ester standards at a concentration of 100 mg/L in a mixture of THF:ACN (1:1) to create a series of standards with concentrations of 0.2, 0.5, 1, 2, and 5 µg/mL, respectively. Also, each of the standard solutions was supplemented with an internal standard, BB, at a concentration of 1 µg/mL.

Sample preparation

1. Weigh 300 mg of the sample and transfer it to a 40 mL vial. Record the mass to the nearest 0.1 mg.
2. Add 10 mL of THF and 30 µL of internal standard BB (1,000 µg/mL) to the vial.
3. Seal the vial tightly and place it in an ultrasonic bath. Sonicate for 30 to 60 minutes until the sample dissolves.
4. After the sample has completely dissolved, let the vial cool to room temperature.
5. Carefully add 20 mL of ACN drop by drop into the vial to precipitate the sample matrix.
6. Let the resulting solution stand at room temperature for 30 minutes.
7. Allow the polymer to settle or filter the mixture through a 0.45 µm polytetrafluoroethylene membrane.
8. Inject on GC/MS.

Instrumentation

The experimental system used in this study was configured (Figure 1) to address and reduce potential challenges arising from the use of hydrogen as the carrier gas and the complexity of the sample matrix in phthalates analysis. The system comprised an 8890 GC configured with an Agilent 7693A automatic liquid sampler (G4513A) and a split/splitless inlet coupled to a 5977C gas chromatography/mass selective detector (GC/MSD) configured with a HydroInert source.

Tables 1 and 2 show the GC and MS conditions for phthalate analysis and the SIM ion parameters used for data acquisition.

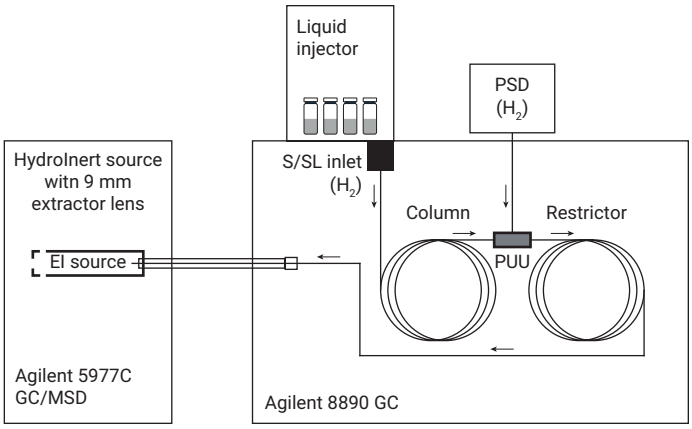


Figure 1. System configuration. S/SL Inlet = split/splitless inlet, PUU = purged ultimate union.

Table 1. GC and MS conditions for phthalate analysis.

Parameter	Value
Agilent 8890 GC	
SSL Inlet	250 °C, Mode: split (5:1)
Liner	Inlet liner, Ultra Inert, split, low pressure drop, glass wool (p/n 5190-2295)
Injection Volume	1 µL
Column	0.7 mL/min, constant flow mode (H_2) Agilent DB-5ms Ultra Inert, 30 m × 0.18 mm, 0.18 µm (p/n 121-5522UI)
Restrictor	1.1 mL/min, constant flow mode (H_2) Purge flow: 3 mL/min Agilent DB-5ms Ultra Inert 5 m × 0.15 mm, 0.15 µm (p/n 165-6626)
Oven Program	110 °C (0.5 min) 30.5 °C/min to 280 °C (0.5 min) 30.5 °C/min to 320 °C (2 min)
Agilent 8890 GC Backflush Parameters	
Inlet Pressure (Backflushing)	2 psi
Backflush Pressure	20 psi
Void Volumes	5.1715
Backflush Time	4 min
Agilent 5977C GC/MSD	
Source Temperature	280 °C
MS Quadrupole	150 °C
MS Transfer Line	280 °C
Acquisition Type	SIM mode
Gain Factor	1.0

Table 2. SIM ion parameters used for data acquisition (same with helium as carrier gas).

Compound	RT (min)	Quantifier Ion (*m/z*)	Qualifier Ions (*m/z*)
BB	5.133	194	105, 212
DIBP	5.395	149	150, 167
DPP	5.724	150	149, 223
DPENP	6.347	149	150, 237
BBP	7.002	149	150, 251
DBP	7.071	149	91, 150
DCHP	7.632	149	150, 167
DEHP	7.666	149	150, 167
DNOP	8.23	149	150, 249
DINP	8.554	293	127, 149
DIDP	8.898	307	149, 167

Results and discussion

Chromatographic performance

IEC 62321-8 describes a 17-minute GC method for the determination of phthalates in polymers by GC/MS. When using hydrogen as the carrier gas, an elution profile similar to previous studies conducted with helium was observed. The combination of hydrogen as the carrier gas and a smaller diameter column resulted in a faster run time compared to helium. The SIM chromatogram of the 1 µg/mL standard solution containing the phthalate is shown in Figure 2. Good chromatographic peak shapes and signal-to-noise ratio (S/N) were obtained for 10 phthalates (excluding the internal standard) in 15 minutes (including backflushing time).

Linearity and range

The linearity and range of the analysis were determined by constructing solvent and standard addition calibration curves for all phthalate compounds within the concentration range of 0.2 to 5 mg/L. Internal standard (BB) concentration was 1 mg/L. The linearity was confirmed by observing R^2 values exceeding 0.996. Figure 3 displays representative calibration plots for four phthalate compounds. Also, the bias percentage for all phthalate compounds at the lowest concentration standard fell within the acceptable range of 80 to 120% of the actual concentration (Table 3).

Table 3. Linear least-squares regression weighted calibration data for 10 phthalates.

Chemicals	Type	Origin	Weight	R^2	%Bias STD 1
BBP	Linear	Include	1/x	0.99921	106.4
DIBP	Linear	Include	1/x	0.99849	104.9
DBP	Linear	Include	1/x	0.99841	111.3
DCHP	Linear	Include	1/x	0.99889	112.1
DEHP	Linear	Include	1/x	0.99902	108.0
DIDP	Linear	Include	1/x	0.99912	996.9
DPENP	Linear	Include	1/x	0.99931	104.9
DNOP	Linear	Include	1/x	0.99802	115.8
DINP	Linear	Include	1/x	0.99935	100.1
DPP	Linear	Include	1/x	0.99920	106.3

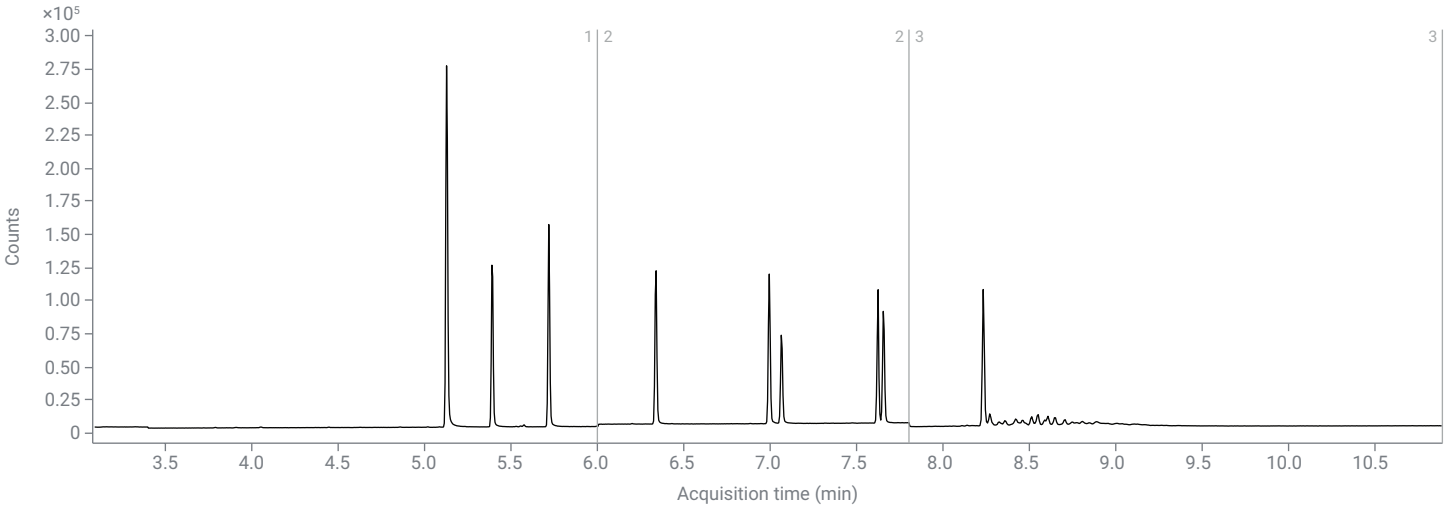
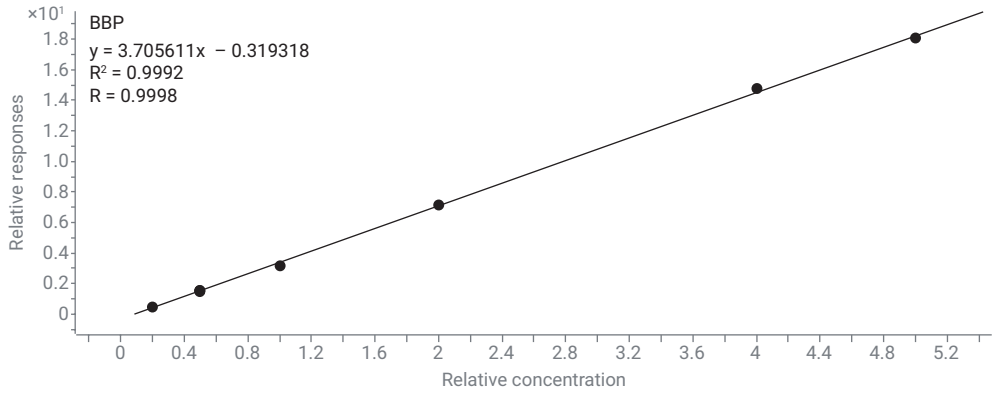
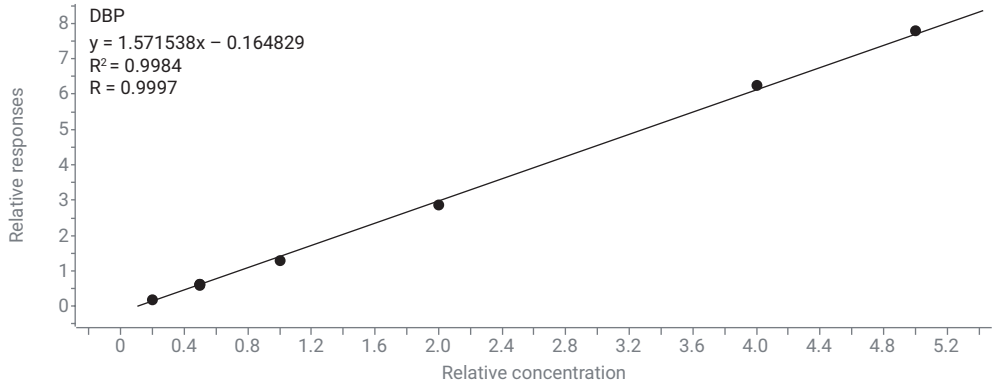
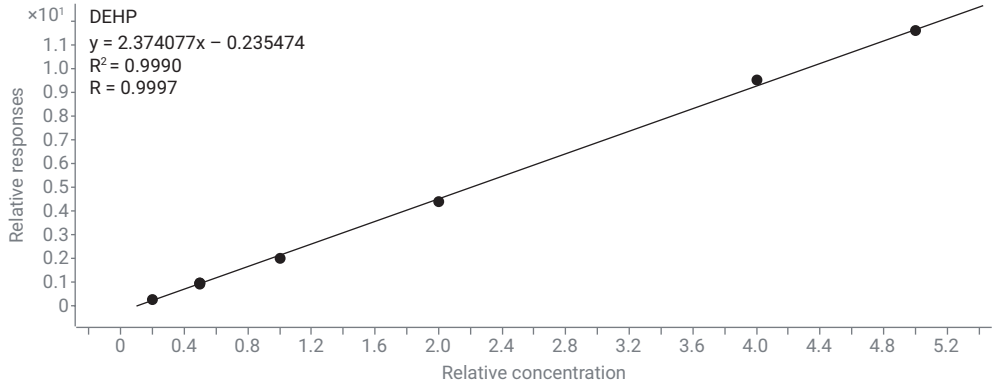


Figure 2. SIM TIC of 10 phthalates at 1 mg/L and BB as internal standard (ISTD) at 1 mg/L.







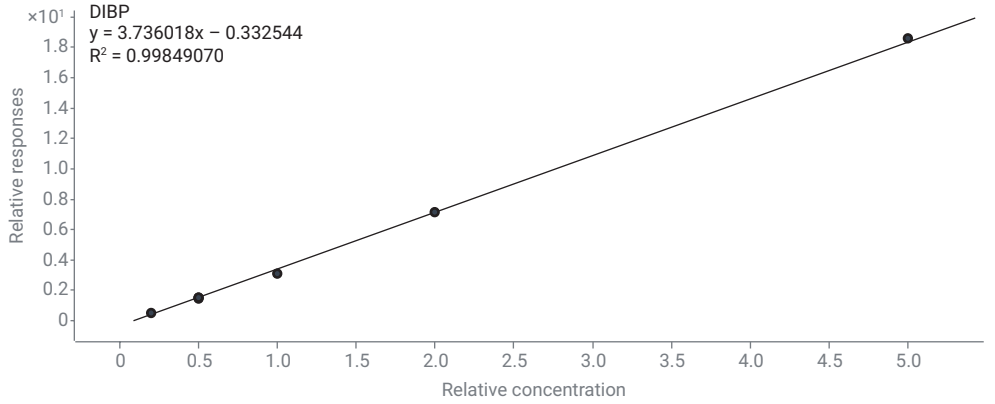


Figure 3. Calibration curves of four phthalates in ROSH 3 from 0.2 to 5 mg/L.

Method detection limit (MDL)

The MDL was estimated based on the standard deviation (SD) of the analysis results of nine spiked polymer samples on two different days at 50 mg/kg. Note that phthalates were not detected in the original polymer samples.

The MDLs for phthalate in the polymer matrix ranged from 1.80 to 3.74 mg/kg, as illustrated in Figure 4. For all 10 phthalate compounds, the calculated relative standard deviations (RSDs) were below 20%. These MDL values meet the low detection limits required for phthalate analysis in electrotechnical products.

Recovery, repeatability, and reproducibility

The recovery, repeatability, and reproducibility were evaluated based on the results of the analysis of 14 spiked samples on two different days. The samples were spiked at concentrations of 50 and 500 mg/kg. The average values of recovery obtained for the spiked samples at different concentrations ranged from 89.6 to 101.1%, as shown in Figure 5. Recovery between 70 and 120% is considered satisfactory based on the limits specified in IEC 62321-8. In addition, the %RSD of the recovery values calculated from 14 spiked samples on three days for each concentration was less than 20%, which satisfies the requirements of IEC 62321-8.

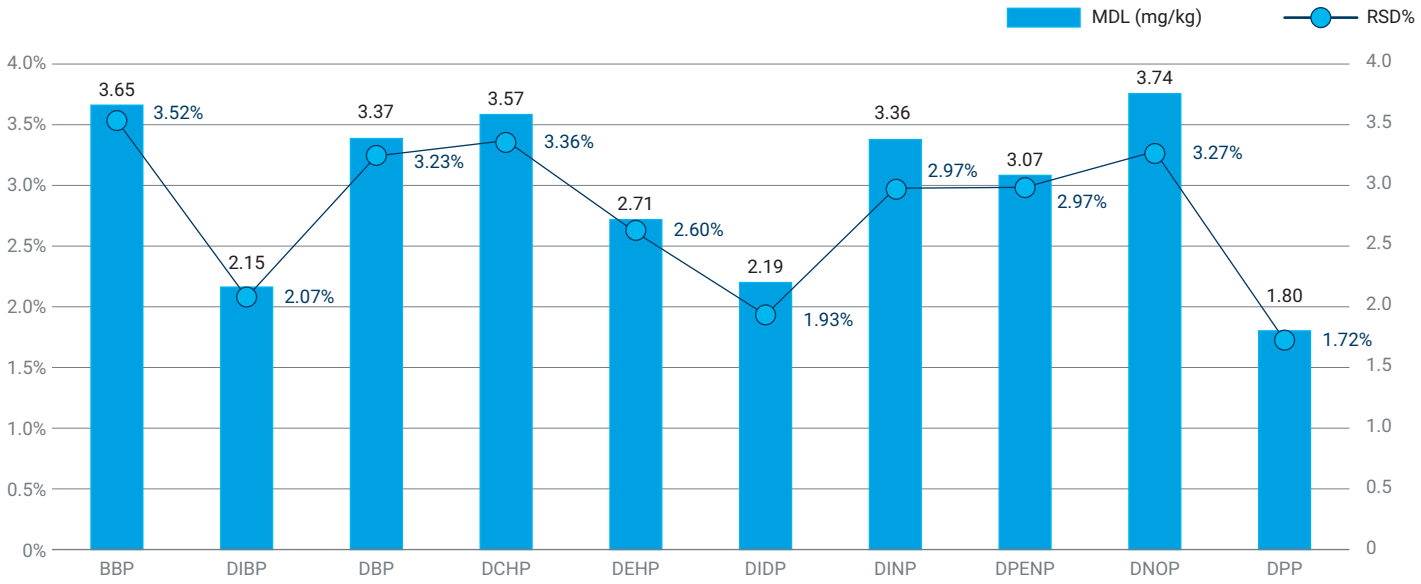


Figure 4. MDLs of phthalates in polymer samples.

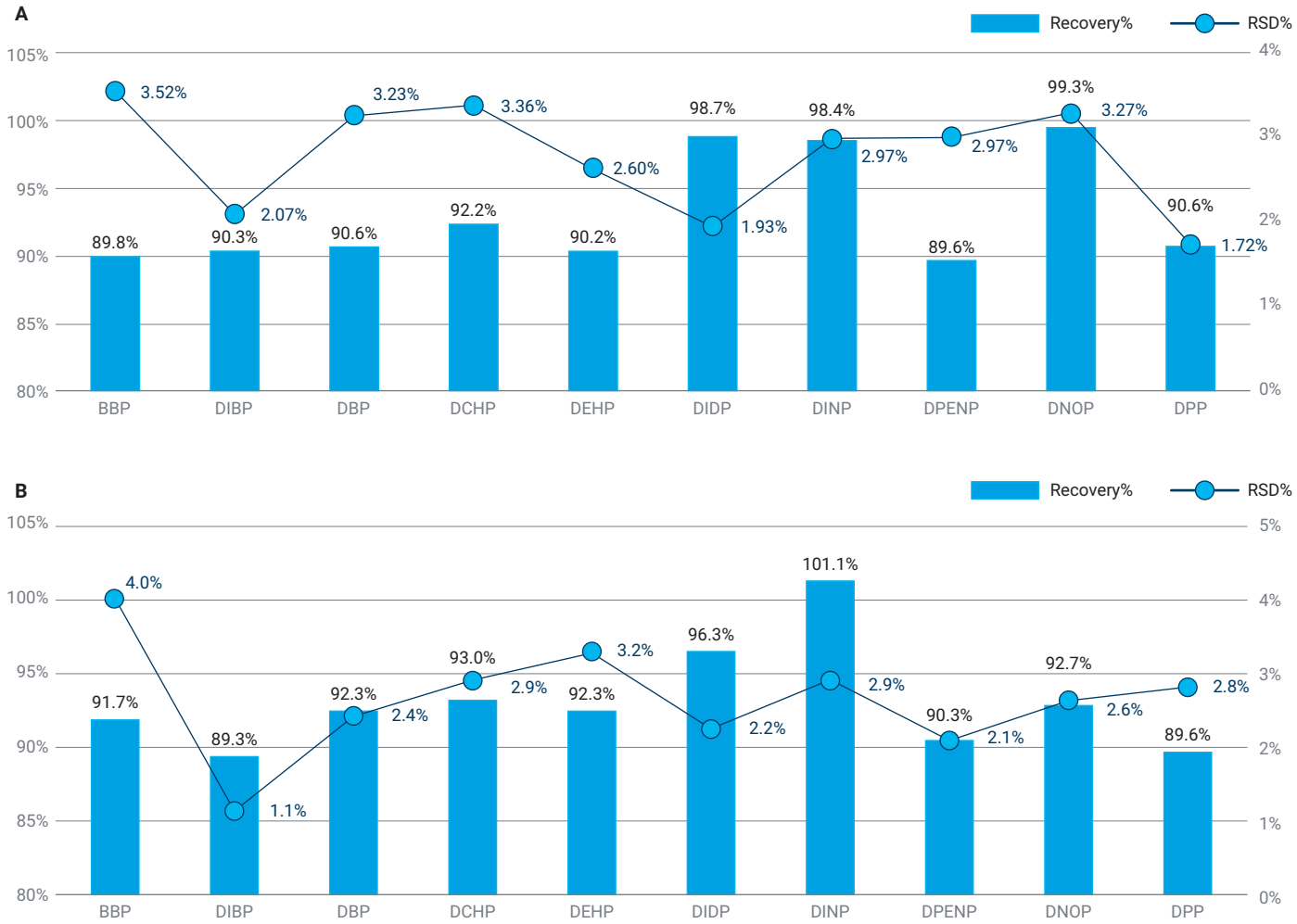


Figure 5. (A) Recovery% at 50 mg/kg of phthalate in polymer samples. (B) Recovery% at 500 mg/kg of phthalate in polymer samples.

Analysis of various cable samples

All MDLs and limits of quantification (LOQs ≈ 3MDLs) were below the lowest calibration point for all compounds. Therefore, the reporting limits for this study were defined as any value greater than or equal to the lowest calibration point for the respective compound. Three real-world cable samples were assessed for the presence of phthalate. The chromatogram and result of three cable samples are presented in Figure 6 and Table 4.

Table 4. Phthalate result analysis of three cable samples.

Compound	Cable 1	Cable 2	Cable 3
DIBP	Detected	Detected	Not detected
DPP	Detected	Detected	Not detected
DPENP	Not detected	Not detected	Not detected
BBP	Not detected	Not detected	Not detected
DBP	Not detected	Not detected	Not detected
DCHP	Not detected	Not detected	Detected
DEHP	Significant level detected	Significant level detected	Not detected
DNOP	Not detected	Not detected	Significant level detected
DINP	Significant level detected	Detected	Not detected
DIDP	Significant level detected	Detected	Not detected

Not detected: below the calibration range tested
Detected: within the calibration range
Significant level detected: above the calibration range

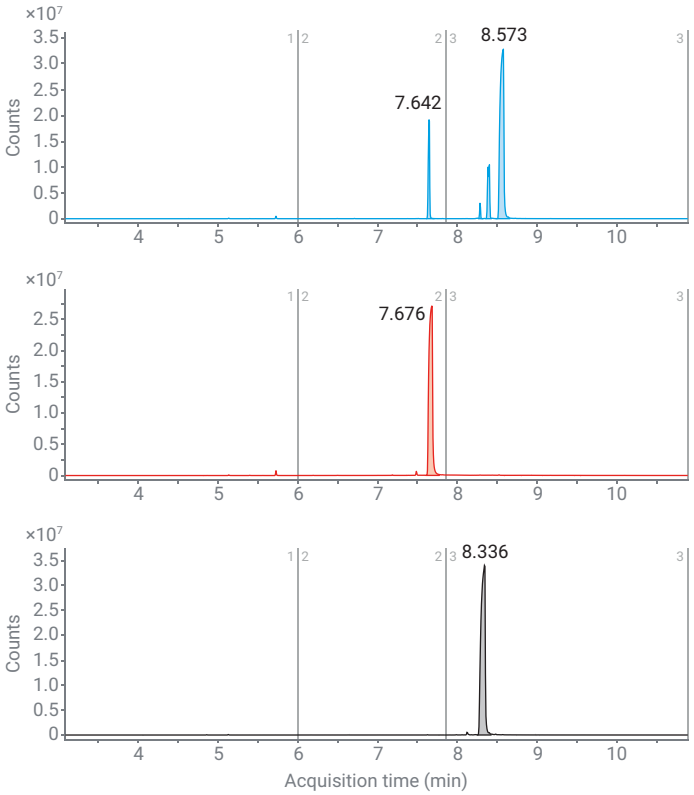


Figure 6. TIC SIM of phthalates in three cable samples.

Method robustness in cable matrix

When using helium gas for phthalate analysis on cable samples, a common issue is a significant reduction on compound responses, which cause high %RSD for certain types of cable matrices. The method robustness when using Agilent HydroInert source with hydrogen carrier gas, in combination with the backflush technique, was assessed through continuous analysis of standards and three different cable sample matrices. The %RSD values for the response of 1 mg/L standard points of phthalates analyzed throughout the analysis of the three cable sample matrices are presented in Table 5.

The %RSD of the response, calculated from the 1 mg/L standard without any maintenance (inlet, ion source), remained below 7% for all compounds across the three cable sample matrices. Also, the %RSD was consistently below 20% for all compounds, showcasing outstanding quantitation stability even when continuously subjected to a complex extract (Figure 7).

Table 5. The %RSD values for the response at 1 mg/L in the method robustness test.

Sample	N (Standard Injection)	RSD Area									
		DIBP	DPP	DPENP	BBP	DBP	DCHP	DEHP	DNOP	DINP	DIDP
Cable 1 (80 injections)	24.000	7.00	6.62	5.74	5.68	6.35	5.85	5.68	5.96	5.67	6.35
Cable 2 (30 injections)	24.000	6.11	6.16	6.08	5.83	5.72	6.30	5.96	5.66	2.41	3.44
Cable 3 (30 injections)	15.000	4.16	3.75	3.73	4.11	3.89	3.98	3.64	3.95	1.96	2.08

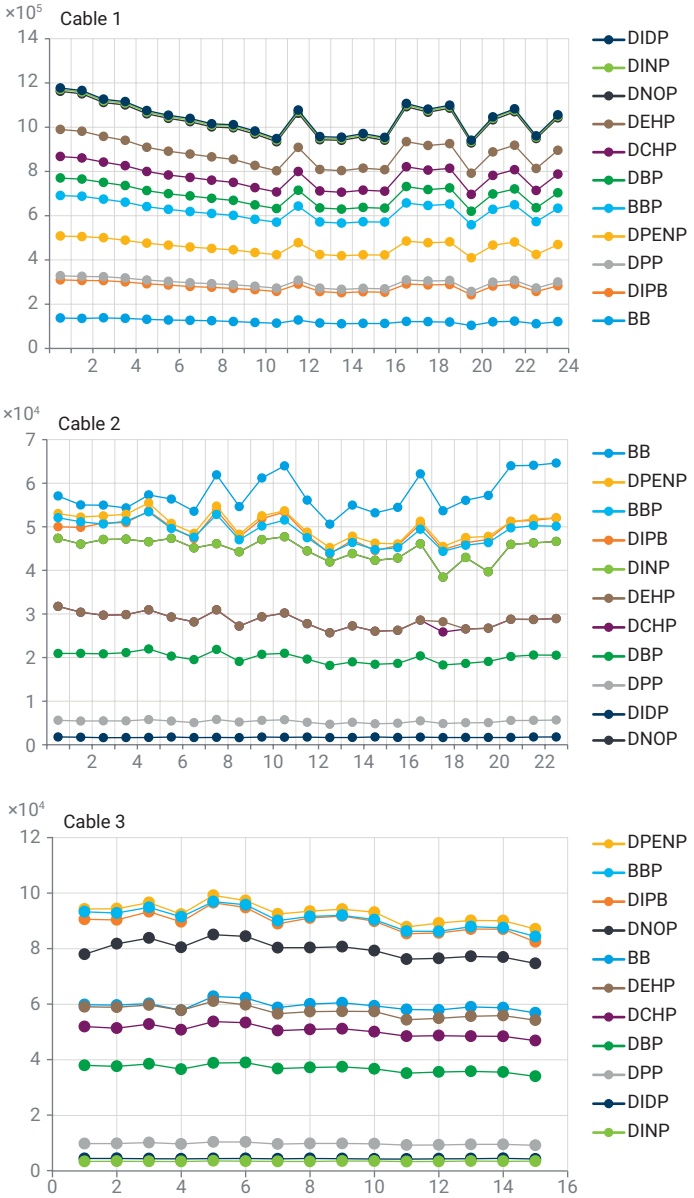


Figure 7. The response values at 1 mg/L in the method robustness test.

Conclusion

This application note demonstrates the configuration of the Agilent 8890 GC coupled with the Agilent 5977C, using an Agilent HydroInert source with hydrogen as the carrier gas in combination with the backflush technique. In addition to fulfilling the requirements of the International Electrotechnical Commission 62321 standard for the analysis of phthalates in electrical products, it also highlights the method's robustness and addresses the issue of instability when analyzing samples with complex components. This is achieved through the self-cleaning properties of hydrogen gas within the ion source and the use of the backflush technique to remove components that are difficult to evaporate.

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Printed in the USA, March 18, 2024
5994-7215EN

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Automated Sample Preparation Using the PAL3 RTC System for EPA 8270E Semivolatile Organic Analysis by GC/TQ

Authors

Gwen Lim Sin Yee,
CTC Analytics AG,
Zwingen, Switzerland

Aimei Zou,
Agilent Technologies, Inc.

Abstract

Growing demand for SVOC analysis at laboratories reveals the analysis bottlenecks, e.g., a lack of practical sample preparation experience, low sample throughput, and high consumption of chemical solvents. Online automated sample preparation is gaining attention as a solution to address these laboratory challenges. An automated workflow solution for quantitation of SVOCs in water samples, combining calibration, sample preparation, and detection was developed on Agilent gas chromatography/triple quadrupole mass spectrometer (GC/TQ) using the PAL3 robotic tool change (RTC) system in this study.

Introduction

Semivolatile organic compounds (SVOCs) analysis is widely implemented at analytical laboratories. The SW-846 Compendium EPA 8270 method provides guidelines on conditions and quality control (QC) checks to ensure successful analysis of SVOCs using gas chromatography/mass spectrometer (GC/MS). Furthermore, the EPA 8270E method, which was released in 2018, includes GC/MS with an MS/MS detector. The MS/MS selectivity ensures better lower limit of quantitation (LLOQ) thus delivers reliable analysis results[1].

Growing demand for SVOC analysis at laboratories reveals the analysis bottlenecks, e.g., a lack of practical sample preparation experience, low sample throughput, and high consumption of chemical solvents. Online automated sample preparation is gaining attention as a solution to address these laboratory challenges. This application note presents a proof-of-concept on a novel automated sample preparation workflow modified based on EPA 3510C method[2] (that is a procedure for isolating organic compounds from aqueous samples), followed by Agilent gas chromatography/triple quadrupole mass spectrometry (GC/TQ) analysis according to EPA 8270E. The novel sample preparation workflow involved liquid-liquid extraction (LLE) of water samples using dichloromethane (DCM) at two specific pH conditions. The combination of these two extracts was then analyzed by the GC/TQ. Surface water samples were prepared automatically and online by the PAL3 robotic tool change (RTC) system prior to GC/TQ analysis. Likewise, working calibration standards, method blank samples, and matrix-spiked QC samples were also prepared automatically by PAL3. Then, 100 analytes of SOVCs were tested and evaluated in this study.

Experimental

Instrumentation

An Agilent 7000 series triple quadrupole mass spectrometer was coupled to an Agilent 8890 GC with a back split/splitless inlet (SSL) and splitless inlet liner (part number 5190-2293, 900 µL, single taper, wool, Ultra). The ion source was equipped with a 9 mm diameter drawout lens (part number G3870-20449). The system was autotuned using the etune algorithm embedded in the Agilent MassHunter software version 10.1. Table 1 contains the conditions and operating parameters for both GC and MS.

A PAL3 Series II RTC system (Figure 1) was used as a liquid handling platform for the calibration/sample preparation and injection onto the GC/TQ system in the study. The PAL3 system was equipped with a vortex mixer and various tray holders and racks (for 2 mL, 10/20-mL vials). Various liquid syringe tools were used fitting different volumes of PTFE coated smart syringe. Solvent module and fast wash module were also configured with the PAL3 system.

The integrated PAL3-GC/TQ system was controlled by Agilent MassHunter Workstation GC/MS Data Acquisition 10.1, offering an easy user experience with a single software system. The operating window is captured in Figure 2.

Table 1. Agilent 8890 GC and 7000 series instrument parameters.

GC Conditions	
Injection Volume	2.0 µL
Column	Agilent J&W DB-UI8270D, 30 m x 250 µm x 0.25 µm (part number 122-9732)
Inlet Temperature	250 °C
Injection Mode	Pulsed splitless
Carrier Gas	Helium, constant flow, 1.2 mL/min
Transfer Line Temperature	320 °C
Oven Program	40 °C hold for 0.5 minutes
	25 °C/minute to 260 °C, hold for 9.3 minutes
	5 °C/minute to 280 °C, hold for 13.3 minutes
	25 °C/minute to 320 °C, hold for 18.9 minutes
MS Parameters	
Acquisition Mode	dMRM
Ion Source Temperature	320 °C
Quadrupole Temperature	150 °C
Ionization	EI mode
EMV Mode	Gain factor (10)
Solvent Delay	1.5 minutes
Cycles Per Second	15

Figure 1. PAL3 RTC system on an Agilent 7000 series GC/TQ.

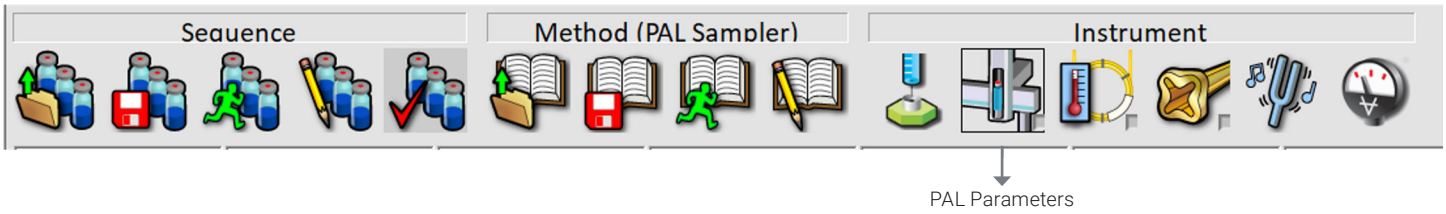

Figure 2. The integrated PAL3-GC/TQ system operating window by MassHunter software 10.1.

Calibration preparation by the PAL3 system

A total of 10 calibration levels were automatically prepared by the PAL3 system for the experimental work in this study. The automated procedure is illustrated in Figure 3. A stock standard solution containing 100 analytes based on the EPA 8270E target list was manually prepared at the concentration of 300 µg/mL (ppm) in DCM. Agilent semi-volatiles internal standard (part number ISM-563-1, 2000 ppm) was manually diluted by DCM at 40 ppm as ISTD. Both stock standard solution and ISTD were loaded onto the predefined vial positions according to the method parameters. As indicated in Figure 3, the 3 intermediate standard solutions were prepared from the stock standard solution by the PAL3 system, and then were used to prepare the 10 levels of working calibration standards from 0.01 to 20 ppm. Lastly, 5 µL of ISTD were then spiked into each vial of calibration standards at a final concentration of 2 ppm.

Sample preparation by the PAL3 system

Surface water was used as a sample to test out the performance of automated sample preparation by the PAL3 system. According to EPA 3510C, manual liquid-liquid extraction (LLE) using a separatory funnel is defined for aqueous samples, which involves large sample size and high chemical/reagents consumption. Based on the LLE described in EPA 3510C, an automated workflow was modified and developed on the PAL3 system in this study. The automated sample preparation workflow is shown in Figure 4.

1 g of NaCl was manually weighed into a 20 mL vial followed by adding 15 mL of the water sample. The vial was capped securely and placed on the sample rack (PAL R60 rack for 10/20-mL vial). The rest of the sample preparation workflow steps were then done by the PAL3 system. The analytes from the water sample were enriched 10-fold during the workflow.

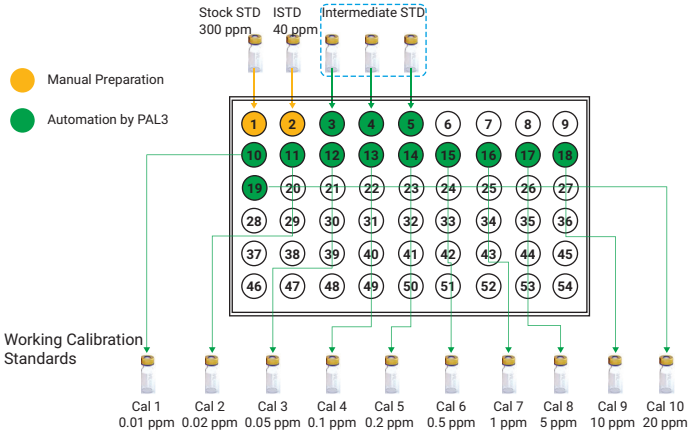

Figure 3. Automated preparation for calibration standards by the PAL3 system.

Figure 4. Automated sample preparation by the PAL3 system.

Online analysis sequence

As illustrated in Figure 5, a batch of online analysis sequence includes working calibration standards, method blank (MB), which is unspiked matrix blank, and matrix-spiked QC samples. First, 10 points of calibration standards were prepared by the PAL3 and subsequently analyzed via GC/TQ. Next, MB was prepared by the PAL3 and immediately injected into GC/TQ for quantitative analysis. In the meantime, PAL3 moved forward to the next sample preparation while GC/TQ was continually working on the analysis of MB. As a result, the integrated PAL3-GC/TQ system allowed sample preparation and sample analysis to proceed in a parallel mode. Thus, the overall lab productivity was increased through automation and eliminating waiting time between runs.

Results and discussion

Compound identification

The acquisition method including multiple reaction monitoring (MRM) transitions, collision energy, and retention time (RT) used for this study was based on the existing well-developed method from a previous application note[3]. Figure 6 shows a representative MRM chromatogram of the 100 analytes at 5 µg/mL (Cal 8) prepared by the PAL3 system. The symmetric sharp peaks demonstrate the efficient chromatographic separation of targets within the retention time window.

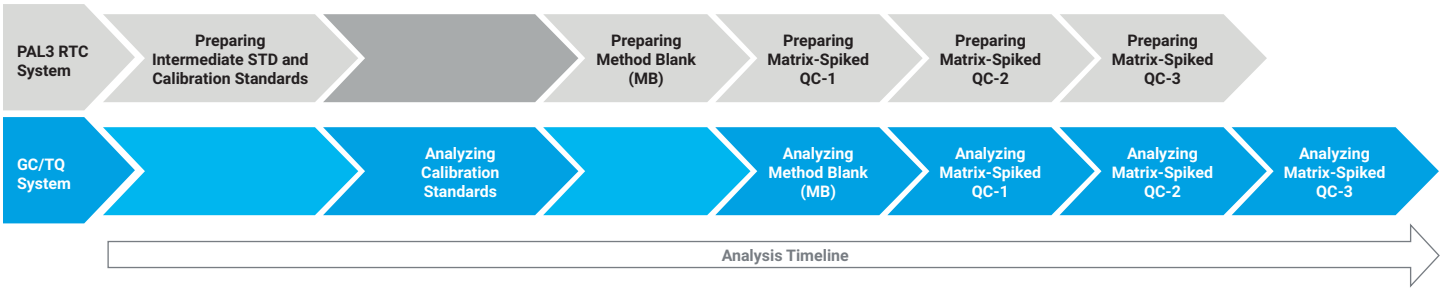

Figure 5. Online analysis sequence on the integrated PAL3-GC/TQ system.

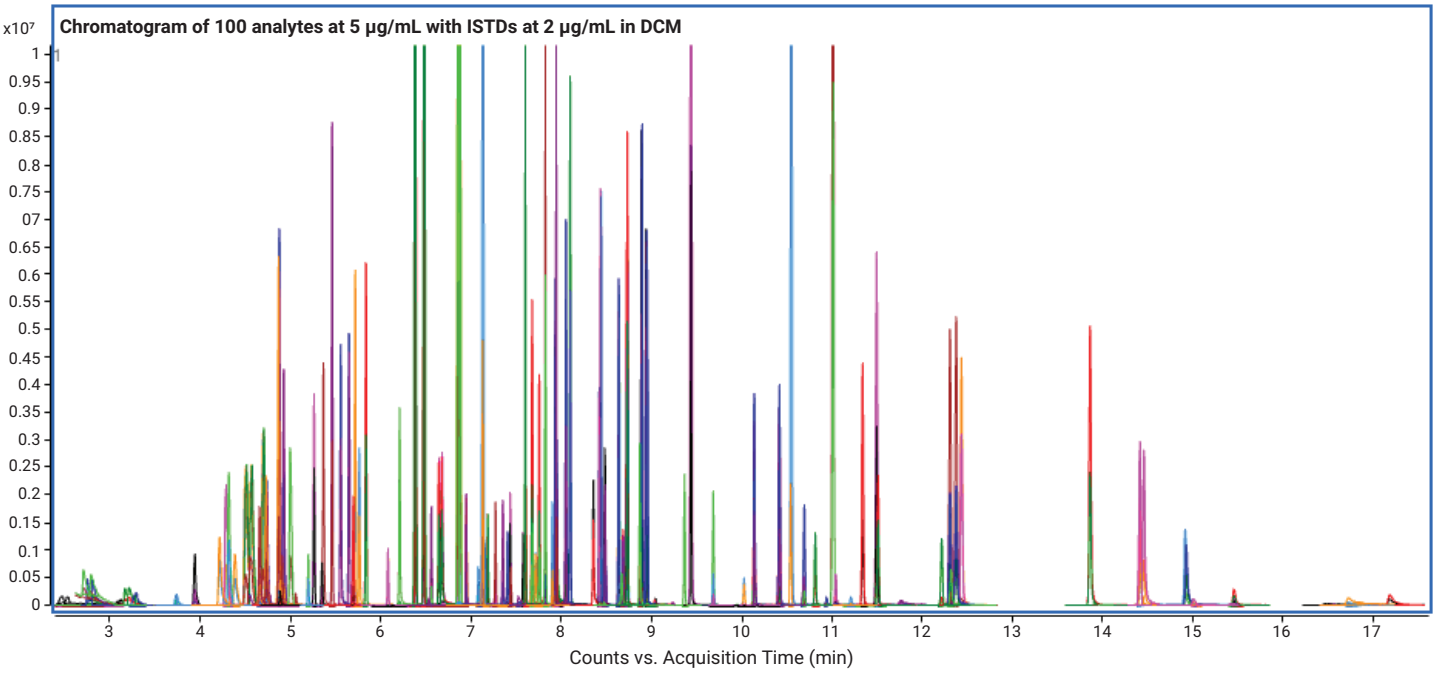

Figure 6. Representative MRM chromatogram for 100 analytes at 5 µg/mL (Cal 8) and ISTDs at 2 µg/mL in DCM prepared by the PAL3 system.

Initial calibration performance

Initial calibration (ICAL) performance was evaluated in terms of linearity, response factor (RF), and accuracy of calibration standards. The results are summarized in Table 2. The overall working range of the method for all analytes was determined to be 0.01 to 20 µg/mL, while some data points for certain compounds may be deleted at the low and high ends of the calibration range to meet the method performance criteria based on EPA 8270E. In this study, 96% of compounds achieved R>0.995 (LR mode) with minimum 5 points and 97% of compounds met the accuracy requirement for each calibration level. The %RSD of RF is within 20% for all analytes, demonstrating the excellent performance done by the integrated PAL3-GC/TQ system for automated calibration preparation and acquisition analysis.

The ISTD was also assessed to determine if the method sensitivity and stability was maintained throughout the whole process. 5 µL of ISTD mixture was added to each calibration level and matrix-spiked QC to reach the final concentration of 2 µg/mL. The absolute RT change for ISTDs was within the regulatory recommendation of ≤ 30 secs. The response of all ISTDs in the individual standard was obtained within 70 to 150% of average response throughout the final calibration range, meeting the EPA performance criteria[1].

Method sensitivity based on LLOQ

The method sensitivity was evaluated based on the LLOQ in this work. The lowest point in the ICAL is defined as LLOQ that met the performance criteria including linearity, RF, and accuracy[1]. The summary of the LLOQ for all analytes is listed in Table 2. The LLOQ of 100 analytes was distributed across 0.01 to 0.5 µg/mL as shown in Figure 7. Overall, 39 out of 100 compounds obtained LLOQ ≤ 0.02 µg/mL, demonstrating the excellent sensitivity of the method developed on the PAL3-GC/TQ.

Method blanks

Method blanks (MBs) must be carried out through all stages of sample preparation and analyzed for the compounds of interest as a safeguard against lab contamination caused from the sample, the reagents used, and the preparation workflow. In this study, duplicate MBs were prepared by the PAL3 system following the same method script except for the addition of analytes/surrogates. Target concentration for all compounds in MBs was obtained less than 50% of the LLOQ, although positive presence was observed for certain compounds, demonstrating that lab contamination was controlled to the desired level.

Table 2. Analytical performance summary for analytes.

Compound Name	Quantifier Transition	Linearity (LR Model)	RT (min)	RF	RSD of RF	LLOQ (µg/mL)	Recovery (%)	RSD of Recovery (n=3)
1,2,4-Trichlorobenzene	179.9 -> 109.0	0.9998	5.64	1.04	0.7%	0.01	115	4%
1,2-Dichlorobenzene	146.0 -> 111.0	0.9999	4.69	1.12	0.5%	0.01	109	5%
1,3-Dichlorobenzene	146.0 -> 111.0	0.9997	4.50	1.12	0.2%	0.01	105	5%
1,3-Dinitrobenzene	168.0 -> 75.0	0.9993	7.16	0.07	6.6%	0.2	102	12%
1,4-Dichlorobenzene	146.0 -> 111.0	0.9998	4.56	1.09	0.5%	0.01	105	8%
1,4-Dinitrobenzene	168.0 -> 75.0	0.9987	7.09	0.04	3.0%	0.2	114	4%
1-Bromo-2-nitrobenzene	156.9 -> 75.9	0.9995	6.56	0.20	0.8%	0.01	122	9%
1-Chloronaphthalene	162.0 -> 127.1	0.9988	6.88	1.45	2.7%	0.02	129	6%
1-Methylnaphthalene	142.0 -> 114.9	0.9998	6.48	1.59	0.7%	0.01	117	3%
1-Naphthylamine	143.1 -> 115.1	0.9951	7.70	0.29	4.3%	0.2	51	30%
2,2'-oxybis[1-chloropropane]	121.0 -> 77.0	0.9998	4.77	0.05	1.7%	0.02	108	16%
2,3,4,6-Tetrachlorophenol	230.0 -> 165.9	0.9980	7.72	0.08	7.5%	0.5	64	3%
2,3,5,6-Tetrachlorophenol	230.0 -> 165.9	0.9988	7.68	0.07	0.6%	0.5	60	4%
2,4,5-Trichlorophenol	195.8 -> 97.0	0.9995	6.70	0.35	10.2%	0.5	63	7%
2,4,6-Trichlorophenol	195.8 -> 97.0	0.9985	6.66	0.46	1.8%	0.05	64	6%
2,4-Dichlorophenol	162.0 -> 63.0	0.9996	5.57	0.98	2.0%	0.01	59	11%
2,4-Dimethylphenol	107.1 -> 77.1	1.0000	5.36	0.99	1.6%	0.01	67	14%
2,4-Dinitrophenol	184.0 -> 79.0	0.9931	7.50	0.01	3.6%	0.5	53	2%
2,4-Dinitrotoluene	165.0 -> 63.0	0.9998	7.59	0.09	3.8%	0.5	100	9%
2,6-Dinitrotoluene	165.0 -> 63.0	0.9990	7.19	0.11	1.4%	0.2	121	9%

Compound Name	Quantifier Transition	Linearity (LR Model)	RT (min)	RF	RSD of RF	LLOQ (µg/mL)	Recovery (%)	RSD of Recovery (n=3)
2-Acetylaminofluorene	222.9 -> 181.1	0.9966	11.82	0.07	6.7%	0.5	119	5%
2-Chloronaphthalene	162.0 -> 126.9	0.9992	6.86	2.48	1.8%	0.05	118	5%
2-Chlorophenol	128.0 -> 64.0	0.9998	4.37	0.35	2.0%	0.01	54	3%
2-methyl-4,6-dinitrophenol	198.0 -> 121.0	0.9949	7.99	0.03	3.6%	0.5	53	7%
2-Methylnaphthalene	142.0 -> 141.0	0.9984	6.39	2.81	0.2%	0.01	117	4%
2-Nitroaniline	138.0 -> 92.0	0.9988	6.96	0.14	1.4%	0.5	104	9%
2-Nitrophenol	138.9 -> 81.0	0.9987	5.34	0.25	1.2%	0.01	61	9%
2-Picoline	93.1 -> 66.0	0.9997	3.20	0.28	2.3%	0.01	40	5%
3-Methylcholanthrene	268.1 -> 252.1	0.9997	15.61	0.81	1.2%	0.5	101	7%
4,4'-DDD	234.8 -> 164.9	0.9986	11.04	1.46	1.5%	0.1	123	6%
4,4'-DDE	245.8 -> 176.0	0.9984	10.56	1.15	1.3%	0.1	116	6%
4,4'-DDT	234.8 -> 164.9	0.9985	11.51	0.89	1.1%	0.05	99	8%
4-Aminobiphenyl	168.1 -> 167.1	0.9986	8.68	0.21	8.6%	0.5	59	11%
4-bromophenyl phenyl ether	248.0 -> 141.0	0.9994	8.44	0.52	1.9%	0.1	112	6%
4-chloro-3-methylphenol	107.0 -> 77.0	0.9998	6.23	0.61	1.1%	0.05	55	13%
4-Chloroaniline	127.0 -> 65.0	0.9991	5.77	0.48	2%	0.02	19	14%
4-Chlorophenyl phenyl ether	141.1 -> 115.1	0.9968	7.94	0.45	0.7%	0.02	123	2%
4-Nitroaniline	138.0 -> 108.1	0.9996	7.97	0.14	6.4%	0.5	76	8%
7,12-Dimethylbenz[a]anthracene	256.1 -> 241.1	0.9998	14.45	1.51	1.5%	0.1	121	5%
Acenaphthene	152.9 -> 77.0	0.9997	7.44	0.17	0.6%	0.01	113	6%
Acenaphthylene	151.9 -> 102.0	0.9998	7.27	0.17	0.3%	0.01	114	8%
Aldrin	262.7 -> 192.6	0.9997	9.69	0.15	1.4%	0.01	105	5%
Aniline	93.0 -> 66.0	0.9999	4.27	0.68	0.9%	0.01	23	15%
Anthracene	177.9 -> 152.0	0.9958	8.94	0.93	4.5%	0.2	118	5%
Azobenzene	77.0 -> 51.0	0.9975	8.10	1.42	2.3%	0.2	119	7%
Benz[a]anthracene	228.1 -> 226.1	1.0000	12.36	1.60	1.2%	0.5	122	7%
Benzo[a]pyrene	252.1 -> 250.1	0.9998	15.04	1.79	3.5%	0.1	113	5%
Benzo[b]fluoranthene	252.1 -> 250.1	0.9997	14.46	2.20	1.4%	0.5	120	4%
Benzo[g,h,i]perylene	276.1 -> 274.1	0.9998	17.39	1.67	3.8%	0.5	112	5%
Benzo[k]fluoranthene	252.1 -> 250.1	0.9979	14.47	1.80	0.1%	0.5	114	5%
Benzyl alcohol	108.0 -> 79.0	0.9999	4.65	0.50	0.8%	0.02	59	22%
BHC-alpha	180.8 -> 144.9	0.9995	8.44	0.50	2.3%	0.02	109	7%
BHC-beta	180.8 -> 144.9	0.9994	8.65	0.38	1.7%	0.1	117	6%
BHC-delta	218.8 -> 182.8	0.9936	8.97	0.39	1.5%	0.1	106	6%
BHC-gamma	218.8 -> 182.9	0.9991	8.74	0.35	3.0%	0.1	112	8%
bis(2-Chloroethoxy)methane	93.0 -> 63.0	0.9998	5.46	1.75	1.2%	0.01	112	10%
bis(2-Chloroethyl)ether	93.1 -> 63.0	0.9999	4.31	0.86	0.5%	0.01	101	19%
Bis(2-ethylhexyl) phthalate	149.0 -> 65.0	0.9997	12.44	1.40	1.7%	0.02	124	5%
Butyl benzyl phthalate	149.0 -> 65.0	1.0000	11.38	0.91	2.4%	0.05	128	6%
Chrysene	226.1 -> 224.1	0.9985	12.38	0.73	7.8%	0.1	111	8%
Dibenz[a,h]anthracene	278.1 -> 276.1	0.9995	16.88	0.86	4.6%	0.5	104	6%
Dibenzofuran	167.9 -> 139.1	0.9959	7.61	1.53	0.2%	0.5	116	7%
Dieldrin	262.9 -> 193.0	1.0000	10.70	0.14	1.9%	0.05	113	6%

Compound Name	Quantifier Transition	Linearity (LR Model)	RT (min)	RF	RSD of RF	LLOQ (µg/mL)	Recovery (%)	RSD of Recovery (n=3)
Diethyl phthalate	149.0 -> 65.0	0.9997	7.83	1.03	0.5%	0.1	114	8%
Dimethyl phthalate	163.0 -> 77.0	0.9999	7.13	0.96	0.4%	0.1	105	11%
Di-n-butyl phthalate	149.0 -> 65.0	0.9923	9.46	3.15	1.5%	0.1	125	5%
Di-n-octyl phthalate	149.0 -> 65.0	0.9993	13.90	1.94	2.7%	0.1	133	5%
Diphenylamine	167.0 -> 166.2	0.9985	8.06	0.80	3.1%	0.2	115	8%
Endosulfan I	241.0 -> 206.0	0.9997	10.41	0.08	2.0%	0.1	121	8%
Endosulfan II	240.7 -> 205.9	0.9999	11.05	0.05	1.0%	0.05	115	6%
Endosulfan sulfate	271.6 -> 236.7	0.9963	11.52	0.21	1.6%	0.02	113	8%
Endrin	262.7 -> 190.5	0.9995	10.94	0.03	0.7%	0.05	94	12%
Ethyl methanesulfonate	109.0 -> 78.9	0.9999	3.91	0.26	1.7%	0.01	79	5%
Fluoranthene	200.9 -> 199.9	0.9996	10.17	0.62	2.3%	0.1	121	7%
Fluorene	166.0 -> 165.1	0.9954	7.95	1.80	0.8%	0.1	121	6%
Heptachlor	273.6 -> 238.7	0.9999	9.37	0.19	0.7%	0.02	104	5%
Heptachlor epoxide	352.7 -> 216.7	0.9996	10.03	0.03	1.8%	0.05	112	7%
Hexachlorobenzene	283.7 -> 213.8	0.9997	8.49	0.51	2.5%	0.1	110	5%
Hexachlorobutadiene	224.7 -> 189.9	0.9998	5.83	1.26	0.9%	0.01	112	1%
Hexachlorocyclopentadiene	236.7 -> 143.0	0.9987	6.53	0.12	0.5%	0.05	87	4%
Hexachloroethane	200.9 -> 165.9	1.0000	4.99	0.88	1.0%	0.01	105	3%
Isophorone	82.0 -> 54.0	1.0000	5.27	0.82	1.4%	0.01	112	11%
Methoxychlor	226.9 -> 211.9	0.9995	12.24	0.23	0.5%	0.05	103	8%
Methyl methanesulfonate	80.0 -> 64.9	0.9999	3.91	0.05	2.0%	0.01	80	5%
Naphthalene	128.1 -> 102.1	0.9998	5.72	1.37	0.6%	0.01	113	5%
N-Nitro-o-toluidine	152.0 -> 106.0	0.9993	7.96	0.10	6.9%	0.5	71	10%
N-Nitrosodiethylamine	102.0 -> 85.0	0.9999	3.70	0.07	2.8%	0.01	97	5%
N-Nitrosodi-n-butylamine	84.1 -> 56.0	0.9998	6.08	0.14	0.2%	0.02	113	12%
N-Nitrosodi-n-propylamine	113.1 -> 71.0	0.9998	4.88	0.05	2.1%	0.02	109	12%
N-Nitrosomethylethylamine	88.0 -> 42.0	0.9999	3.25	0.11	2.3%	0.01	66	5%
N-Nitrosomorpholine	116.0 -> 86.0	0.9999	4.90	0.10	2.1%	0.05	55	13%
N-Nitrosopiperidine	114.0 -> 84.1	0.9998	5.19	0.14	2.8%	0.02	106	7%
N-Nitrosopyrrolidine	100.1 -> 55.1	0.9996	4.87	0.07	0.7%	0.05	69	13%
p-Dimethylaminoazobenzene	225.1 -> 120.1	0.9995	10.82	0.26	2.2%	0.5	144	6%
Pentachloronitrobenzene	248.8 -> 213.8	0.9997	8.70	0.17	1.2%	0.1	105	6%
Phenanthrene	177.9 -> 152.0	0.9985	8.91	1.32	2.6%	0.2	119	3%
Phenol	94.0 -> 66.1	0.9996	4.21	0.50	0.5%	0.01	19	18%
Pronamide	173.0 -> 145.0	0.9976	8.73	1.07	1.2%	0.5	116	6%
Pyrene	201.1 -> 200.0	0.9997	10.45	0.84	1.2%	0.2	119	7%
Thionazin	143.0 -> 79.0	0.9997	7.91	0.13	2.8%	0.1	118	8%
1,4-Dichlorobenzene-d4 (ISTD)	149.9 -> 114.9	N.A.	4.55	N.A.	N.A.	N.A.	N.A.	N.A.
Acenaphthene-d10 (ISTD)	161.9 -> 159.9	N.A.	7.40	N.A.	N.A.	N.A.	N.A.	N.A.
Chrysene-d12 (ISTD)	240.0 -> 235.9	N.A.	12.36	N.A.	N.A.	N.A.	N.A.	N.A.
Naphthalene-d8 (ISTD)	135.9 -> 107.9	N.A.	5.70	N.A.	N.A.	N.A.	N.A.	N.A.
Perylene-d12 (ISTD)	263.9 -> 259.9	N.A.	15.04	N.A.	N.A.	N.A.	N.A.	N.A.
Phenanthrene-d10 (ISTD)	187.9 -> 160.0	N.A.	8.88	N.A.	N.A.	N.A.	N.A.	N.A.

Matrix-spiked QC recovery

Three technical replicates of matrix-spiked QC (n=3, 2 µg/mL in the final extract) were prepared by the PAL3 system in order to evaluate the reproducibility and robustness of the automated sample preparation. Each QC was analyzed by GC/TQ in duplicates account for the homogeneity of the QC solution and the repeatability of spiked recovery. The recovery values and %RSD are summarized in Table 2. Overall, 96% of compounds met recovery 50 to150%, and 98% of compounds obtained RSD of recovery ≤20% as shown in Figure 8A and 8B, respectively. The obtained results indicate that this automated protocol developed on the PAL3-GC/TQ is suitable, offering good reproducibility and robustness for SVOC analysis according to EPA 8270E.

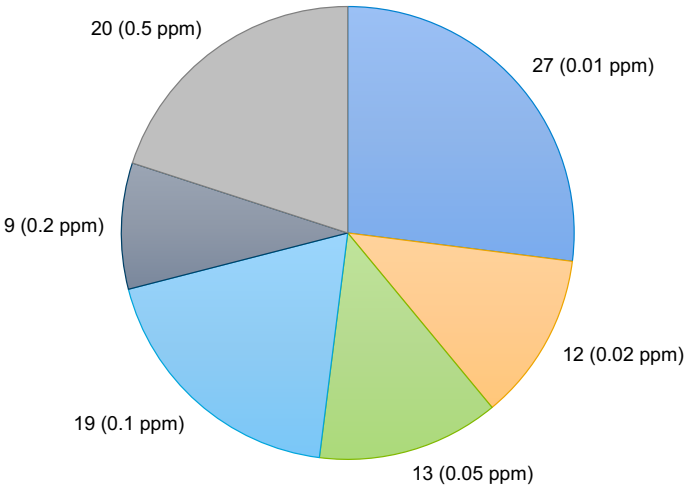

Figure 7. LLOQ distribution of 100 compounds.

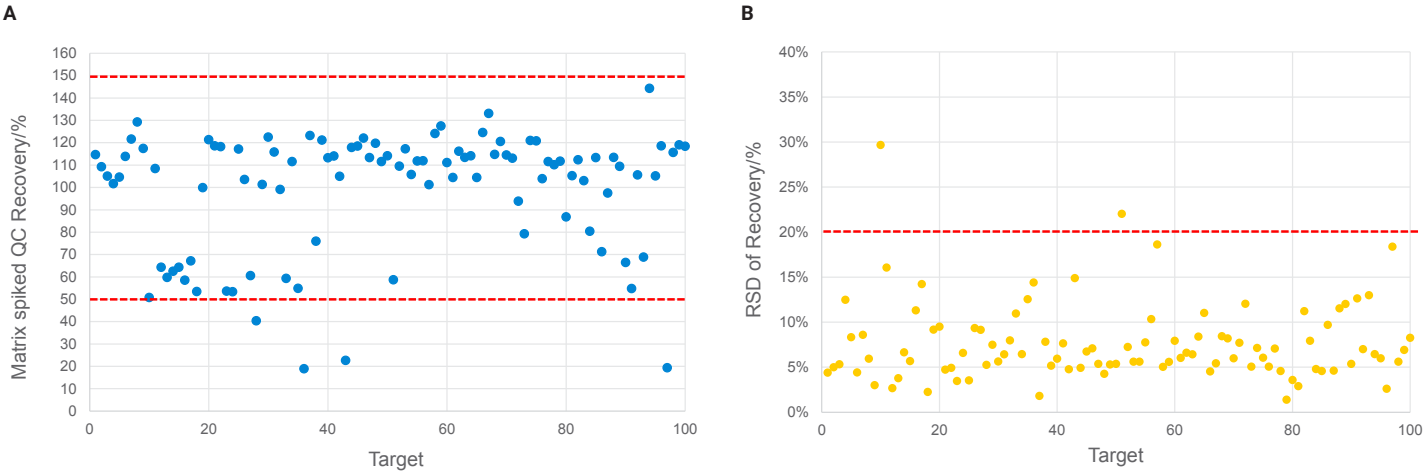

Figure 8. Matrix-spiked QC recovery **(A)** at 2 µg/mL in the final extract and %RSD of recovery **(B)**.

Conclusion

An automated workflow solution for quantitation of SVOCs in water samples, combining calibration/sample preparation and detection was developed on Agilent gas chromatography/ triple quadrupole mass spectrometer (GC/TQ) using the PAL3 robotic tool change (RTC) system in this study. The analytical performance parameters were evaluated based on EPA 8270E, meeting acceptance criteria for more than 90 out of 100 compounds. The PAL3 system provides various tools and modules enabling the automated preparation of calibration standards and samples to meet diverse customer needs, resulting in less manual work for the user. Agilent 7000 series triple quadrupole mass spectrometer coupled to 8890 GC offers excellent selectivity and sensitivity to target analytes. This newly developed automated workflow on the integrated PAL3-GC/TQ system offers an easy to use and more environmentally friendly solution for users by reducing chemicals/standards consumption as well as waste. This automated solution will enhance lab productivity and reduce costs significantly.

References

1. Method EPA 8270E: Semivolatile Organic Compounds by Gas Chromatography/Mass Spectroscopy, Revision 6, June **2018**.
2. EPA Method 3510C: Separatory Funnel Liquid-Liquid Extraction
3. A Fast Method for EPA 8270 in MRM Mode Using the 7000 Series Triple Quadrupole GC/MS, *Agilent Technologies application note*, 5994-0691EN, **2019**

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DE14313123

Published in the USA, February 1, 2024
5994-7138EN

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Novel Column Chemistry Raises the Bar on Sensitivity and Data Accuracy in the Analysis of Semivolatile Organic Compounds

Authors

Vanessa Abercrombie,
Frans Biermans,
Anastasia Andrianova,
Joel Ferrer, and Ashlee Gerardi
Agilent Technologies, Inc.

Abstract

As ionization sources continue to advance, lowering limits of detection and increasing confidence in analyte identification, column technologies can also be used in conjunction to push the practical limits of sensitivity and data accuracy. Increases in the detection of analyte response also result in unwanted increases in the detection of background noise. Gas chromatography/mass spectrometry (GC/MS) column technology that lowers interfering column bleed ions and elevated bleed baselines, maintains peak shape for active compounds, and can withstand aggressive thermal cycling can greatly enhance the performance and productivity of GC/MS methods. This application note examines how column attributes like bleed, inertness, and thermal stability can further benefit the sensitivity and accuracy of an MS. This study illustrates achievable data parameters, like sensitivity limits at trace levels, retention time consistencies, and data accuracy for active semivolatile organic compounds (SVOCs), when the Agilent 7010D triple quadrupole GC/MS (GC/TQ) system is used.

Introduction

Governmental regulatory authorities have established method and performance criteria for GC/MS measurement of SVOCs that are identified as pollutants in environmental and industrial matrices. The United States Environmental Protection Agency (U.S. EPA) method 8270, for example, contains a list of over 200 compounds, some of which can be susceptible to unwanted chemical activity in the instrument flow path, resulting in data quality degradation. If the performance criteria of method 8270 are not met, system maintenance is often needed, such as liner replacement followed by column trimming or replacement, resulting in unplanned instrument downtime .

Monitoring of the DFTPP tuning standard, which contains 4,4'-DDT, pentachlorophenol, and benzidine, validates the suitability of the flow path and monitors when maintenance should be performed. The breakdown of 4,4'-DDT to 4,4'-DDE, and 4,4'-DDD, as well as the tailing factors of benzidine and pentachlorophenol, tests the flow path inertness, indicating the activity of susceptible acidic and basic analytes. GC columns contribute the largest surface area in the sample flow path and, therefore, are a critical factor in controlling interferences in the analytical path. Agilent Ultra Inert (UI) GC liners, along with an inert GC column phase, can improve the robustness of SVOCs analyses.[1]

Stationary phases used in GC/MS analysis of SVOCs are typically comprised of liquid polymers with a polysiloxane backbone. When heat is applied to the column during routine use, the terminal end of the stationary phase polymer can bend back and attack itself; this is called "backbiting." Ring structures, which are thermodynamically stable, are liberated from the stationary phase, increasing background noise and raising the baseline; this can be problematic for low signal-to-noise (S/N) analytes. Peak integration can become less repeatable, lowering quantitation accuracy. In addition, the increase in freed ring structures—which fragment in the ion source—and analytes can cause spectral interference in extracted mass spectra and decrease the qualitative score of a library spectral hit. The Agilent J&W HP-5Q and DB-5Q GC columns have an increased thermal stability at upper temperature limits, allowing for less spectral interference, lower levels of column bleed, and better data quality, especially for heavier analytes that may suffer from issues with lower S/N.[2]

The new Agilent high-efficiency ion source (HES) 2.0, as seen in Figures 1 and 2, is equipped with a novel dipolar RF lens that redirects carrier gas and low mass ions by > 95%. The deflected ions land on adjacent lenses and are pumped out before entering the mass analyzer, providing reduced noise and extended instrument robustness while maintaining sensitivity. A ramped RF amplitude versus mass is implemented to avoid spectrum tilt. The reduction of noise allows for a further increase of sensitivity to attogram-level detection limits. Built-in intelligence features such as SWARM autotune and early maintenance feedback further enhance instrument performance and diagnostic capabilities. The DB-5Q GC column and the HES 2.0 work in concert to increase the robustness of difficult analyses such as that of SVOCs.[3,4]

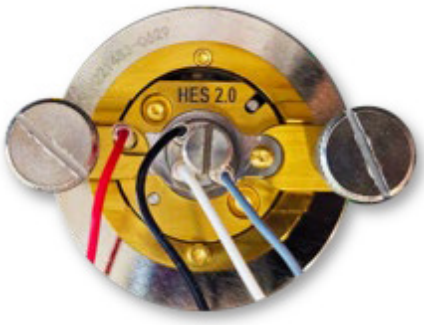


Figure 1. Front view of the Agilent HES 2.0.

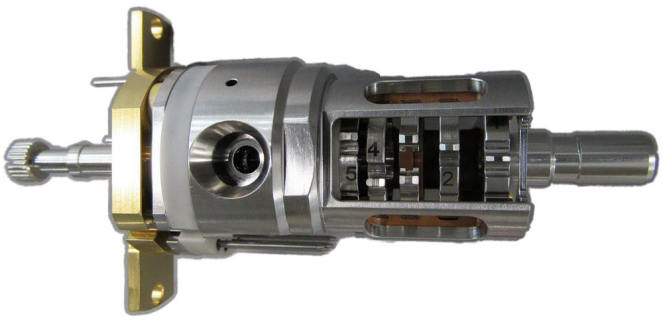


Figure 2. Side view of the Agilent HES 2.0.

Experimental

The Agilent 8000 Series semivolatiles standard (part number SVM-8270-1)—a representative mixture of semivolatile acids, bases, and neutrals—was prepared in dichloromethane (DCM) for calibration standards to be analyzed at 10 to 1,000 pg on column. A semivolatile internal standard mix (part number CRM48902) was procured from Sigma-Aldrich (Saint Louis, MO, U.S.).

The tuning standard, containing a mixture of benzidine, pentachlorophenol, 4,4'-diphenyltrichloroethane (4,4'-DDT), and decafluorodiphenyltrichloroethane (DFTPP) at 25 µg/mL, was used to obtain MS calibration and tuning settings.

A composite mixture of soils extracted with DCM prepared for method 8270 analysis, which is a representative matrix residue that is typically encountered in the lab, was procured from Pace Analytical (Mt. Juliet, TN, U.S.).

An Agilent 8890 GC coupled with an Agilent 5977B GC/MSD and Inert Extractor source, as well as an 8890 GC coupled with a 7010D triple quadrupole GC/MS (GC/TQ) system, upgraded with the HES 2.0, were used for the analysis.

Table 1. GC parameters for the Agilent 8890 GC.

Parameter	Value
Agilent 8890 GC	
Inlet	300 °C, Pulsed splitless mode
Injection Volume	0.5 mL
Inlet Liner	Agilent UI inlet liner, split, low pressure drop (p/n 5190-2295)
Injection Pulse Pressure	30 psi until 0.6 min
Purge Flow to Split Vent	50 mL/min at 0.6 min
Septum Purge Flow	3 mL/min
Oven	40 °C (0.5 min), ramp 10 °C/min to 100 °C, ramp 25 °C/min to 260 °C, ramp 5 °C/min to 280 °C, 15 °C/min to 320 °C (5 min), 10 °C/min to 330 °C (10 min), 10 °C/min to 340 °C (10 min)
Column	
Carrier Gas	Helium, 1.3 mL/min, constant flow
Column	– Agilent J&W DB-5Q, 30 m × 0.25 mm, 0.25 µm (p/n 122-5532Q) – Agilent J&W DB-5ms UI, 30 m × 0.25 mm, 0.25 µm (p/n 122-5532UI) – 5ms-Type column X, 30 m × 0.25 mm, 0.25 µm – 5ms-Type column Y, 30 m × 0.25 mm, 0.25 µm
Inlet Connection	Split/splitless inlet
Outlet Connection	MSD

Table 2. MS parameters for the Agilent 5977B GC/MSD.

Parameter	Value
Agilent 5977B GC/MSD	
Source	Agilent Inert Extractor source
Mode	Scan (35 to 500 amu)
Solvent Delay	2.5 min
Source Temperature	300 °C
Quadrupole Temperature	175 °C
Gain	1.0

Table 3. MS parameters for the Agilent 7010D GC/TQ.

Parameter	Value
Agilent 7010D GC/TQ	
Source	Agilent HES
Mode	Dynamic multiple reaction monitoring (dMRM)/scan
Solvent Delay	2.5 min
Source Temperature	300 °C
Quadrupole Temperature	175 °C
Gain	1.0

Table 4. Quantitative/qualitative transitions (dMRM based) for Agilent 7010D GC/TQ acquisition parameters.

	SVM-8270-1		Quantitative				Qualitative			
Peak	RT	Compound	Precursor Ion	Product Ion	Dwell	CE	Precursor Ion	Product Ion	Dwell	CE
1	3.043	NDMA	74	44	56.07	6	74	42	56.07	14
2	6.411	Phenol	94	66.1	14.17	15	94	65.1	14.17	20
3	6.670	Chlorophenol-2	128	64	9.22	15	128	63	9.22	30
4	6.940	1, 3-Dichlorobenzene	146	111	8.33	15	146	75	8.33	30
4.5	6.960	1, 4-Dichlorobenzene-d_4	150	115	9.31	15	150	78	9.31	3
5	7.074	1, 4-Dichlorobenzene	146	111	13.21	15	146	75	13.21	30
6	7.313	1, 2-Dichlorobenzene	146	111	9.63	15	146	75	9.63	30
7	7.700	Nitrosodi-n-propylamine N-	113.1	71	7.14	10	101	70	7.14	0
8	7.450	Methylphenol-2 (Cresol o-)	108	107	7.96	15	107	77	7.96	15
9	7.500	bis(2-Chloro-1-methylethyl)ether	121	77	7.68	5	121	49	7.68	30
10	7.700	Methylphenol-4 (Cresol p-)	108	107.1	8.36	15	107	77.1	8.36	15
11	7.800	Hexachloroethane	200.9	165.9	7.19	15	118.9	83.9	7.19	35
12	7.900	Nitrobenzene	123	77	8.28	10	77	51	8.28	15
13	8.250	Isophorone	138	82	10.46	5	82	54	10.46	5
14	8.370	Nitrophenol, 2-	138.9	81	11	15	109	81	11	10
15	8.450	Dimethylphenol 2,4- (2, 4-xylenol)	122.1	107	12.24	10	107.1	77.1	12.24	15
16	8.600	bis(2-Chloroethoxy)methane	95	65	10.37	5	93	63	10.37	5
17	8.700	Dichlorophenol, 2,4-	163.9	63	9.65	30	162	63	9.65	30
18	8.800	Trichlorobenzene, 1,2,4-	179.9	145	10.32	15	179.9	109	10.32	30
18.5	8.805	Naphthalene-d_8	136.1	108.1	9.59	20	136.1	84.1	9.59	25
19	8.900	Naphthalene	128.1	102.1	12.38	20	128.1	78.1	12.38	20
20	8.980	Chloroaniline,4-	127	92	13.18	15	127	65	13.18	20
21	9.070	Hexachlorobutadiene	226.9	191.9	28.71	15	224.8	189.9	28.71	1
22	9.570	Phenol 4-chloro-3-methyl-	142	107	34.24	15	107	77	34.24	15
23	9.750	Methylnaphthalene, 2-	142.1	141.1	22.34	15	141.1	115.1	22.34	15
24	9.940	Hexachlorocyclopentadiene	237	143	17.85	20	237	119	17.85	20
25	10.060	Trichlorophenol, 2,4,5-	197.9	97	16.66	25	195.9	97	16.66	25
26	10.100	Trichlorophenol, 2,4 6-	198	97	17.45	30	196	97	17.45	30
27	10.300	Chloronaphthalene, 2-	162	127.1	21.04	20	162	77	21.04	35
28	10.400	Nitroaniline, 2-	138	92	25.89	15	138	65	25.89	25
29	10.600	Dimethyl phthalate	163	92	23.89	30	163	77	23.89	20
30	10.670	Dinitrotoluene, 2,6-	165	90.1	20.02	15	165	63	20.02	25
31	10.740	Acenaphthylene	152.1	102.1	14.15	30	151.1	77	14.15	25
32	10.840	Nitroaniline, 3-	138	92	11.18	15	138	80	11.18	5
32.5	10.826	Acenaphthene-d_{10}	164.1	162.1	10.43	15	162.1	160.1	10.43	20
33	10.910	Acenaphthene	154.1	127	10.43	40	153.1	77	10.43	45
34	10.950	Phenol, 2,4-dinitro-	184	107	12.76	25	184	79	12.76	25
35	11.010	Nitrophenol, 4-	138.9	109	12.71	5	109	81	12.71	10
36	11.120	Dibenzofuran	168.1	139.1	13.47	25	139.1	63	13.47	35
37	11.080	Dinitrotoluene, 2,4-	165	119	12.55	5	165	63	12.55	45
38	11.340	Diethyl phthalate	149	93	12.42	15	149	65	12.42	20
39	11.460	Fluorene	166.1	165.1	10.14	15	165.1	163.1	10.14	35
40	11.470	Chlorophenyl phenyl ether, 4-	204	77	10.34	30	141.1	115.1	10.34	20

Peak	SVM-8270-1		Quantitative				Qualitative			
	RT	Compound	Precursor Ion	Product Ion	Dwell	CE	Precursor Ion	Product Ion	Dwell	CE
41	11.480	Nitroaniline, 4-	138	108.1	10.1	5	108	80	10.1	15
42	11.510	DNOC (2-methyl-4 6-dinitrophenol)	198	167.9	13.44	5	198	121	13.44	10
43	11.630	Azobenzene	105	77.1	11.37	5	77	51	11.37	15
44	11.970	4-Bromophenyl phenyl ether	250	141	22.75	20	248	141	22.75	20
45	12.020	Hexachlorobenzene	283.8	213.9	28.52	30	248.9	214	28.52	15
46	12.220	Pentachlorophenol	265.9	167	30.92	25	165	130	30.92	25
47	12.430	Phenanthrene	178.1	152.1	22.36	25	176.1	150.1	22.36	25
47.5	12.360	Phenanthrene-d10	188.3	160.2	18.93	20	188.3	158.2	18.93	35
48	12.490	Anthracene	178.1	152.1	18.72	25	178.1	151.1	18.72	30
49	12.650	Carbazole	167	139	33.75	45	167	89	33.75	60
50	13.000	Di-n-butyl phthalate	149	121	74.98	15	149	65	74.98	25
51	13.690	Fluoranthene	202.1	152.1	26.52	30	201.1	200.1	26.52	15
52	13.980	Pyrene	202.1	151	21.27	45	201.1	200	21.27	15
53	14.900	Butyl benzyl phthalate	149	65	55.86	25	91	65	55.86	15
54	15.860	Benz[a]anthracene	228.1	226.1	23.12	30	226.1	224.1	23.12	35
54.5	15.842	Chrysene-d_{12}	240.2	236.2	16.17	3	236.1	232.1	16.17	40
55	15.930	Chrysene	226.1	224.1	23.59	40	113.1	112.1	23.59	10
56	16.000	bis(2-Ethylhexyl) phthalate	167	149	23.09	5	149	65	23.09	25
57	17.450	Di-n-octyl phthalate	149	93	27.51	20	149	65	27.51	25
58	18.050	Benzo[b]fluoranthene	252.1	250.1	18.69	35	126	113.1	18.69	10
59	18.150	Benzo[k]fluoranthene	252.1	250.1	18.56	30	126.1	113.1	18.56	10
60	18.700	Benzo[a]pyrene	252.1	250.1	21.83	35	125	124.1	21.83	10
60.5	18.754	Perylene-d_{12}	264.2	260.1	16.16	35	260.1	256.1	16.16	40
61	20.580	Indeno[1,2,3-cd]pyrene	276.1	274.1	30.66	40	137	136	30.66	15
62	20.660	Dibenz[a,h]anthracene	278.1	276.1	24.37	35	276.1	274.1	24.37	35
63	21.080	Benzo[g,h,i]perylene	276.1	274.1	45.33	45	138	137	45.33	1

Results and discussion

Reduced bleed stabilizes GC/MS baselines

According to EPA method 8270, the GC/MS system must meet the required performance criteria to confirm suitability before samples can be analyzed. The system suitability results, along with the chromatographic resolution criteria of closely eluting structural isomer pairs, have been described previously for an Agilent UI glass wool liner and UI 5ms-type column.[1] The chromatographic performance of the J&W DB-5Q GC column was tested for the suitability of method 8270 by GC/MS, and a representative chromatogram is demonstrated in Figure 3. When comparing the J&W DB-5Q to a conventional 5ms-type GC column, a significant decrease in column bleed stabilized the chromatographic baseline, which is ideal when analyzing low concentrations of compounds at high temperatures (Figure 4).

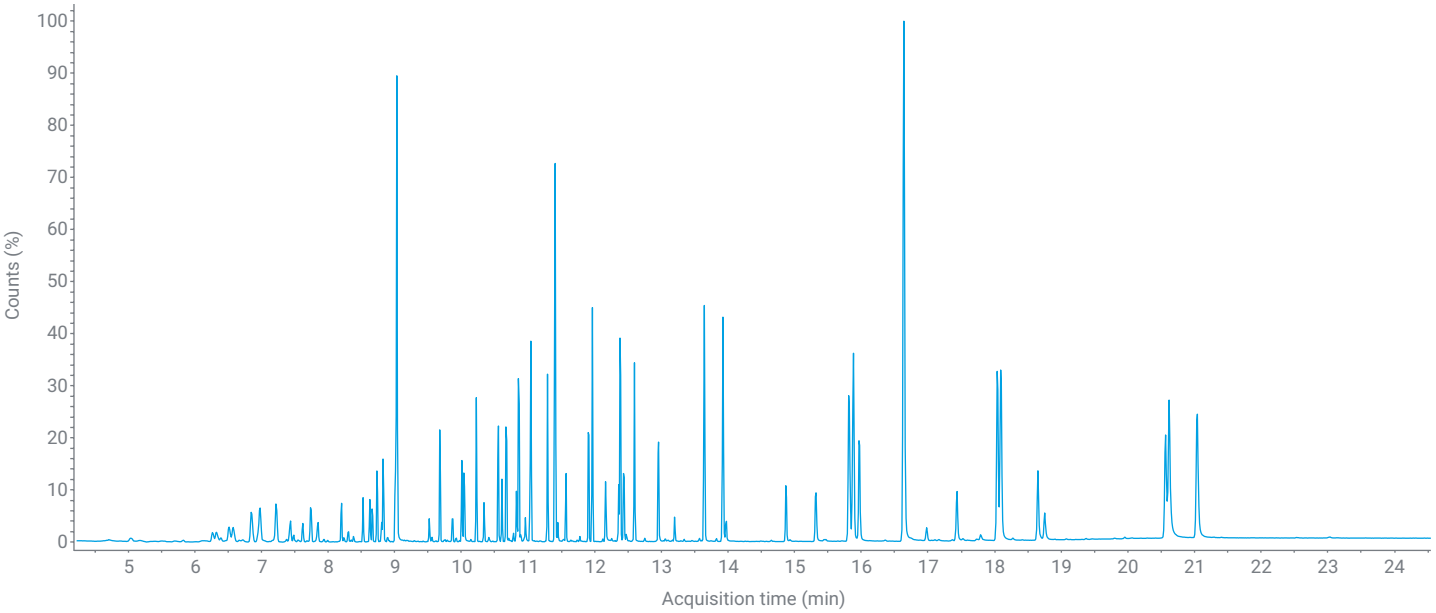


Figure 3. A representative chromatogram of 8,270 compounds analyzed on an Agilent J&W DB-5Q GC column and collected using an Agilent 5977B GC/MSD.

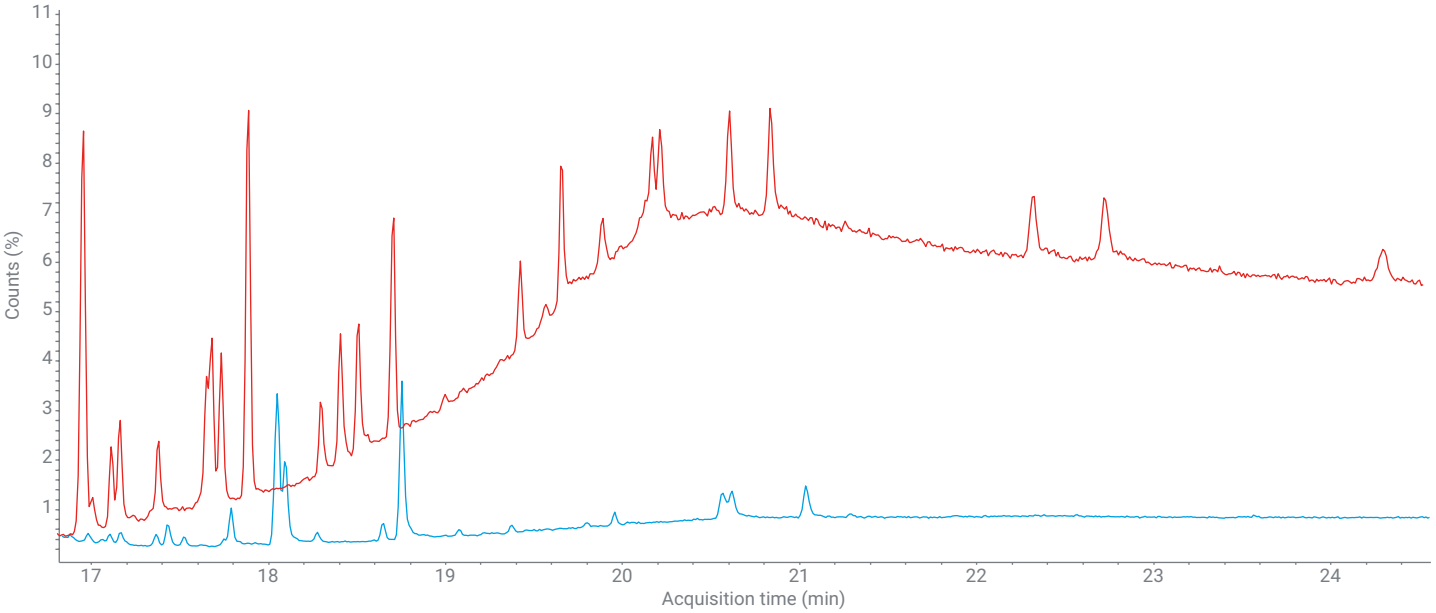


Figure 4. A standard of 50 pg on column of 8,270 compounds, analyzed on an Agilent J&W DB-5Q (blue) and the 5ms-type column Y (red), and collected on an Agilent 5977B GC/MSD.

Reduced column activity improves peak symmetry of problematic analytes

In EPA method 8270, column activity is observed as increased peak tailing, resulting in S/N loss. This is commonly observed with the more active compounds in the analyte panel. In Figure 5, the peak symmetry and S/N ratio for 2,4-dinitrophenol, a problematic analyte, was compared at 250 pg on column, analyzed on both a DB-5Q column and a conventional 5ms-type column. The column activity of the 5ms-type column X resulted in a loss of peak symmetry that significantly decreased analyte sensitivity. Also, the peak shape of another problematic compound, 2-methyl-4,6-dinitrophenol, was compared through analysis on two conventional 5ms-type columns (marked as X and Y) and the DB-5Q, as shown in Figure 6. The inertness of the DB-5Q allowed for better S/N, leading to better sensitivity for this difficult phenolic compound.

Lastly, Figure 7 demonstrates the comparison of pentachlorophenol on the DB-5Q and a conventional 5ms-type column. Again, when analyzed at the same concentration, the tailing factor of pentachlorophenol increased, leading to less S/N response. When working with difficult analytes, such as those in EPA method 8270, inert column chemistries, such as those observed on the DB-5Q, will optimize sensitivity.

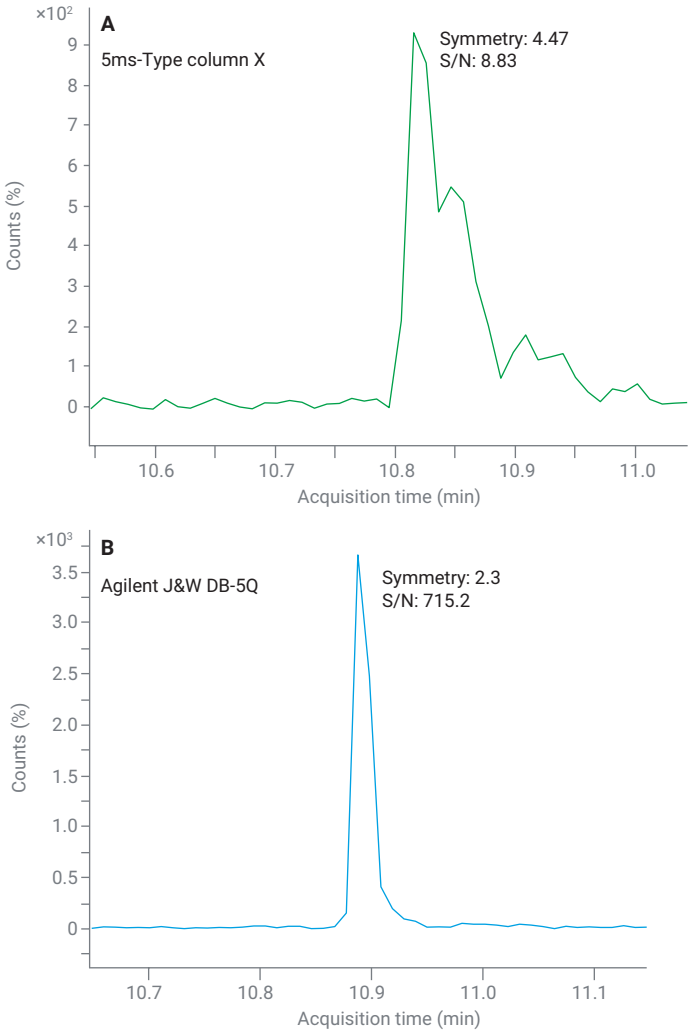


Figure 5. Integrated peak 250 pg on column 2,4-dinitrophenol, analyzed on the 5ms-type column X and an Agilent J&W DB-5Q GC column.

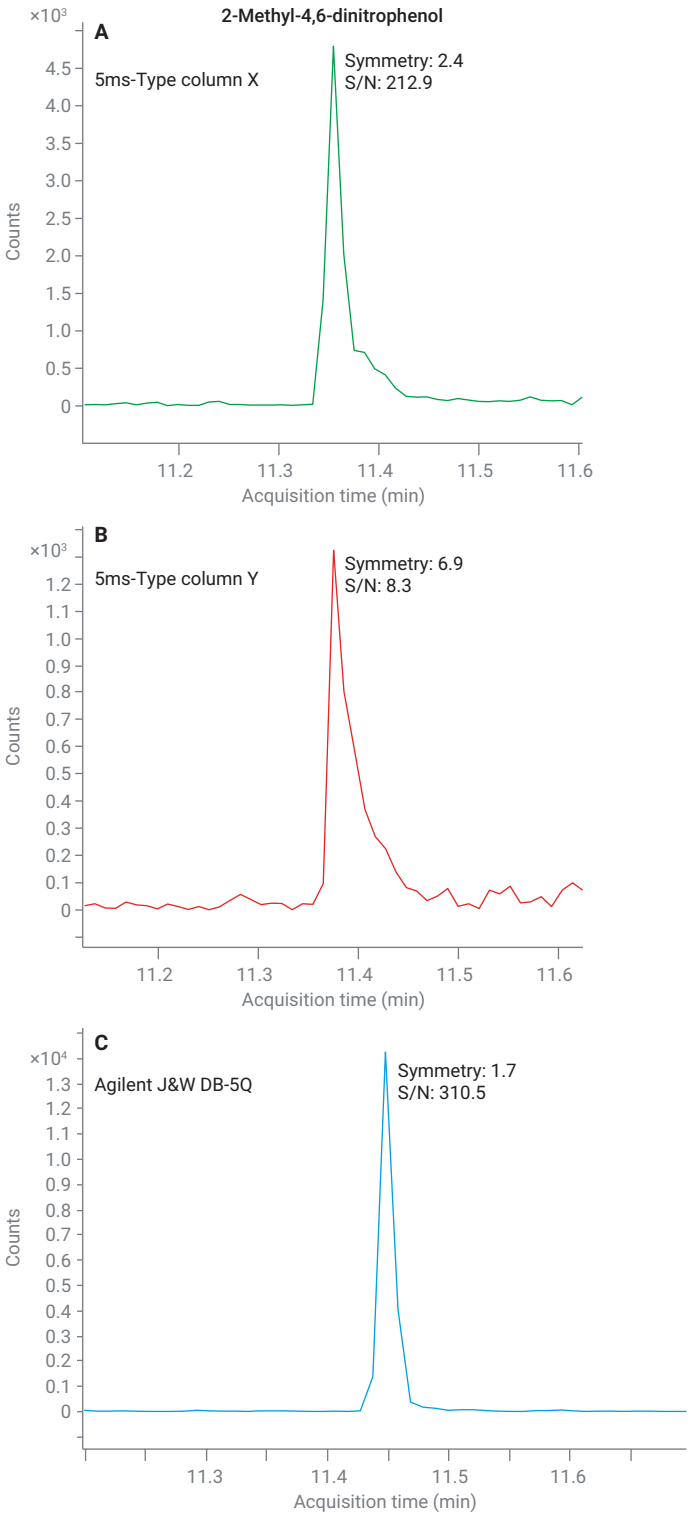


Figure 6. Integrated peak 250 pg on-column 2-methyl-4,6-dinitrophenol, analyzed on the 5ms-type columns X and Y, and an Agilent J&W DB-5Q GC column.

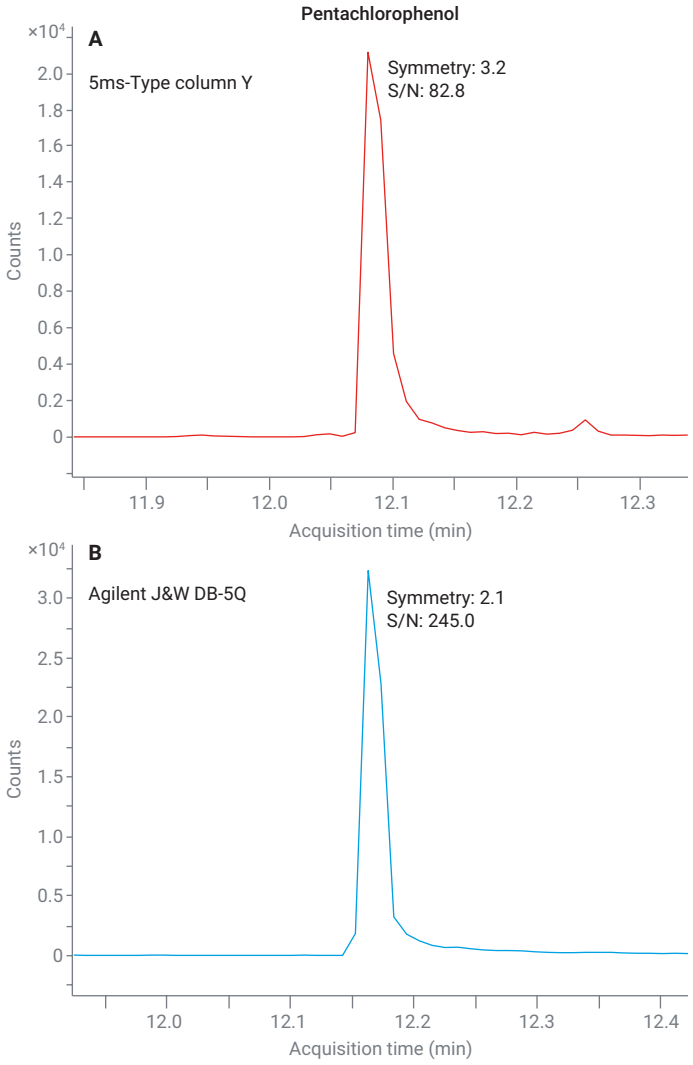
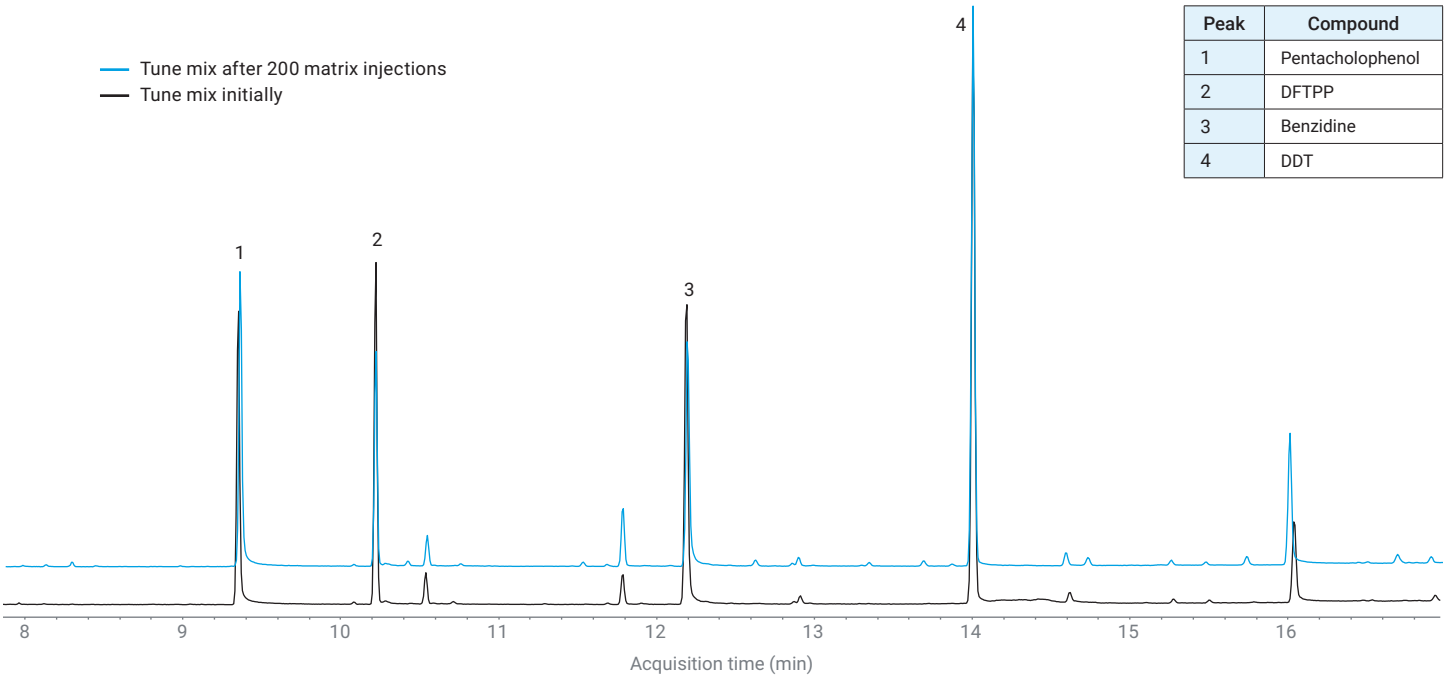


Figure 7. Integrated peak 250 pg on-column pentachlorophenol, analyzed the 5ms-type column Y and an Agilent J&W DB-5Q GC column.

Improved durability leads to consistent chromatography

To stress the robustness of the GC system in a real-world application, a heavy soil matrix was diluted in DCM and analyzed over multiple thermal cycles. DFTPP tuning standard was analyzed every five matrix injections, using %DDT breakdown as an indicator to replace the inlet liner. The inlet liner and septum were replaced every 20 matrix injections, as they failed the method criteria after %DDT breakdown. The peak shapes of pentachlorophenol and benzidine were used as indicators of increased column activity resulting from matrix accumulation and thermal degradation. Figure 8 demonstrates that after 200 matrix injections, the retention times and peak shapes for all test compounds were consistent with minimal data quality reduction. The DB-5Q column is durable to withstand routine, high-throughput cycling shape, even when working with difficult matrices such as soil extracts.



Peak	Compound
1	Pentacholophenol
2	DFTPP
3	Benzidine
4	DDT

Figure 8. DFTPP tune mix initially (black) and after 200 matrix injections (blue) on an Agilent J&W DB-5Q column.

Optimal sensitivity and acquisition flexibility

The improved sensitivity of the HES 2.0 ion source, with the triple off-axis detector configuration, allows for fast MRM speeds. Data can now be acquired in dMRM and scan modes simultaneously. This improvement allows for targeted and untargeted analyses at the same time. The dMRM is useful in setting up MRM methods, as once the retention time is inputted into Agilent MassHunter acquisition software, the dwell time is calculated automatically. While this streamlines the method setup process, if retention times shift from matrix accumulation or thermal instability, it can be helpful to collect scan data and dMRM in the same acquisition method. In Figures 9 and 10, a 10 pg on-column standard was analyzed using dMRM/scan collection mode. Figure 9 demonstrates the extracted scan chromatogram, which is zoomed-in on later-eluting compounds to display their S/N ratios. With the combination of the improved HES 2.0 ion source, along with the improved thermal stability of the DB-5Q column, it is possible to simultaneously acquire selective methods, such as dMRM and scan mode.

Selectivity matching eases column adoption

A standard EPA method 8270 analysis was conducted at 1,000 pg on-column, using the same instrumentation and method conditions, on a DB-5ms UI and a DB-5Q column. The similar selectivity allows for upgrading analytical methods without the need for more development, as seen in Figure 11. Also, with the same selectivity, there is no need to update retention times, which makes the DB-5Q compatible with existing retention time locking libraries.

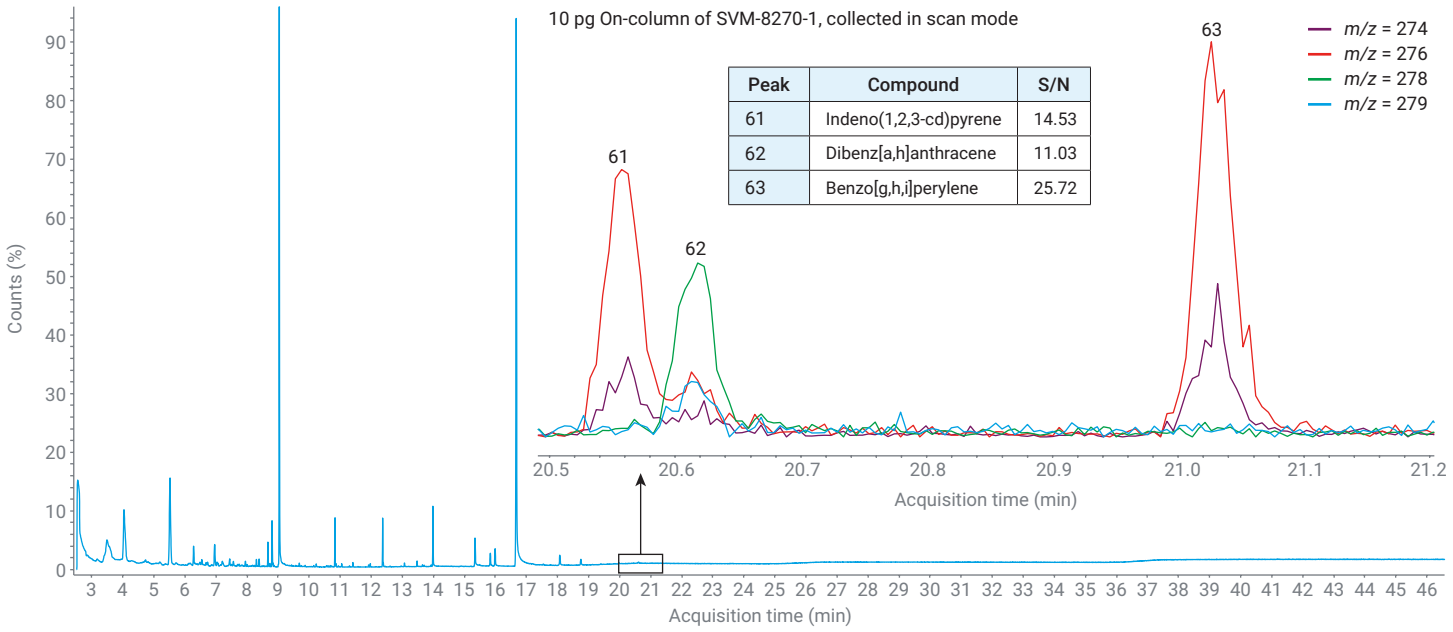


Figure 9. A standard of SVOCs analyzed at 10 pg on column, collected by dMRM/scan mode with the extracted scan data.

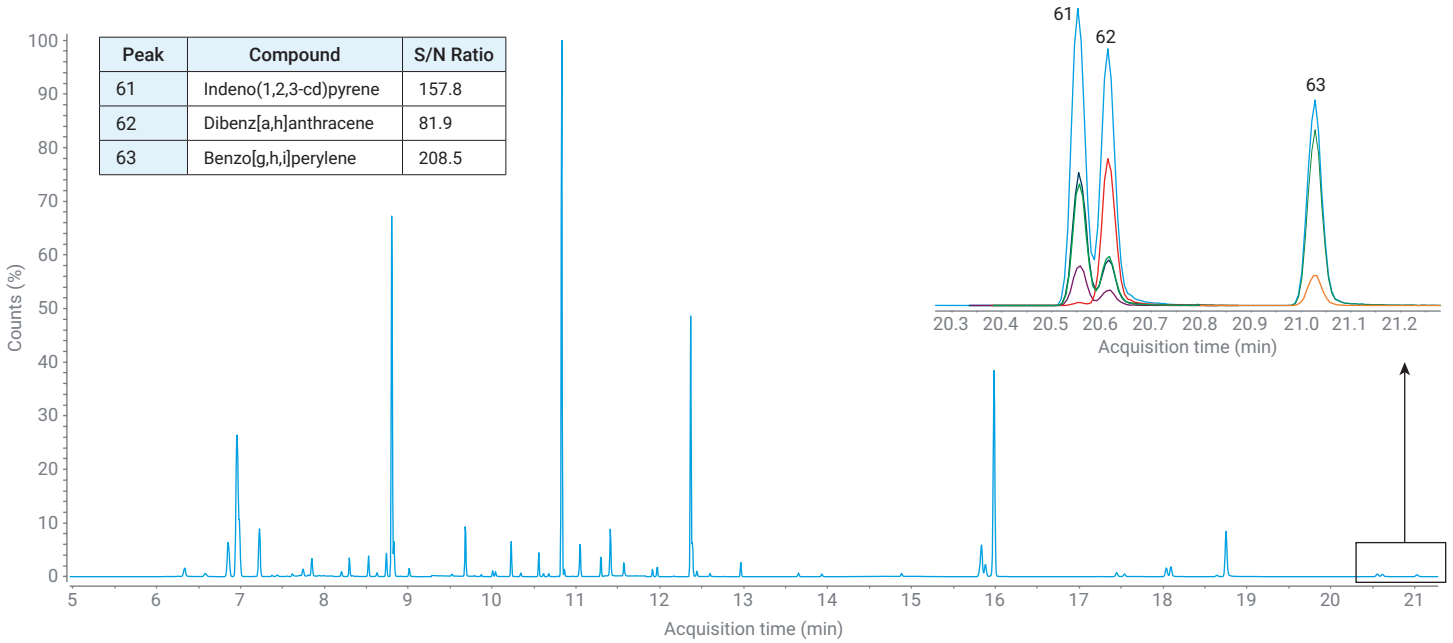


Figure 10. A standard of SVOCs analyzed at 10 pg on column, collected by dMRM/scan mode with the extracted MRMs.

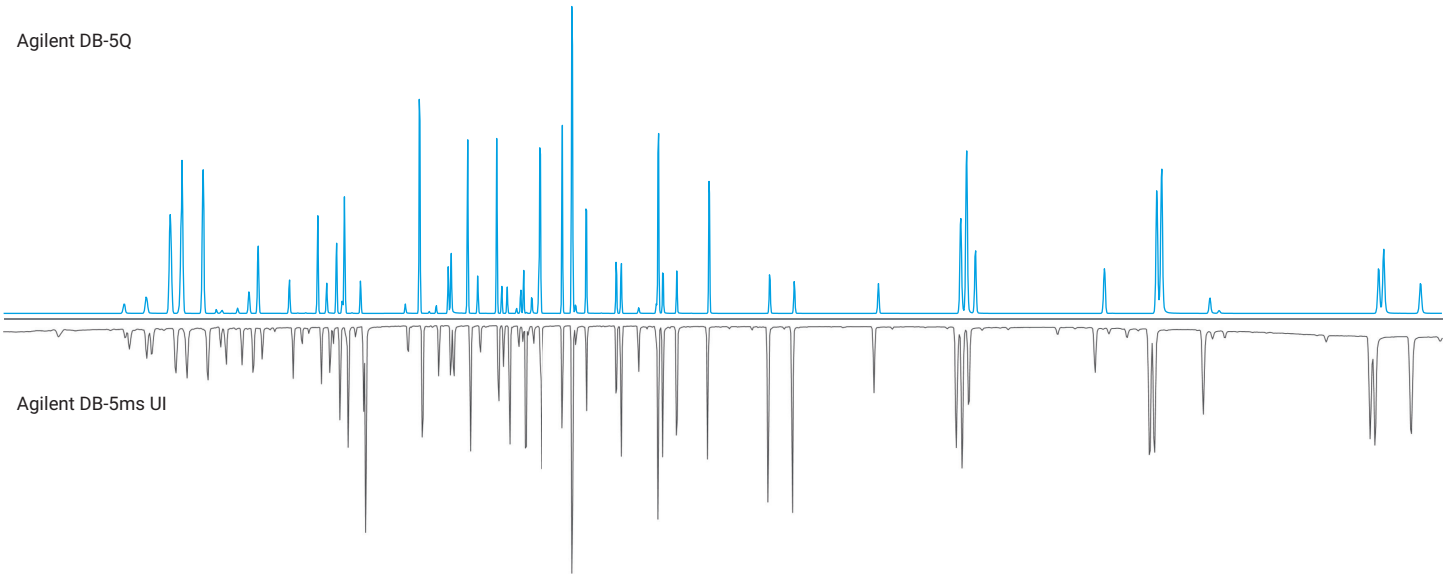


Figure 11. The Agilent J&W DB-5Q has similar selectivity to an Agilent J&W DB-5ms UI, as demonstrated in the analysis of 8,270 compounds.

Conclusion

This application note demonstrates that the Agilent J&W DB-5Q GC column can exceed the performance requirements of EPA method 8270. Agilent Ultra Inert chemistry across the sample flow path will maintain peak symmetry of problematic analytes, leading to improved limits of detection and accurate integration. Ultralow-bleed chemistry stabilizes baselines and reduces interfering bleed ions. High-temperature stability allows for the repeated temperature cycling needed for high throughput methods, even when analyzing heavy, complex soil matrices. The matching selectivity of the J&W DB-5Q compared to the Agilent J&W DB-5ms UI allows for seamless adoption, including compatibility with existing retention time locking libraries. The analytical performance of the DB-5Q coupled with the upgraded Agilent HES 2.0 allows for optimal sensitivity, as well as the ability to perform targeted and nontargeted analyses in tandem.

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DE-000135

Printed in the USA, August 14, 2024
5994-7686EN

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Accurate Mass Library for PFAS Analysis in Environmental Samples and Workflow for Identification of Pollutants in Drinking Water Using GC/Q-TOF

Authors

Luann Wong, Gabrielle Black, and Thomas Young
Department of Civil and Environmental Engineering, University of California, Davis, CA, U.S.

Sofia Nieto
Agilent Technologies, Inc.

Abstract

Development of accurate mass libraries in environmental applications is key in expanding the scope of monitored compounds and allowing for target/suspect detection with high confidence. It also provides the opportunity to use a targeted data analysis approach that offers higher sensitivity and flexibility compared to nontarget screening.

This application note describes the development and use of an accurate mass personal compound database and library (PCDL) of per- and polyfluoroalkyl substances (PFAS) for the Agilent 7250 GC/Q-TOF and demonstrates how the PCDL can be applied in both target as well as nontarget screening approaches using environmental samples, such as drinking water extracts. This study also demonstrates the benefits of using the high-resolution accurate mass GC/Q-TOF in nontarget screening using NIST23 and third-party libraries for identifying a substantial number of other contaminants of industrial origin in drinking water.

Introduction

PFAS are emerging contaminants of increasing concern due to their environmental persistence, toxicity, and capability of bioaccumulation. There are currently thought to be over 6,000 PFAS that have been commercially produced[1], and recent studies have shown that many emerging PFAS detected in the environment can be volatile or semivolatile in nature[2–4]. Therefore, many analytical techniques are necessary for PFAS detection. Gas chromatography/mass spectrometry (GC/MS) is typically used for detecting volatile and semivolatile nonpolar PFAS compounds. In this study, the 7250 GC/Q-TOF system was used to take advantage of its high-resolution for detecting compounds with mass defects that are different from that of complex environmental matrixes.

To ensure the most sensitive and reliable detection of PFAS, an accurate mass library that includes over 150 electron ionization (EI) PFAS spectra and contains both retention times (RTs) and retention indices (RIs) was created.

The PFAS PCDL was further tested using both target and nontarget approaches when analyzing the drinking water extracts. In addition, to fully benefit from the GC/Q-TOF accurate mass capability combined with full-spectrum acquisition, enabling nontarget detection, NIST23 and the third-party library MassBank of North America (MassBank.us[5]) were also used to identify other contaminants in drinking water, with the false positives being effectively removed based on accurate mass information. Thus, many pollutants were identified in drinking water, including disinfection by-products (DBPs), industrial chemicals originated from personal care products, pharmaceuticals, as well as pesticide residues.

Experimental

Sample preparation

The drinking water samples were collected at two different locations in California, U.S. and represented two different water source categories: a small surface water (Weaverville) and a mixed surface and ground water (Irvine). Water samples (2.4 L) were extracted on a multimode solid phase extraction (SPE) using HLB, WAX, WCS, and Isoelut ENV sorbents, and eluted with 5% methyl tert-butyl ether (MTBE) in methanol (MeOH), dichloromethane (DCM), 0.5% NH_4OH in 1:1 ethyl acetate (EtAc):MeOH, and 1.7% formic acid in 1:1 EtAc:MeOH. The combined extracts were concentrated, solvent exchanged to EtAc, and diluted tenfold.

Table 1. Data acquisition parameters.

GC and MS Conditions	Agilent DB-5ms	Agilent DB-624
MS	Agilent 7250 GC/Q-TOF	
GC	Agilent 8890 GC	
Inlet	Agilent multimode inlet, Ultra Inert 4 mm liner, single taper with wool	
Inlet Temperature	70 °C for 0.01 min; 300 °C/min to 250 °C	
Injection Volume	1 µL	
Column	Agilent J&W DB-5ms Ultra Inert (UI), 30 m × 0.25 mm, 0.25 µm	Agilent DB-624 Ultra Inert, 30 m × 0.25 mm, 1.4 µm
Oven Temperature Program	35 °C for 2 min; 7 °C/min to 210 °C, 20 °C/min to 300 °C, 4 min hold	30 °C for 2 min; 3 °C/min to 75 °C, 2 °C/min to 110 °C, 10 °C/min to 210 °C, 20 °C/min to 240 °C, 2 min hold
Column Flow	1.2 mL/min constant flow	1 mL/min constant flow
Carrier Gas	Helium	
Transfer Line Temperature	250 °C	
Quadrupole Temperature	150 °C	
Source Temperature	200 °C	
Electron Energy	70 eV	
Emission Current	Variable by time segment, 0.01 to 5 µA	
Spectral Acquisition Rate	5 Hz	
Mass Range (Tune)	50 to 1,200 *m/z*	

Data acquisition and data processing

GC/MS analysis was performed using an Agilent 8890 GC coupled to an Agilent 7250 GC/Q-TOF using the data acquisition parameters described in Table 1. PFAS accurate mass spectra of GC-amenable compounds were acquired from individual PFAS standards.

The chromatographic deconvolution and library search were performed in Agilent MassHunter Unknowns Analysis software, version 11.1. Accurate mass electron ionization (EI) fragments were converted to the theoretical *m/z* using Agilent MassHunter Qualitative Analysis software, version 10.0, and the spectra were exported into the accurate mass Agilent Personal Compound Database and Library (PCDL) Manager, version 8.0. The Agilent GC/Q-TOF Pesticide PCDL, PFAS PCDL, NIST23, as well as MassBank.us were used to perform initial compound identification. Prior to performing the library search with MassBank.us, the spectra, along with metadata information from this database, were exported in the PCDL format using Agilent ChemVista software, version 1.0, as described elsewhere.[6–7] RIs and accurate mass information were used to confirm the compound identification. Statistical analysis was performed in Agilent Mass Profiler Professional (MPP), version 15.1.

Results and discussion

Accurate mass library for PFAS

To create an accurate mass GC/MS PCDL, spectra were collected for over 100 volatile and semivolatile PFAS compounds. Accurate mass fragment ions were automatically annotated with formulas based on accurate mass information and isotope ratios using MassHunter Qualitative Analysis software (Figure 1). The fragment formula annotations were verified, corrected when necessary, and automatically converted to the theoretical *m/z*.

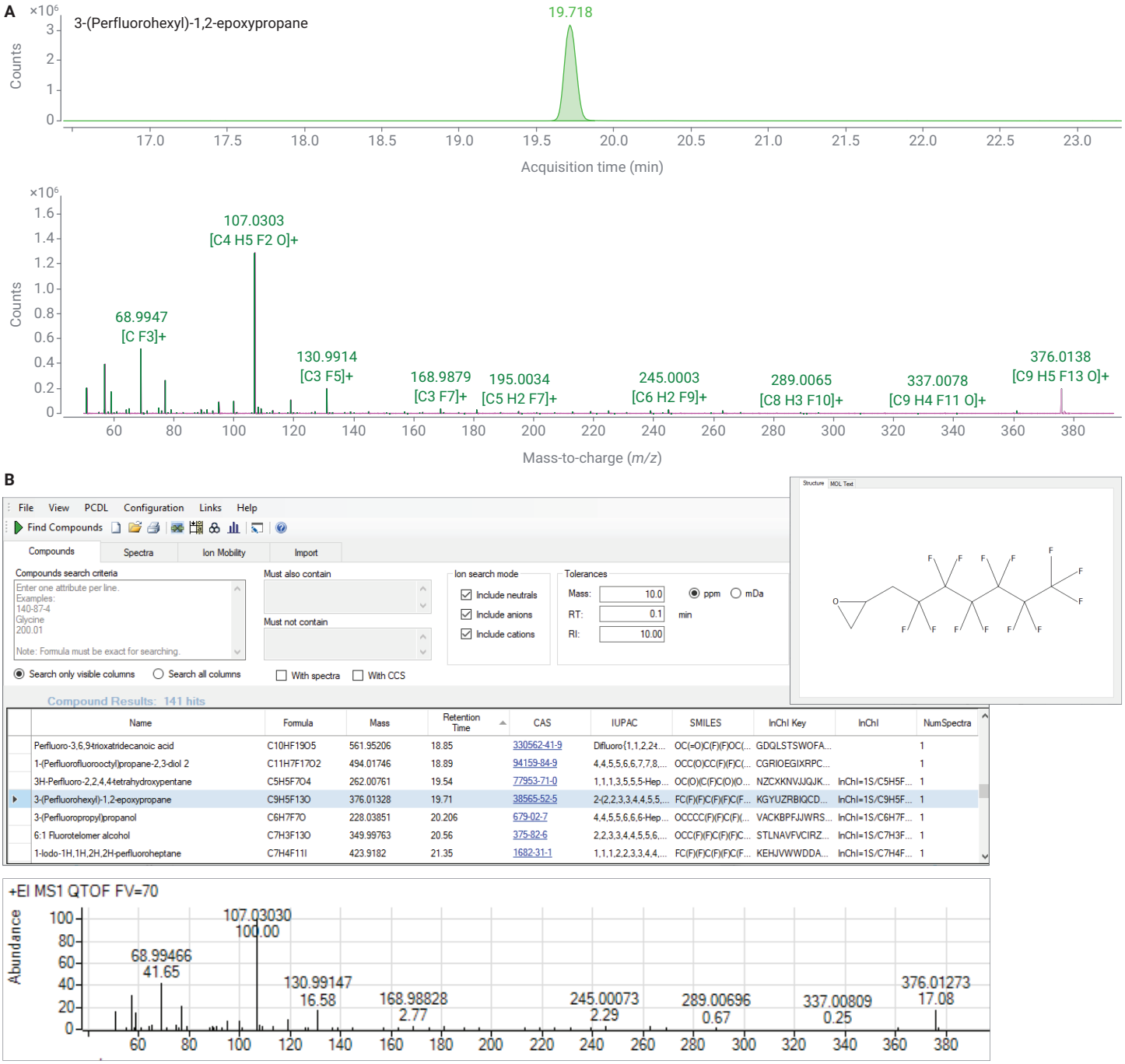


Figure 1. (A) Extracted ion chromatogram (EIC) of the molecular ion and fragment formula annotation of spectrum for one of the PFAS compounds in Agilent MassHunter Qualitative Analysis software. (B) The PFAS PCDL contains EI spectra as well as the metadata, including molecular structure and database identifiers.

The PFAS compound classes include perfluoroalkyl iodides (PFAIs), fluorotelomer iodides (FTIs), fluorotelomer alcohols (FTOHs), fluorotelomer olefins (FTOs), fluorotelomer acrylates (FTACs), fluorotelomer methacrylates (FTMACs), fluorotelomer carboxylic acids (FTCA), fluorotelomer unsaturated carboxylic acids (FTUCA), perfluoroalkane sulfonamides (FASA), and more (Figure 2).

To acquire spectra for the PFAS PCDL, the mid-polar DB-624 GC column (30 m × 0.25 mm, 1.4 µm) was used to ensure the best retention and separation of the challenging volatile PFAS. In addition to the RTs, RIs for the mid-polar column phase were also calculated for all compounds. Inclusion of RIs provides the flexibility of the GC method when using the PFAS PCDL as soon as GC column phase stays the same.

The remaining metadata, including compound structures and database identifiers, were added using PCDL Manager.

Compound overlap of the volatile and semivolatile PFAS classes between the accurate mass PFAS PCDL and NIST23 library is shown in Table 2.

Table 2. Compound overlap between the PFAS PCDL and NIST23 library.

	Percent Unique to PCDL	Total Number
All	53	158
PFCA	55	29
FTO	50	6
PFAI and FTI	17	6
FTCA and FTUCA	67	9
FTAC and FTMAC	25	8
FTOH	40	15
FASA	8	12

A significant number of the PFAS compounds (over 50%) were found to be present uniquely in the PFAS PCDL. In particular, the spectra of many per- and polyfluorinated carboxylic acids, fluorotelomer olefins, and fluorotelomer alcohols were found to be unique to the PCDL, thus highlighting the value of the accurate mass PFAS library in PFAS research.

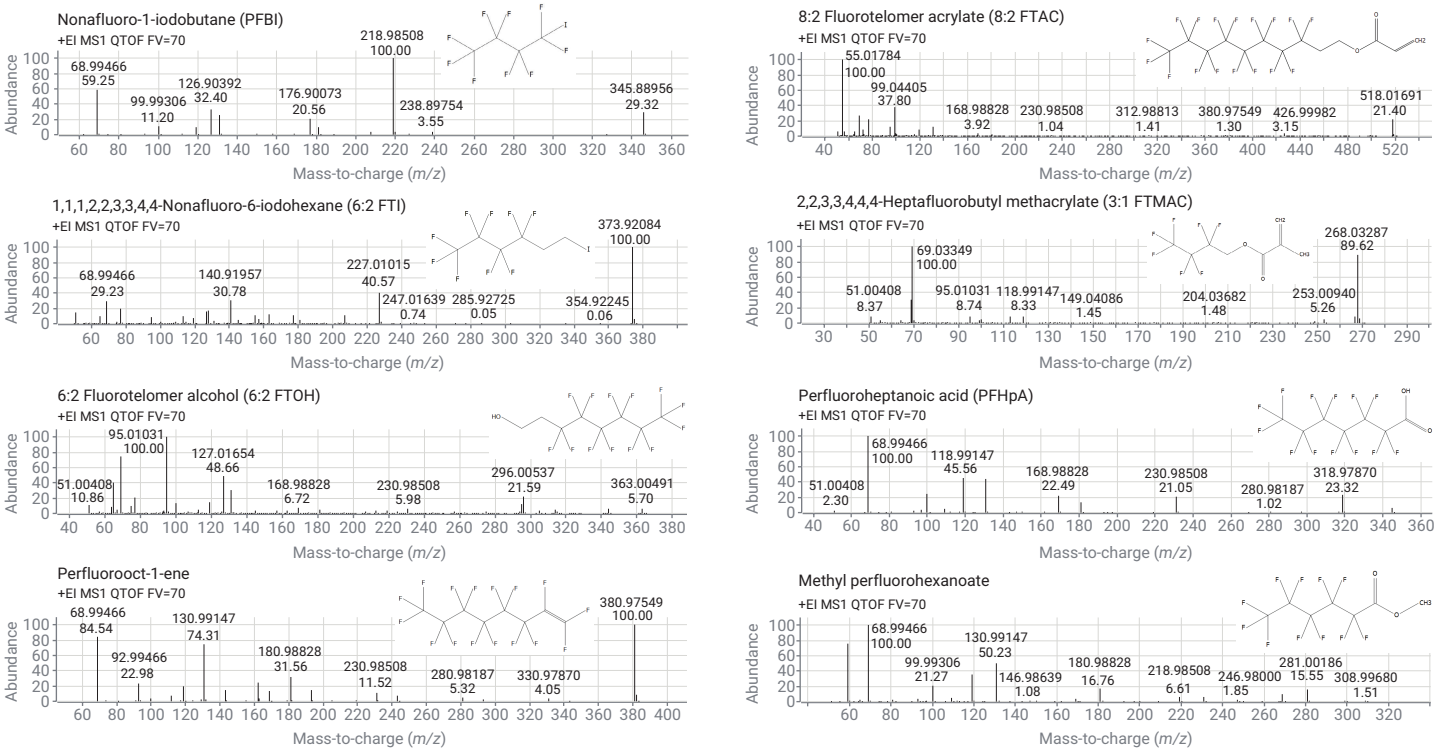


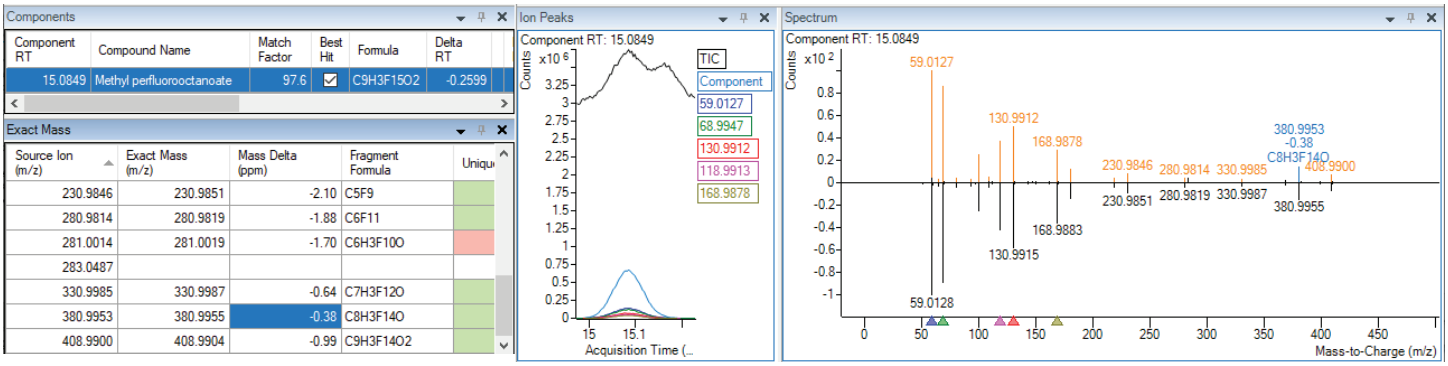
Figure 2. Examples of different PFAS compound classes from the PFAS PCDL.

PFAS in drinking water extracts

For PFAS detection, the extracts of drinking water were separated on a DB-624 column and analyzed using the 7250 GC/Q-TOF. To be able to detect early-eluting volatile PFAS, the emission current was set up by segment, as shown in Table 1, thus excluding the solvent peak from the detection.

Both target and nontarget approaches were evaluated using the PFAS PCDL. When performing the nontarget analysis, the chromatographic deconvolution was carried out in the MassHunter Unknowns Analysis software using a SureMass algorithm, which is optimized for complex, high-resolution EI data. The PFAS PCDL then was used to search the deconvoluted spectra with RT matching. One of the PFAS—a transformation product of the perfluorocarboxylic acid—was identified in drinking water extract using this approach (Figure 3A). An additional benefit of nontarget screening is the use of multiple libraries (including libraries containing unit mass spectra) that can all be searched simultaneously.

A



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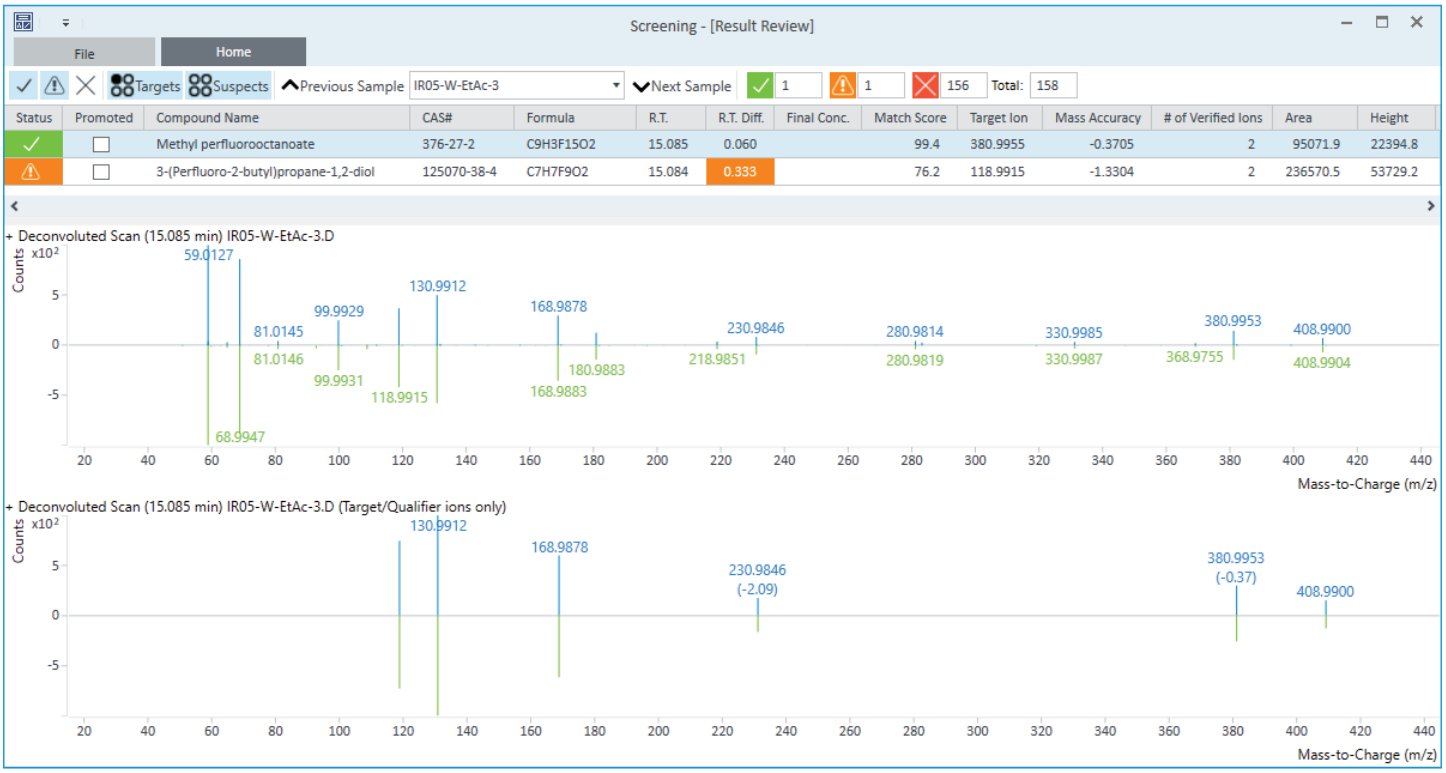

Figure 3. Example of PFAS (methyl perfluorooctanoate) identified in drinking water samples using PFAS PCDL in a nontarget approach using (A) Agilent MassHunter Unknowns Analysis software, and (B) a target GC/Q-TOF screening approach.

One of the advantages of the target approach based on the GC/Q-TOF Screener tool of the MassHunter Quantitative Analysis software (described in detail previously[8]) and PCDL is that all the parameters could be set up individually for every compound in the method. This approach allows for a substantial flexibility when performing the screening method optimization at the data processing level, enabling the highest degree of sensitivity and specificity. Another significant benefit of this approach is that it saves the time usually spent reviewing the results. The GC/Q-TOF Screener algorithm validates quantifier and qualifier ions based on outliers, and for most compounds, either confirms or rejects their presence automatically. Only a few compounds remain highlighted to indicate that a manual review might be necessary for confirmation of compound identity.

The same PFAS compound—methyl perfluorooctanoate—that was identified in drinking water extracts using a nontarget approach was also detected using the GC/Q-TOF Screener with library match scores (LMS) of > 99 (Figure 3B). It has previously been reported that perfluoroalkyl carboxylic acids can be converted to corresponding methyl esters in the presence of methanol[9], thus plausibly explaining the presence of the methyl ester of PFOA in drinking water extracts.

Identification of other contaminants in drinking water samples

To screen for additional contaminants in drinking water samples in a nontarget manner, the GC/Q-TOF Pesticide PCDL, NIST23 library, and MassBank.us were used. The choice of the DB-5ms UI column enabled RI matching while searching the NIST23 library, thus enhancing the confidence in compound identification. The ExactMass tool in MassHunter Unknowns Analysis software was used to eliminate the false positives based on the accurate mass information and molecular formula of the hit. This is particularly practical when using unit mass libraries such as NIST23 (Figure 4A) and MassBank.us.

Over 100 contaminants were identified and confirmed using accurate mass information (Figure 4A and 4B, and Tables 3 and 4) from the sample without re-injection.

Among the identified contaminants, one of the significant groups was disinfection by-products, formed when chlorine and bromine interact with organic matter. These compounds included halomethanes and haloacetic acids, which are the most common disinfection by-products. Other prominent groups of contaminants included compounds originating from industrial processes (such as those used in cleaning products and manufacturing of plastics, dyes, and pharmaceuticals), PAHs and their derivatives, as well as pesticides.

Approximately 400 hits per sample with LMS > 70 were detected using MassBank.us, including over 20 contaminants—mostly DBPs and PAHs. Since this library does not contain RI information for most of the compounds, many hits could potentially be false positives. One such example is shown in Figure 5. The hit from MassBank.us was 1-bromooctane with a high LMS of 88.3 and an RI of 1,134, according to NIST23. This would make a difference of over 400 RI units between the hit and the compound in question, indicating that the ID is likely incorrect. The difference between the compound RI and the NIST23 hit was only 10 RI units (Figure 5B). Note that the hits from both NIST23 and MassBank.us perfectly match the accurate mass information displayed in the ExactMass tables (Figure 5).

Figure 4. Examples of the contaminants identified in drinking water extracts using (A) NIST23 and (B) Agilent GC/Q-TOF Pesticide PCDL. The ExactMass tool (outlined in orange) helped to provide additional confirmation of unit mass library hits based on accurate mass. Compound ions are highlighted in the mirror plot when *m/z* corresponds to the library hit formula.

Table 3. Contaminants identified in drinking water using the NIST23 library with LMS > 75. *The cases where delta RI was calculated considering predicted RIs rather than experimental (experimental not available) are denoted by an asterisk. Some of the prominent disinfection by-products are highlighted in red.

RT	Compound Name	Match Score	Formula	RI Difference
4.79	Bromodichloromethane	95.4	$CHBrCl_2$	−56
4.81	Chloral	78.8	C_2HCl_3O	−9
4.91	Dichloroacetonitrile	86.4	C_2HCl_2N	−76
4.95	Chloromethylmethyl sulfide	94	C_2H_5ClS	−59*
5.11	Dimethyl disulfide	98.4	$C_2H_6S_2$	−35
5.35	Methyldiallylamine	85.7	$C_7H_{13}N$	−50*
5.47	Bromoacetonitrile	82.8	C_2H_2BrN	1
5.95	Dibromochloromethane	95.5	$CHBr_2Cl$	−25
6.01	Tetrachloroethylene	96.5	C_2Cl_4	−12
6.04	1,1-Dimethyl-3-chloropropanol	88.4	$C_5H_{11}ClO$	7
6.34	Bromoacetone	87.8	$C_2HBrClN$	−3
6.59	Dichloroacetic acid methyl ester	89.2	$C_3H_4Cl_2O_2$	−7*
7.67	Tribromomethane	98.2	$CHBr_3$	−10
8.24	Methyl bromo(chloro)acetate	77.4	$C_3H_4BrClO_2$	−3
8.31	Dibromoacetonitrile	86.6	C_2HBr_2N	−15
10.63	2,2-Dichloroacetamide	83.5	$C_2H_3Cl_2NO$	−4*
10.73	1,2-Dichlorbenzene	98.5	$C_6H_4Cl_2$	9
14.12	Naphthalene	81.9	$C_{10}H_8$	−4
15.45	Caprolactam	89.6	$C_6H_{11}NO$	3
16.43	2-Methylnaphthalene	89.3	$C_{11}H_{10}$	−1
16.64	Phthalic anhydride	92.5	$C_8H_4O_3$	5
16.98	Benzamide	82.8	C_7H_7NO	18
18.05	Biphenyl	83.2	$C_{12}H_{10}$	−1
18.18	Benzeneacetamide	84.3	C_8H_9NO	13
19.27	Dimethyl phthalate	75.1	$C_{10}H_{10}O_4$	8
19.96	Acenaphthene	91.2	$C_{12}H_{10}$	−4
20.18	4-Methylbiphenyl	79.4	$C_{13}H_{12}$	−4
20.28	2,4-Di-tert-butylphenol	90.2	$C_{14}H_{22}O$	10
20.56	Dibenzofuran	92.4	$C_{12}H_8O$	−5
21.23	1-Bromododecane	75.7	$C_{12}H_{25}Br$	−10
21.45	Diethyltoluamide (DEET)	78.1	$C_{12}H_{17}NO$	10
21.69	Diethyl phthalate	96	$C_{12}H_{14}O_4$	8
21.71	Fluorene	75.3	$C_{13}H_{10}$	−4
22.01	2-(Methylmercapto)benzothiazole	77.8	$C_8H_7NS_2$	2
22.43	Benzophenone	94.8	$C_{13}H_{10}O$	4
22.60	Tributyl phosphate	93	$C_{12}H_{27}O_4P$	7
23.51	Hexachlorobenzene	97.2	C_6Cl_6	9

RT	Compound Name	Match Score	Formula	RI Difference
24.19	9H-Fluoren-9-one	97.1	$C_{13}H_8O$	8
24.26	9H-Fluoren-9-ol	81.5	$C_{13}H_{10}O$	9*
24.90	Anthracene	94.4	$C_{14}H_{10}$	0
24.91	Tris(2-chloroisopropyl)phosphate	82.2	$C_9H_{18}Cl_3O_4P$	27
25.02	Benzo[h]quinoline	88.2	$C_{13}H_9N$	−2
25.53	2,4-Diphenyl-4-methyl-2(E)-pentene	76.5	$C_{18}H_{20}$	8
25.55	Benzo[f]quinoline	91.1	$C_{13}H_9N$	−1
25.73	Carbazole	76.8	$C_{12}H_9N$	−4
25.96	Di-sec-butyl phthalate	90.8	$C_{16}H_{22}O_4$	−2
26.09	3,3-Diphenyl-2-propenenitrile	82.7	$C_{15}H_{11}N$	18*
26.27	3-Methyldibenzothiophene	80.8	$C_{13}H_{10}S$	−5
26.66	3-Methylphenanthrene	84	$C_{15}H_{12}$	1
27.00	2-Methylanthracene	88.5	$C_{15}H_{12}$	−27
27.31	Dibutyl phthalate	92.4	$C_{16}H_{22}O_4$	9
27.59	9,10-Anthracenedione	93.2	$C_{14}H_8O_2$	−27
28.21	Octachlorostyrene	88.6	C_8Cl_8	−7
28.36	Cyclic octaatomic sulfur	93.1	S_8	−18
28.51	Drometrizole	82.3	$C_{13}H_{11}N_3O$	−5
28.53	Fluoranthene	97.8	$C_{16}H_{10}$	−12
28.54	Phenindione	79	$C_{16}H_{10}$	−28*
28.91	Dibenzothiophene sulfoxide	87.2	$C_{12}H_8OS$	−41*
28.94	Pyrene	89.3	$C_{16}H_{10}$	−25
29.02	1-Azapyrene	78.4	$C_{15}H_9N$	2
29.34	Bisphenol A	84.1	$C_{15}H_{16}O_2$	26*
29.37	2-Amino-9-fluorenone	83.3	$C_{13}H_9NO$	2*
29.69	Bis(4-chlorophenyl) sulfone	77	$C_{12}H_8Cl_2O_2S$	−1
29.87	2,2'-Methylene-bis-(4-methyl-6-t-butylphenol)	87.5	$C_{23}H_{32}O_2$	2
30.79	Benzo[b]naphtho[1,2-d]thiophene	77.2	$C_{16}H_{10}S$	13
30.90	7H-Benz[de]anthracen-7-one	89.2	$C_{17}H_{10}O$	85
30.94	Benzo[b]naphtho[2,1-d]thiophene	81.3	$C_{16}H_{10}S$	−16
31.04	Phthalic acid, di(2-propylpentyl) ester	94.8	$C_{24}H_{38}O_4$	−5
31.38	Bis[3,4-dichlorophenyl]sulfone	82.3	$C_{12}H_6Cl_4O_2S$	3*
31.48	Bumetrizole	78.6	$C_{17}H_{18}ClN_3O$	57
31.51	Benz(a)anthracene-7,12-dione	76.1	$C_{18}H_{10}O_2$	−48*
31.82	Bis(2-ethylhexyl) isophthalate	84.7	$C_{24}H_{38}O_4$	−35
32.37	Decachlorobiphenyl	94.3	$C_{12}Cl_{10}$	−81

Table 4. Additional contaminants identified in drinking water using the Agilent GC/Q-TOF Pesticide PCDL.

RT	Compound Name	Match Factor	Formula
6.17	2-Picoline	96.7	C_6H_7N
6.90	Methanesulfonate-methyl	79.6	$C_2H_6O_3S$
8.17	PPD/p-Phenylenediamine	80.0	$C_6H_8N_2$
8.40	o-Toluidine	82.3	C_7H_9N
8.93	Thanite	83.9	$C_{13}H_{19}NO_2S$
9.17	Benzaldehyde	98.5	C_7H_6O
9.52	Phenol	89.6	C_6H_6O
11.46	Acetophenone	94.3	C_8H_8O

RT	Compound Name	Match Factor	Formula
11.99	2,4,5-Trimethylaniline	82.7	$C_9H_{13}N$
12.90	2-Nitrophenol	77.1	$C_6H_5NO_3$
22.29	DPA/Diphenylamine (DFA)	84.7	$C_{12}H_{11}N$
22.44	Isoxadifen	93.3	$C_{16}H_{13}NO_3$
24.64	Benzylbenzoate	83.0	$C_{14}H_{12}O_2$
25.96	DIBP/Diisobutyl phthalate	86.1	$C_{16}H_{22}O_4$
27.00	1-Methylphenanthrene	85.3	$C_{15}H_{12}$

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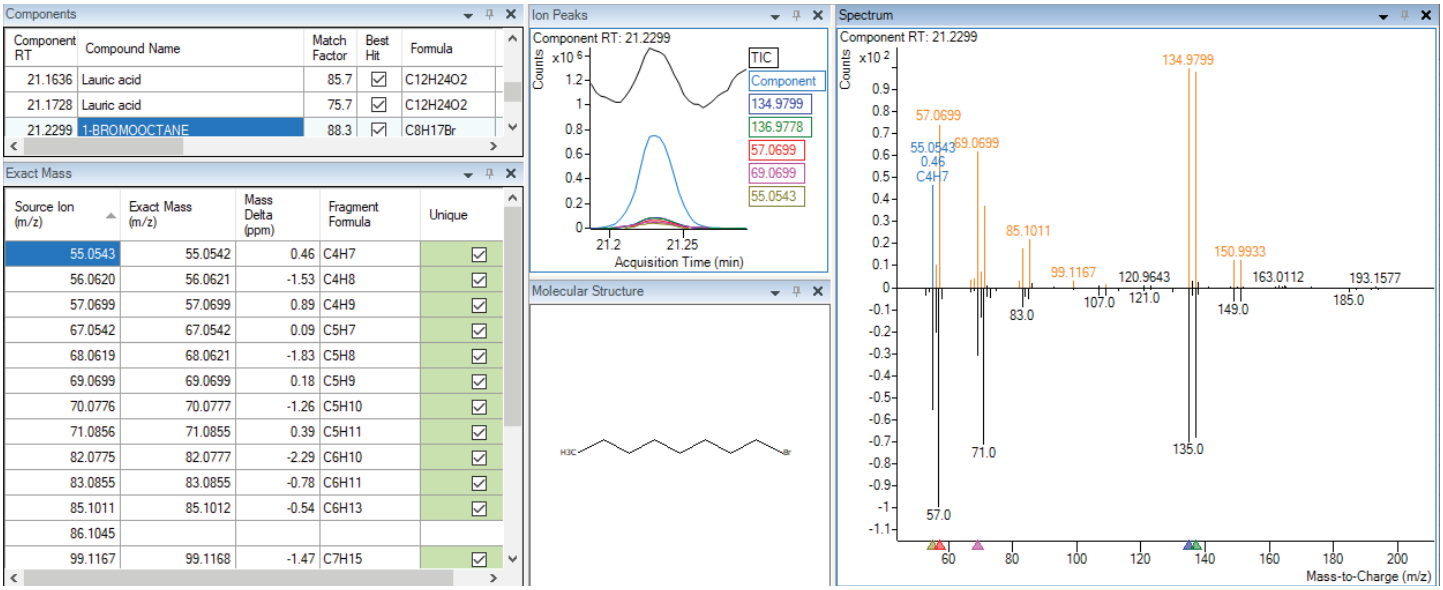

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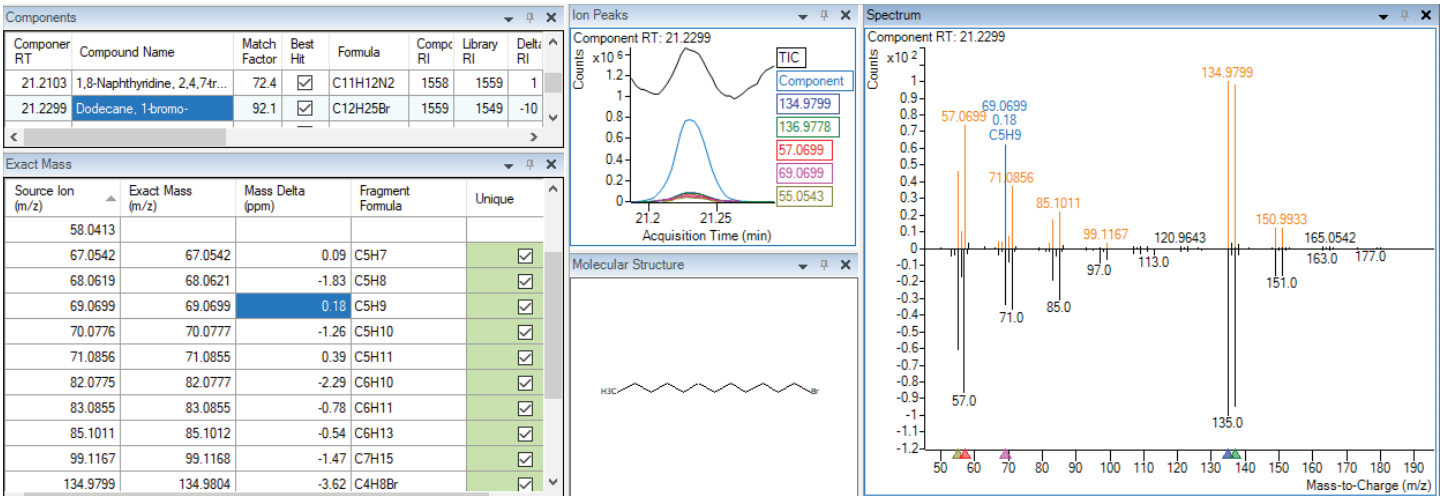

Figure 5. Compound misidentified by MassBank.us due to lack of the RI information. (A) MassBank.us hit. (B) NIST23 hit.

Additionally, an interesting case was observed for a compound with an RI of 1,858, whereby MassBank.us likely provided correct ID (with the LMS of 85.8) while NIST23 did not (Figure 6), proving the value of including third-party libraries in a compound identification workflow. The compound's most likely ID is thioxanthene, with a NIST23 experimental RI of 1,977, and an AI-predicted RI of 1,876. The experimental RI for this compound, used for the NIST23 library search, provided a significant RI delta of 124 RI units. However, the experimental RI for this compound was only based on one data point, and thus may not be accurate. The AI-predicted NIST23 RI generated a significantly smaller RI delta (18 RI units). Due to the large RI difference between the compound RI and the NIST23 experimental RI of thioxanthene, another NIST23 hit, 1-methyldibenzothiophene with the lower LMS of 81.1 was chosen (Figure 6B).

Among the contaminants with the highest response, which were identified in drinking water extracts using all three libraries, were mostly PAHs, DBPs, and phthalates (Figure 7). Individual samples of drinking water from the same group represented different households.

The contaminants in drinking water extracts were detected at a wide range of concentrations, estimated to be from low- and sub-ppb levels (for pesticides) to hundreds of ppb (in the case of PAHs), suggesting that an extended dynamic range might be desirable for this application.

Statistical analysis was performed in the MPP software, where the differences between Weaverville and Irvine water sources (n = 5 per group) were evaluated and displayed on a volcano plot (Figure 8). The volcano plot displays fold change versus statistical significance, and is used to quickly detect differences between the two groups. Compounds that were present in higher concentrations in Irvine water compared to Weaverville are colored in red and shown in the upper-right quadrant. Compounds that were found at higher concentrations in Weaverville water extracts compared to Irvine are colored in blue and displayed in the upper-left quadrant. Most contaminants occurred at higher levels in drinking water from Irvine (a more densely populated urban area) compared to Weaverville.

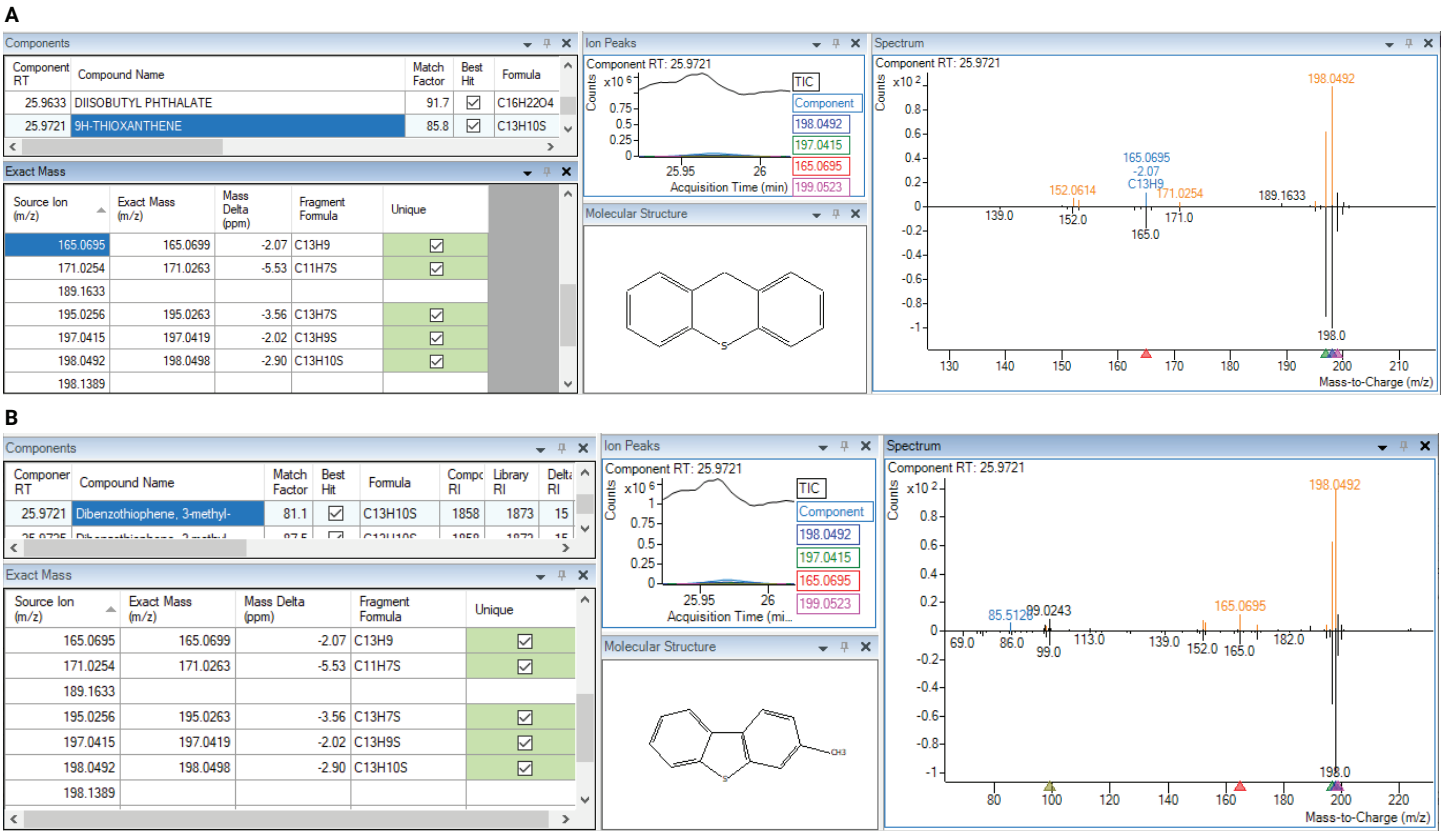

Figure 6. Compound likely misidentified by NIST23 due experimental RI information available for only one data point. (A) MassBank.us hit. (B) NIST23 hit.

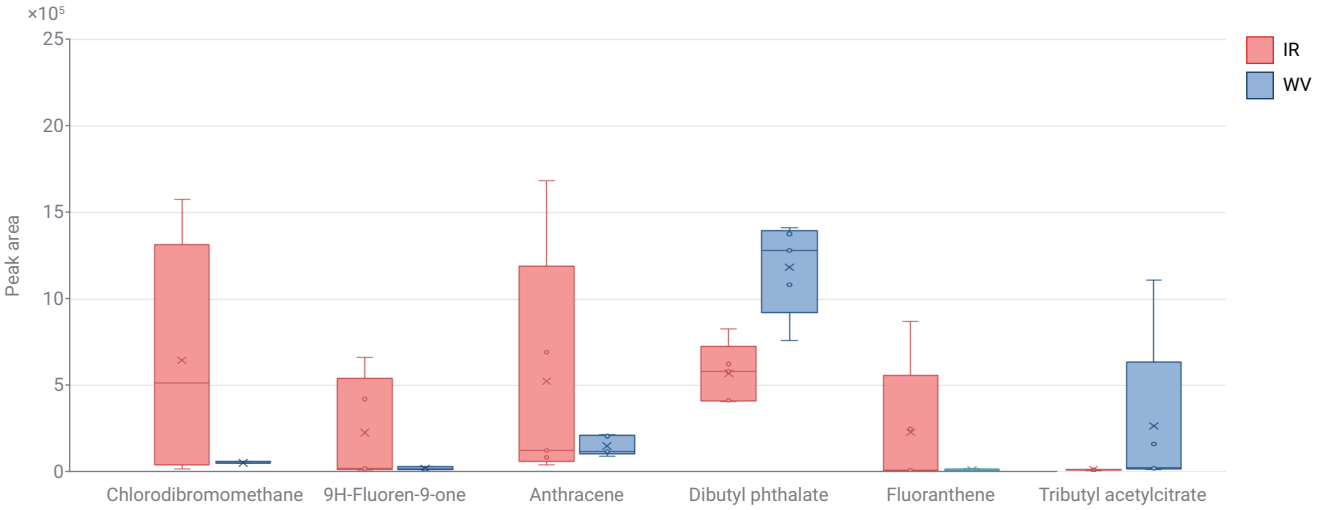

Figure 7. High-level contaminants identified in the drinking water of different households (n = 5 for each group) from Irvine (IR) and Weaverville (WV).

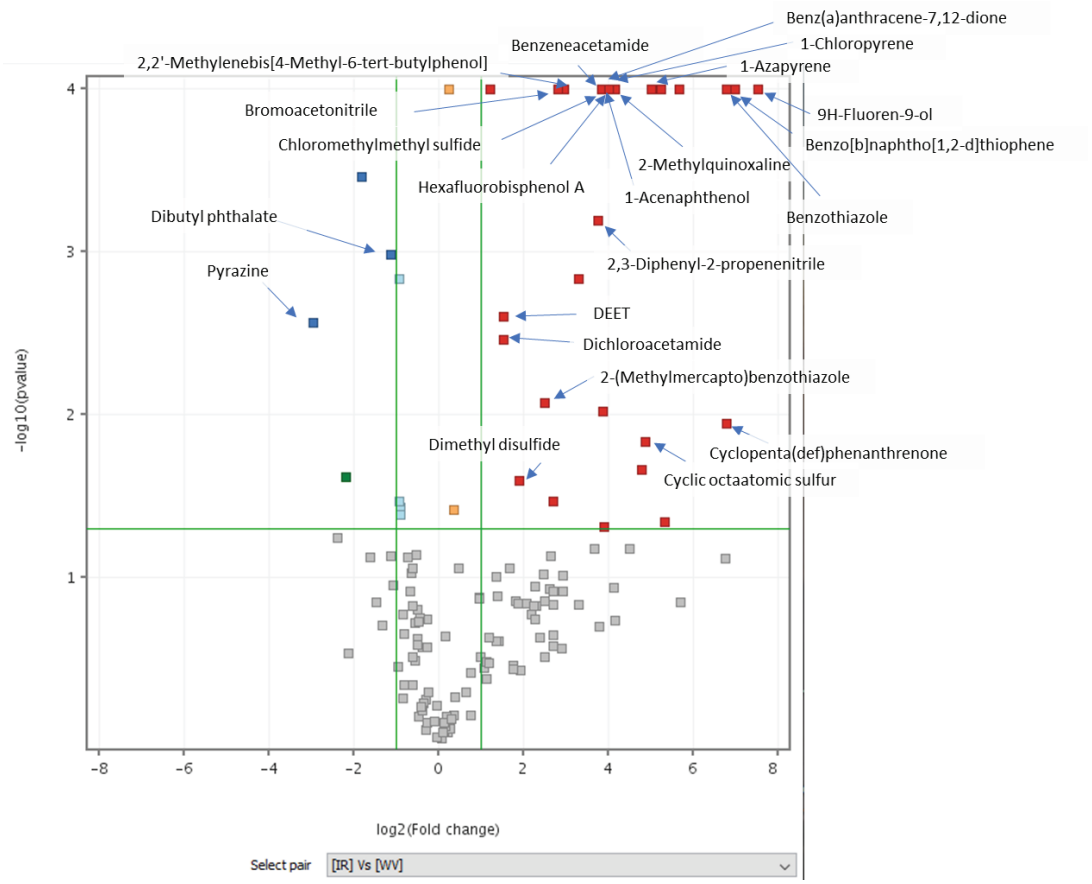

Figure 8. Comparison of water sourced in Irvine versus Weaverville on volcano plot, showing $\log_2$ of fold change (FC) versus $-\log_{10}$ of p-value.

Conclusion

Accurate mass libraries of environmental contaminants (such as PFAS) broaden the scope of suspects screened in environmental samples and increase confidence in the identification of pollutants. The accurate mass library described in this application note, containing over 150 PFAS EI spectra, including several emerging volatile PFAS, enabled identification of PFAS in drinking water samples using both nontarget and target workflows.

Additional contaminants were identified in drinking water from two different source categories, including disinfection by-products, PAHs, pesticides, and other industrial contaminants. Two drinking water sources were compared, and a higher number of contaminants were identified in the water extracts from Irvine compared to Weaverville.

Acknowledgement

The authors would like to thank the U.S. Environmental Protection Agency (EPA) for preparing the PFAS standards and providing them to Agilent Technologies through a material transfer agreement.

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DE50093873

Printed in the USA, December 5, 2023
5994-6966EN

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Application Note

Environmental

Enhanced Longevity and Revolutionized Robustness for the Sensitive Detection of 190 Pesticides over 800 Injections

Author

Brooke C. Reaser
Agilent Technologies, Inc.

Abstract

Multiresidue pesticide analysis has become one of the most difficult but important analytical challenges for those using gas chromatography and mass spectrometry. The Agilent 8890 gas chromatograph (GC) coupled with the Agilent 7010 triple quadrupole mass spectrometer (GC/TQ) with a high efficiency source 2.0 (HES 2.0) upgrade is an analytically accurate, robust, and reproducible instrument for multiresidue pesticide analysis of complex samples. The analysis of 190 pesticides in a spinach extract was conducted using an Agilent QuEChERS extraction kit across 800 injections. Only GC inlet maintenance was required over the duration of the injections. The instrument configuration that enabled robust performance included a multimode inlet, a mid-column backflushing configuration, and the HES 2.0. No degradation of the analytical method, sensitivity, or instrument performance occurred, allowing for the high-throughput, accurate, robust, and sensitive detection of pesticides in spinach.

Introduction

Multiresidue pesticide analysis remains an important analytical challenge for food safety.[1-7] Pesticides used to improve crop yield can end up in the final product, raising concerns for consumer safety. As a result, multiple governing bodies worldwide have published requirements for the maximum legal residue limit (MRL) or tolerance for pesticides allowed in a product. However, the number of pesticides used in food products continues to grow as novel chemicals are introduced, which in turn increases the complexity of the multiresidue pesticide analysis.

For especially difficult matrices, QuEChERS, which stands for quick, easy, cheap, effective, rugged, and safe, has become widely accepted as a sample preparation technique for multiresidue pesticide analysis.[1] Agilent QuEChERS extraction kits provide prepackaged dispersive and extraction products, extraction salts, and ceramic homogenizers in easy-to-use kits. The kits are ready-made for various methods, including methods of the Association of Official Agricultural Chemists (AOAC)[2] and European Standard (EN).[3]

QuEChERS extracts of food commodities can be analyzed by either GC or high performance liquid chromatography (HPLC) combined with a mass spectrometer (MS) or tandem mass spectrometers (MS/MS).[4,5] Depending on the extent of sample cleanup and the food commodity being analyzed, QuEChERS extracts can cause contamination of the instrument, resulting in poor data quality.[6] This contamination can exhibit as loss of sensitivity, retention time shifting, poor peak shape, and more. Regular maintenance of the instrument, including GC inlet maintenance, GC column trimming, and ion source cleaning, is required to ensure the robustness and accuracy of the method results.

Backflushing is one of the key practices in which GC/MS/MS analyses of complex matrices can be improved.[7] Backflushing refers to the reversing of flows in the capillary column so that unwanted matrix components are flushed out of the GC split vent instead of proceeding to the detector. Backflushing can provide improved method robustness and help minimize the required maintenance of the mass spectrometer.

Agilent has introduced the new HES 2.0 ion source as part of the 7010D triple quadrupole mass spectrometer (TQ), and it is also available as an upgrade to 7010A/B/C GC/TQ instruments. The HES 2.0 ion source provides improved system robustness, allowing the analysis of hundreds of injections of pesticides in food matrices with only GC maintenance and ion source cleaning necessary. The HES 2.0 delivers the same unparalleled analytical sensitivity for ultratrace-level analysis as the original HES.

Multiresidue pesticide analysis in spinach extract was carried out using a 7010B GC/TQ upgraded with the HES 2.0. Matrix-matched standards were used to analyze and quantify over 400 injections of baby spinach extract spiked with 50 ppb of multiresidue standards, demonstrating both method and instrument robustness. The multimode inlet (MMI) and backflushing between two 15 m columns allowed for minimal downtime for GC inlet maintenance. Sensitivity and quantitative accuracy were maintained without any maintenance performed on the mass spectrometer.

Experimental

GC/TQ analysis

An 8890 GC with a 7010B TQ system upgraded with the HES 2.0 was used for analysis. The instrument and method were configured as outlined in a previous application note[7], as shown in Figure 1.

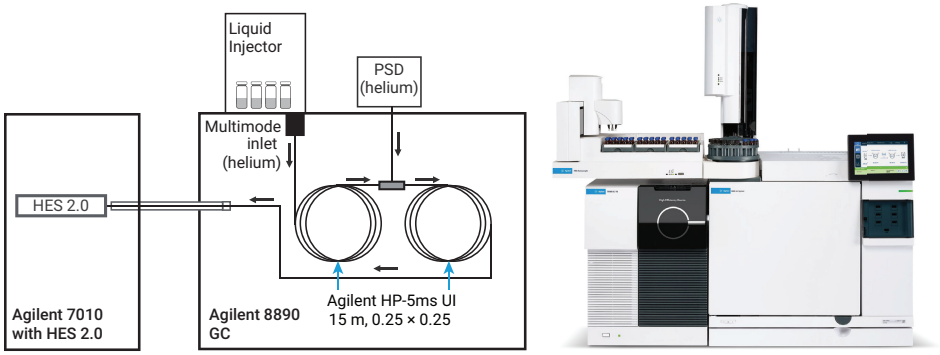

Figure 1. The Agilent 8890/7010B GC/TQ system upgraded with the HES 2.0 and system configuration.

The GC was equipped with an Agilent 7650A automatic liquid sampler (ALS) and 50-position tray. The GC used an MMI to achieve a temperature-programmed splitless injection. Mid-column backflush was carried out using an Agilent Purged Ultimate Union (PUU) installed between two identical 15 m columns; the 8890 GC pneumatic switching device (PSD) module allowed for fewer occurrences of regular maintenance. The method parameters are listed in Table 1.

Table 1. Agilent 8890 GC and Agilent 7010B upgraded with the HES 2.0 ion source conditions for pesticide analysis.

GC	
Instrument	Agilent 8890 with Fast Oven, Auto Injector and Tray
Inlet	Multimode Inlet (MMI)
Mode	Splitless
Purge Flow to Split Vent	15 mL/min at 0.75 min
Septum Purge Flow	3 mL/min
Septum Purge Flow Mode	Switched
Injection Volume	1.0 µL
Injection Type	Standard
L1 Air Gap	0.1 µL
Gas Saver	Off
Inlet Temperature	60 °C for 0.1 min, then to 280 °C at 600 °C/min
Postrun Inlet Temperature	310 °C
Postrun Total Flow	25 mL/min
Carrier Gas	Helium
Inlet Liner	Agilent Ultra Inert 2 mm dimpled liner (p/n 5190-2297)
Oven	
Initial Oven Temperature	60 °C
Initial Oven Hold	1 min
Ramp Rate 1	40 °C/min
Final Temperature 1	170 °C
Final Hold 1	0 min
Ramp Rate 2	10 °C/min
Final Temperature 2	310 °C
Final Hold 2	3 min
Total Run Time	20.75 min
Postrun Time (Backflushing)	1.5 min
Equilibration Time	3 min

Column 1	
Type	Agilent HP-5ms UI (p/n 19091S-431UI)
Length	15 m
Diameter	0.25 mm
Film Thickness	0.25 µm
Control Mode	Constant flow
Flow	1.00 mL/min
Inlet Connection	Multimode inlet (MMI)
Outlet Connection	PSD (PUU)
PSD Purge Flow	5 mL/min
Postrun Flow (Backflushing)	–7.873 mL/min
Column 2	
Type	Agilent HP-5ms UI (p/n 19091S-431UI)
Length	15 m
Diameter	0.25 mm
Film Thickness	0.25 µm
Control Mode	Constant flow
Flow	1.200 mL/min
Inlet Connection	PSD (PUU)
Outlet Connection	MSD
Postrun Flow (Backflushing)	8.202 mL/min
MSD	
Model	Agilent 7010B
Source	Agilent HES 2.0
Vacuum Pump	Performance turbo
Tune File	Atunes.eihs.tune.xml
Solvent Delay	3 min
Quad Temp (MS1 and MS2)	150 °C
Source Temperature	280 °C
Mode	dMRM or Scan
He Quench Gas	4 mL/min
N_2 Collision Gas	1.5 mL/min
MRM Statistics	
Total MRMs (dMRM mode)	552
Minimum Dwell Time (ms)	2.63
Minimum Cycle Time (ms)	82.42
Maximum Concurrent MRMs	48
EM Voltage Gain Mode	10

The Agilent Pesticide and Environmental Pollutant (P&EP) database (P&EP 4, part number G9250AA) was used to easily and rapidly create the dynamic multiple reaction monitoring (dMRM) method. This method, which enabled the analysis of 190 pesticides with a total of 552 MRMs, resulted in a maximum of 48 concurrent MRMs, as shown in Figure 2.

QuEChERS sample preparation

The sample preparation procedure is summarized in Figure 3. A bag of frozen organic baby spinach was homogenized using a spice grinder. Then, eight replicates were prepared. For each replicate, 15 g of the homogenized spinach were weighed into a 50 mL test tube. Then, two of the replicates were designated as samples, while the remaining six were designated for pooled matrix-matched standards. Fifteen microliters of the internal standard mixture (part number 5190-0502) diluted to 50 ng/µL was added to the two spinach samples. To all eight replicates, 15 mL of 1% acetic acid in acetonitrile was added and the mixture was vortexed until well mixed. To each

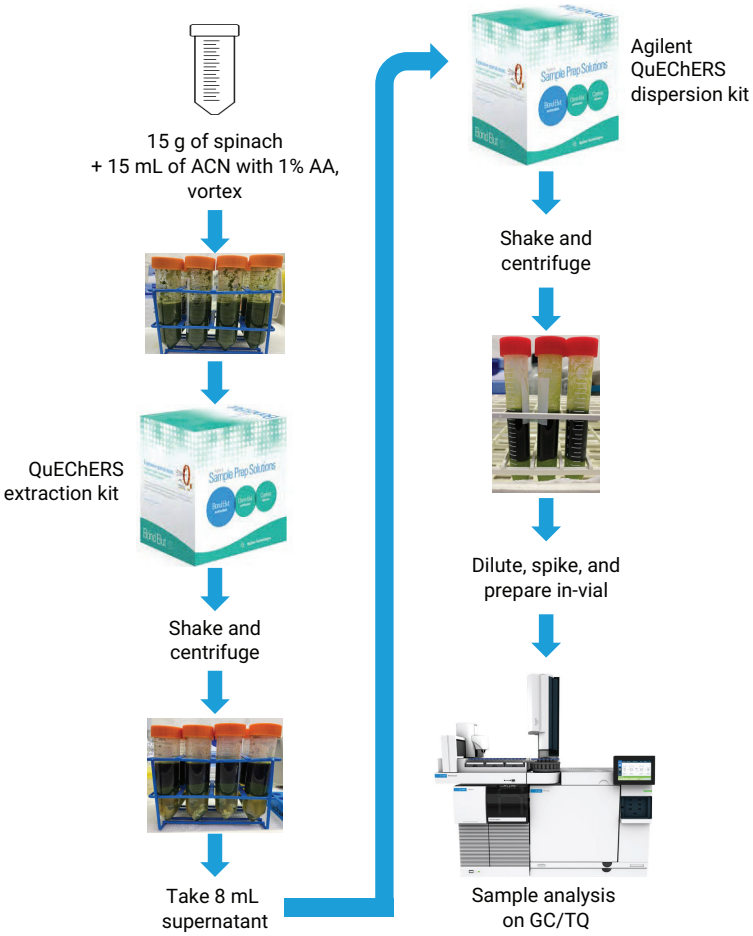

Figure 3. Sample preparation workflow.

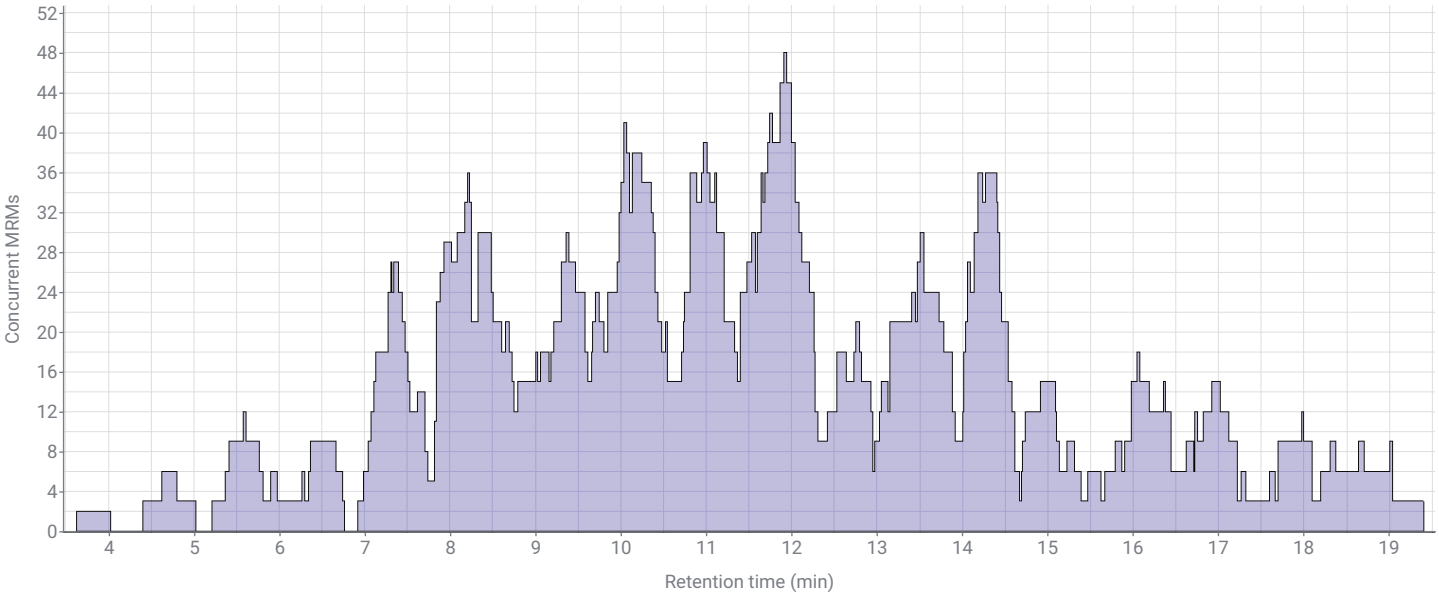

Figure 2. Concurrent MRMs versus retention time.

50 mL tube, the salt packet and two homogenizers from the QuEChERS kit (part number 5982-5755) were added for extraction. These were shaken for 1 minute, then centrifuged at 4,000 rpm with a maximum radius of 17.4 cm for 5 minutes. For the samples and the pooled standards, 8 mL of the supernatant was transferred to a tube with salt for dispersion using the QuEChERS kit (part number 5982-5058). These were shaken for 30 seconds then centrifuged as before for 5 minutes. The supernatant for each of the samples was removed and placed in an amber glass vial. For the pooled standards, the supernatant of all replicates was removed and mixed in a large amber jar for standard preparation.

Standard preparation

The multiresidue matrix-matched standards were created from the FDA analytical reference standards kit (part number PSM-101). Mixes A, B, C, D, E, L, M, N, O, and P from the PSM-101 pesticides mix were combined to create a 10 ppm stock standard of 190 pesticide residues. The stock solution was then diluted in acetonitrile down to the following nominal concentrations: 1,000, 100, 10, and 1 ppb. In a GC vial, the standards were combined with the spinach extract. The internal standard mixture of parathion-d_{10} and alpha-BHC-d_6, and acetonitrile were combined until the nominal concentration of the internal standard was 50 ppb and the nominal concentrations of the matrix-matched standards in a total volume of 1,500 µL were as follows: 0.1, 0.5, 1, 5, 10, 50, 100, 253.3, 500, and 1,000 ppb. Extra vials of the 50-ppb matrix-matched standard were made for quantification to test robustness. The samples were diluted by a factor of three in the vials to match the matrix concentration in the standards. This 3x dilution factor was determined by analyzing the matrix alone in full scan mode as described in a previous application note[7] to ensure that the instrument was not overloaded or saturated by the matrix. Vials with 250 µL glass inserts were used with 100 µL of each standard or sample in-vial for GC analysis.

Sequence

Each sequence included 102 injections of spinach extract, either as a sample or matrix-matched standard. Additional injections of blank acetonitrile were used to evaluate system cleanliness by ensuring no analyte carryover or additional background contamination. A representative MRM chromatogram of the 50-ppb matrix-matched standards in spinach extract is shown in Figure 4A with a zoomed in portion shown in Figure 4B. The sequence included:

- Matrix-matched calibration curve (10 pts)
- Two spinach samples
- Matrix-matched calibration curve (10 pts)
- Matrix-matched standards (50 ppb) × 60 times
- Matrix-matched calibration curve (10 pts) × 2 times, each standard in duplicate

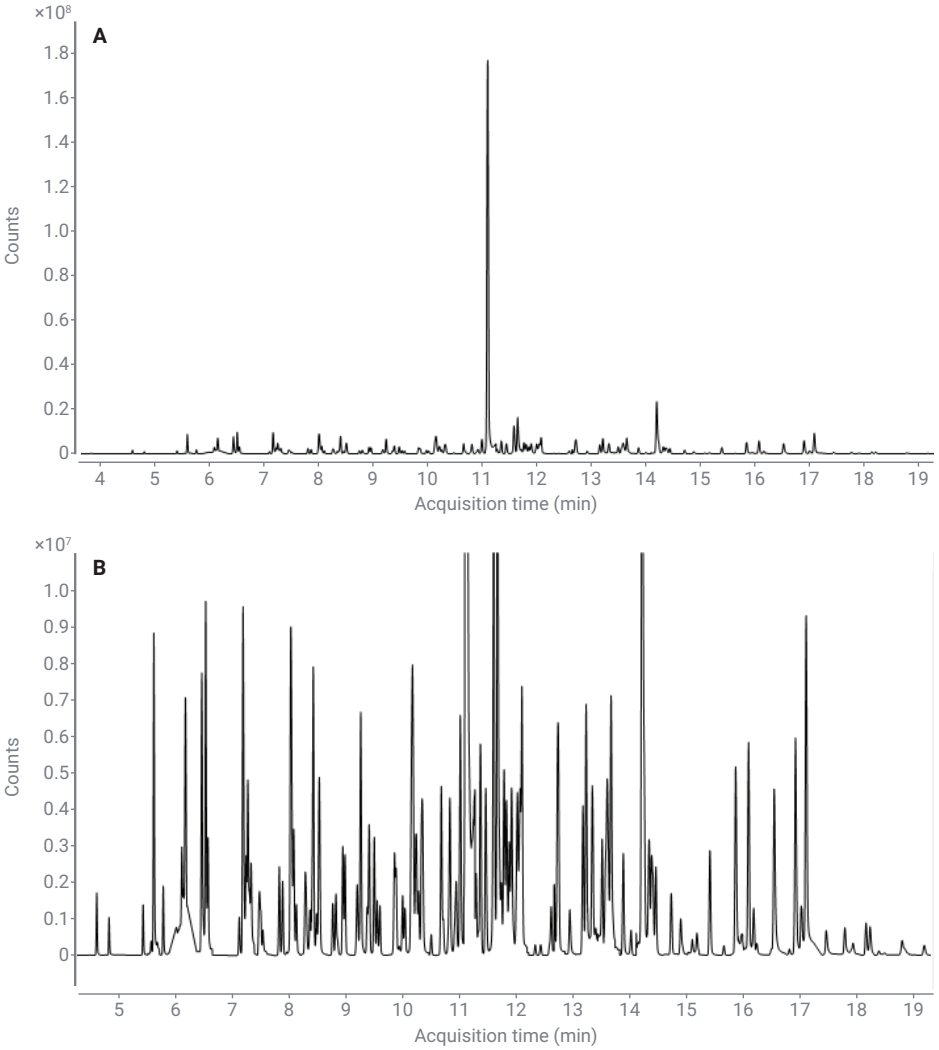

Figure 4. A representative chromatogram (A) and a zoomed in portion of the chromatogram (B).

To achieve more than 400 injections of the 50 ppb matrix-matched standard, seven sequences were run for a total of 819 injections, of which 714 were spinach matrix injections. After each sequence, the GC inlet liner and septum were changed, and the 5 µL syringe was changed as necessary. Also, the GC vials were refreshed with samples and standards that had been stored in the freezer. No additional instrument maintenance was performed.

Results and discussion

Of the 190 pesticide residues analyzed by GC/TQ, 114 were selected for further study based on their analytical response and performance. These 114 residues were chosen because their calibration curves were either linear or quadratic throughout the seven sequences. These curves did not require extensive analyst intervention, such as manual integration, and had calibration curves that encompassed the 50 ppb point so that the 50 ppb matrix-matched standards for robustness could be calculated as samples. Any points on the calibration curve that had signal-to-noise (S/N) < 3, were interfered with by contaminants, or had accuracy greater than or equal to ± 25% were excluded (≥ ± 25%). A table summarizing these residues can be found at the end of the application note in Table 2.

Many of the pesticide residues could accurately be quantified down to 0.5 or 0.1 ppb while maintaining S/N > 10 and quantification accuracy of < 25%. For example, Figures 5A, 5B, and 5C show the 0.1 ppb peak, corresponding qualifiers, and calibration curve of DCPA, respectively. The data are defined well by a quadratic calibration curve over the calibration range 0.1 to 1,000 ppb through four orders of magnitude. DCPA has many MRLs as defined by the US FDA down to 50 ppb in various fruits and vegetables.

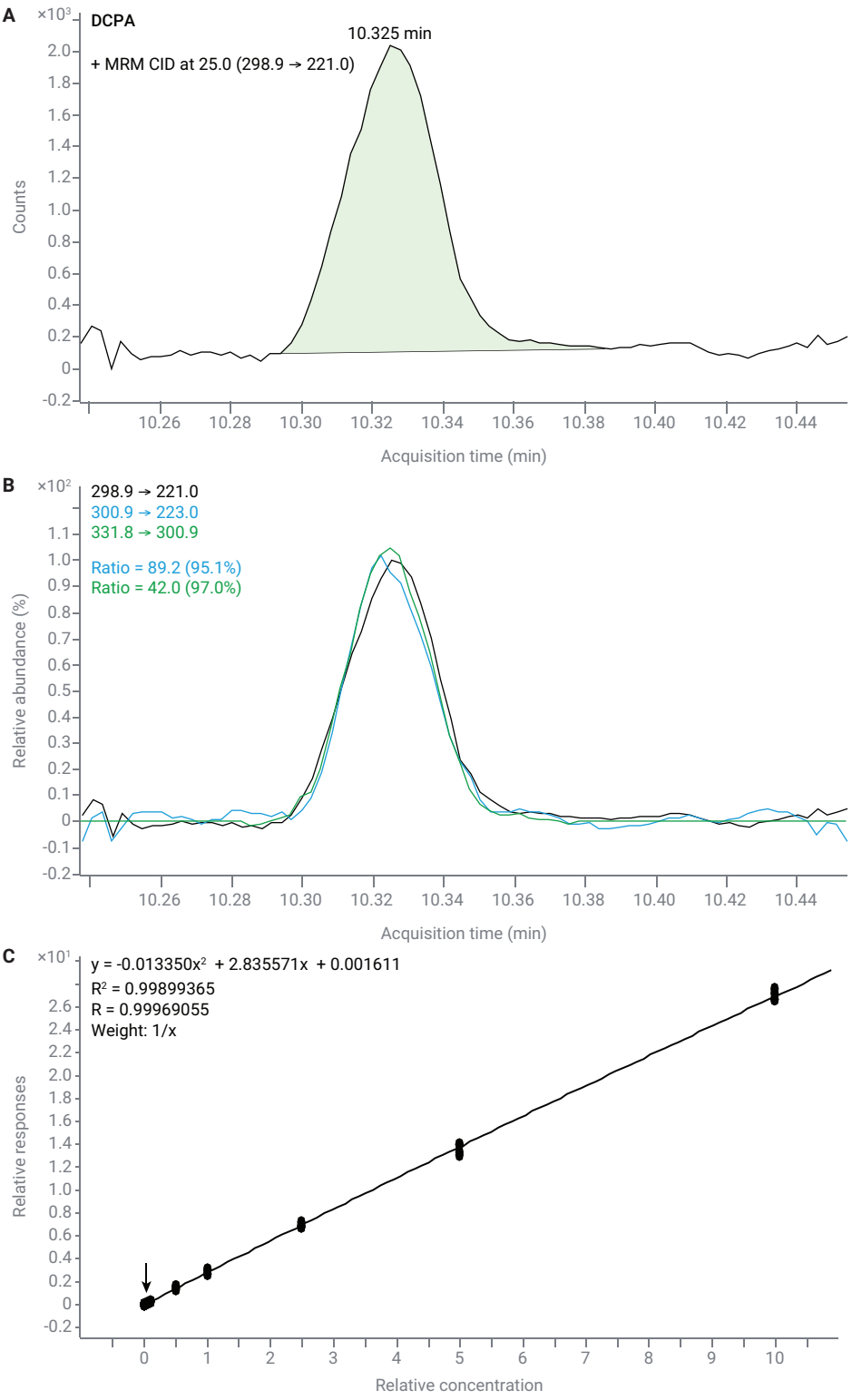

Figure 5. Integrated peak (A) and qualifiers (B) of the 0.1 ppb peak of DCPA as well as the full calibration curve (C).

Figures 6A and 6B show the chlorpyrifos 0.5 ppb peak and qualifiers (respectively), while Figure 6C shows the corresponding calibration curve. The curve is linear through 3.5 orders of magnitude and chlorpyrifos has MRLs in various food commodities, the lowest of which is 0.01 ppm in food items such as egg, fig, grape, and apple.

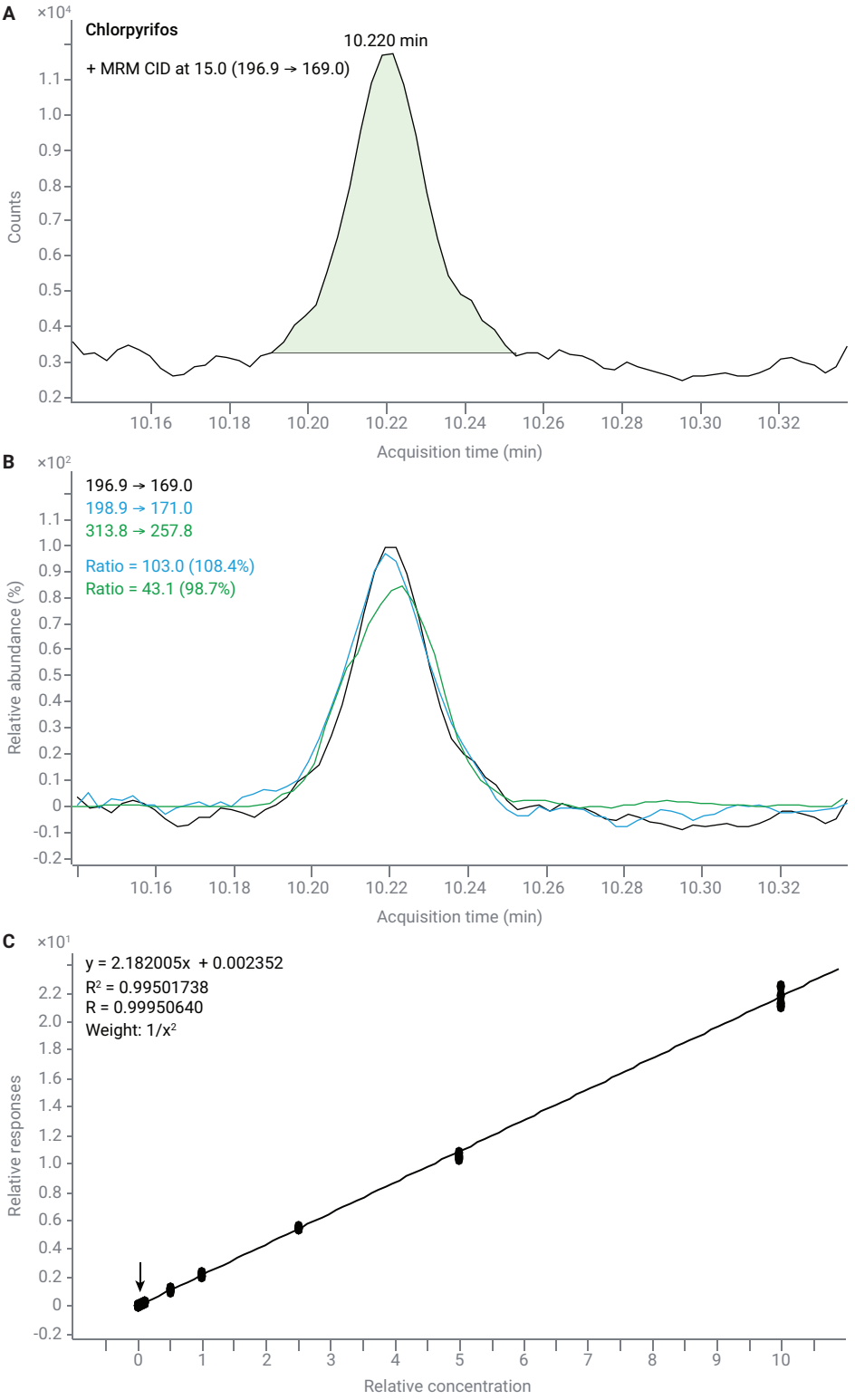

Figure 6. Integrated peak (A), qualifiers (B) of the 0.5 ppb peak of chlorpyrifos as well as the full calibration curve (C).

In terms of spinach MRLs, Figures 7, 8, and 9 provide three examples. Figure 7 shows the results for bifenthrin, which is quadratic through 3.5 orders of magnitude down to 0.5 ppb and has an MRL of 0.2 ppm in spinach. Diazinon, linear through 3.5 orders of magnitude down to 0.5 ppb, is shown in Figure 8, with an MRL in spinach of 0.7 ppm.

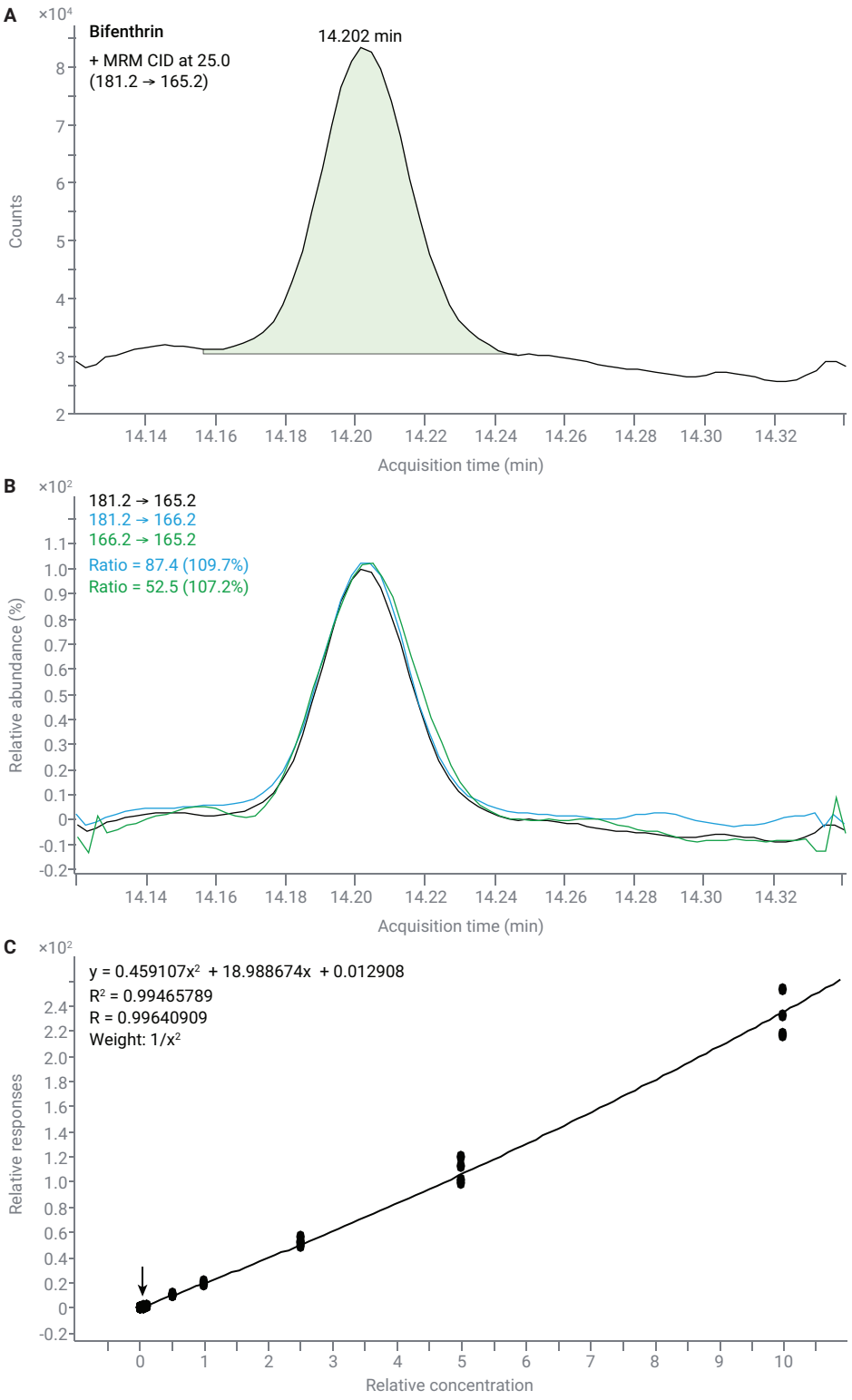

Figure 7. Integrated peak (A), qualifiers (B) of the 0.5 ppb peak of bifenthrin as well as the full calibration curve (C).

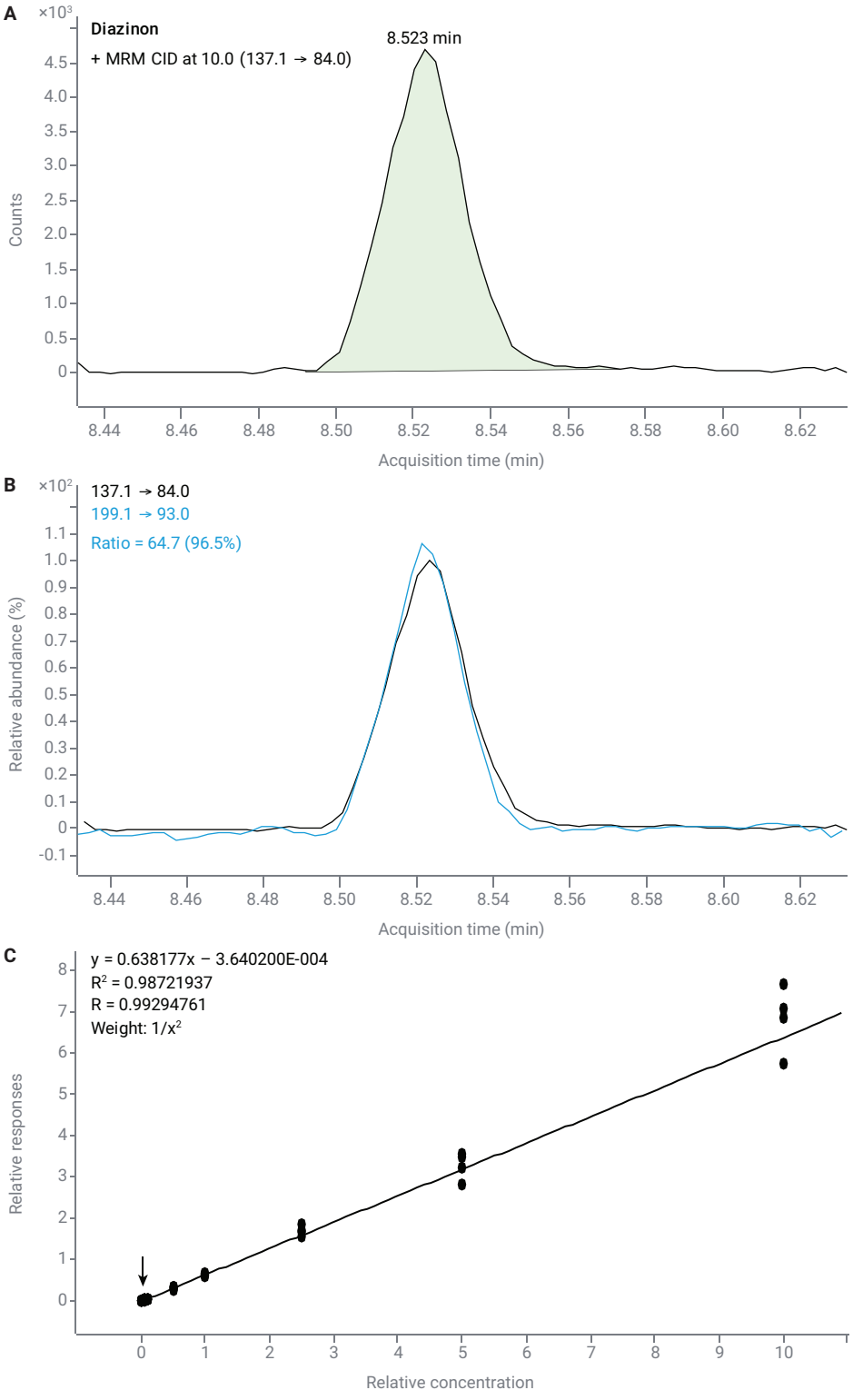

Figure 8. Integrated peak (A), qualifiers (B) of the 0.5 ppb peak for diazinon as well as the full calibration curve (C).

Lastly, boscalid is shown in Figure 9 with an MRL of 1 ppb; it is easily defined by a quadratic curve with four orders of magnitude down to 0.1 ppb. In the spinach sample, all three residues fell below the limit of quantification (LOQ) of the analytical method, and below their respective spinach MRLs. In the spinach samples, both boscalid and bifenthrin fell below the limit of detection (LOD) as no peak was detected for either residue. However, a small amount of diazinon was detected that was not present in the solvent blank. However, the area of the peak fell well below the LOQ and had an S/N very close to 3, and therefore approached or fell below the LOD.

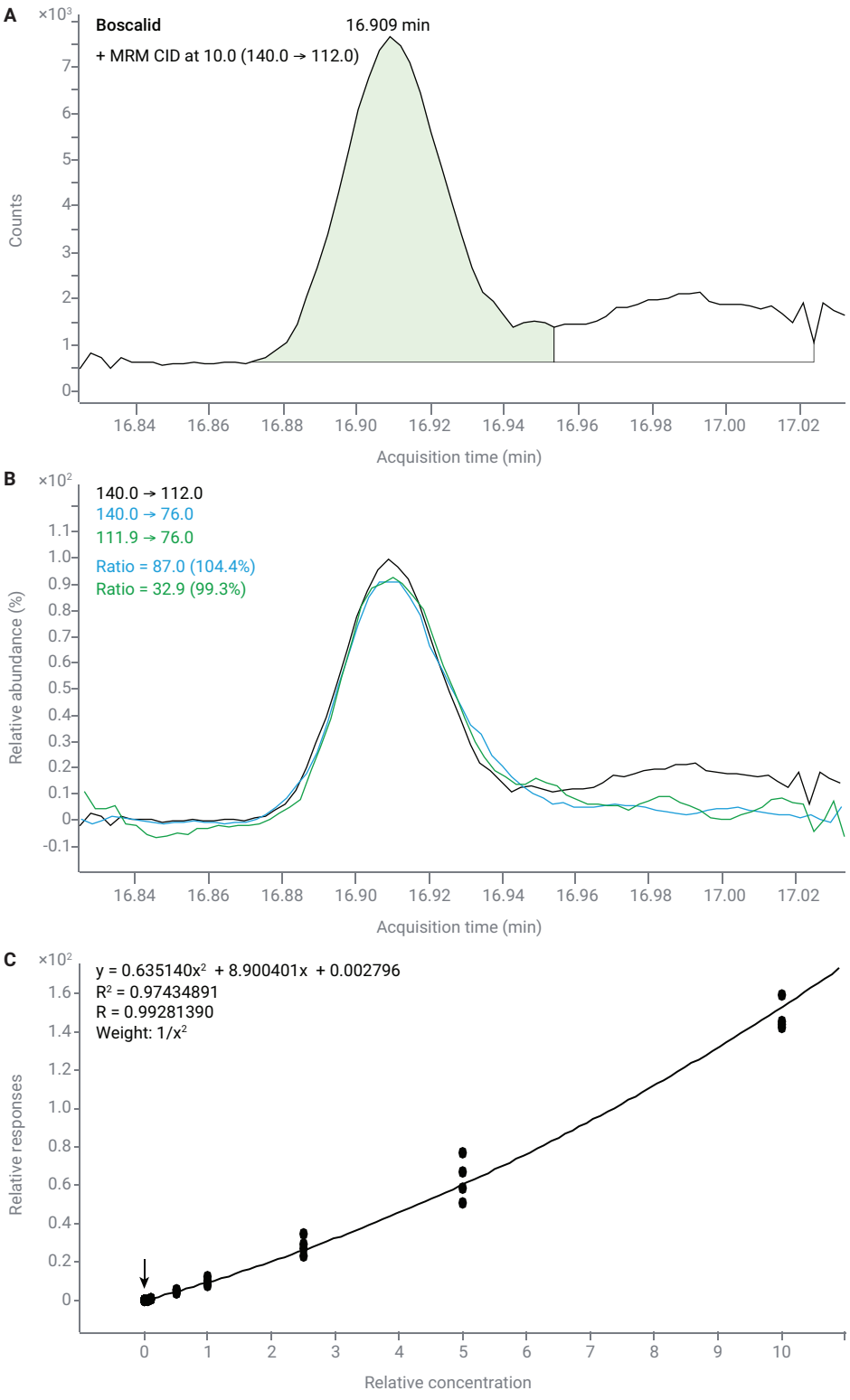

Figure 9. Integrated peak (A), qualifiers(B) of 0.1 ppb peak of boscalid as well as the full calibration curve (C).

The innovative HES 2.0 source enabled enhanced response stability for the analyzed pesticides over 400 replicate injections of the matrix-matched calibration standard at 50 ppb analyzed within a sequence with over 800 total injections. Figure 10 shows the 400 replicates of the 50 ppb matrix-matched standard, calculated as samples from the corresponding calibration curves across six sequences. The X-axis on the top corresponds to the number of injections of just the 50 ppb matrix-matched standard for robustness. The lower X-axis corresponds to the total injection number of spinach QuEChERS matrix, and therefore excludes blank injections. The concentration in ppb versus injection number plot shows how robust and accurate the analysis was. The results showed all but one point falling within ± 20% error, the usual % error given by GC/TQ data, and most points well within ± 10% of the actual. The %RSD for 400 replicates of all 114 residues are summarized in Table 2, with nearly 80% of the 114 residues having %RSD < 10%, and only five having %RSD above 20%. The robustness of the analysis and the instrument is clearly shown, with only inlet maintenance required between sequences and no further maintenance of the instrument needed.

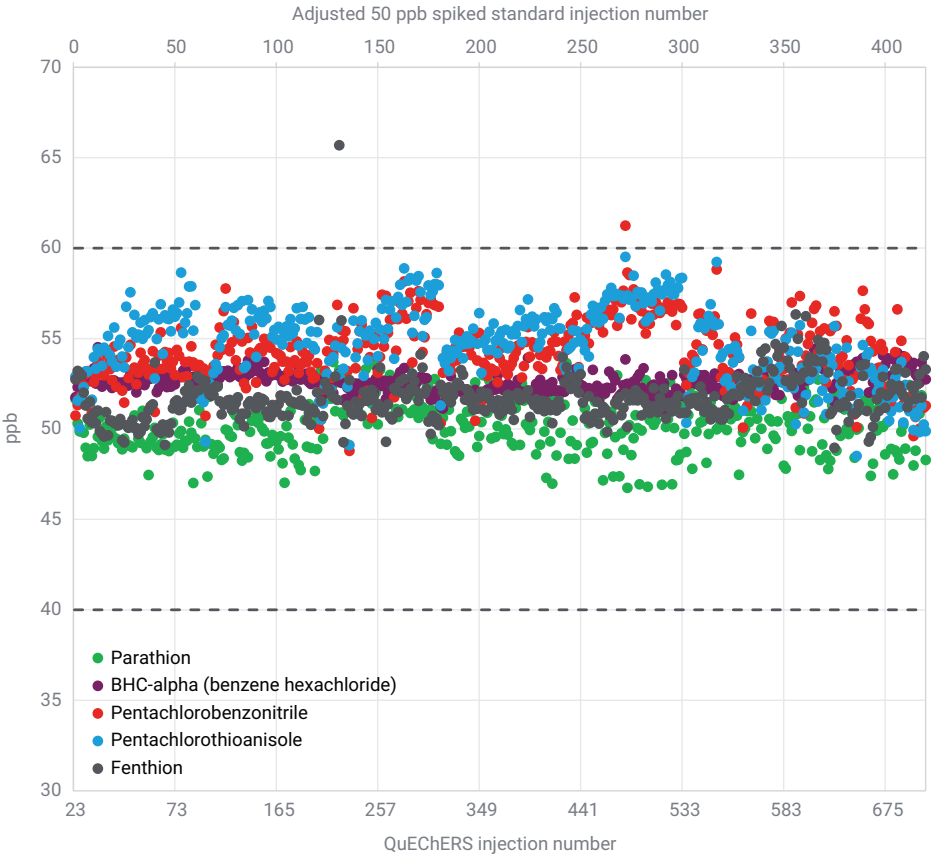

Figure 10. Calculated concentration of pesticides in 50 ppb matrix-matched standard over the course of 714 injections of spinach QuEChERS extract.

Table 2. Summary of 114 residues, including calibration range, calibration curve fit, and %RSD over the 400 injections of 50 ppb matrix-matched standard injections for robustness. Those results with %RSD > 20% are highlighted in red.

Name	Retention Time (minutes)	Min Cal (ppb)	Max Cal (ppb)	Curve Fit	Curve Weight	%RSD
Ethiolate	4.609	0.5	1,000	Linear	$1/x^2$	7.6
Dichlorvos	4.826	0.5	1,000	Quadratic	1/x	10.4
Nicotine	5.424	0.5	1,000	Linear	$1/x^2$	11.9
Biphenyl	5.615	1	1,000	Linear	$1/x^2$	6.2
2-Phenylphenol	6.457	5	1,000	Quadratic	1/x	7.5
Pentachlorobenzene	6.566	0.1	1,000	Linear	$1/x^2$	4.8
Tecnazene	7.118	0.1	1,000	Linear	$1/x^2$	3.9
Diphenylamine	7.186	0.1	1,000	Quadratic	$1/x^2$	6.5
Ethoprophos	7.238	5	1,000	Quadratic	1/x	12.3
2,3,5,6-Tetrachloroaniline	7.297	0.5	1,000	Linear	$1/x^2$	4.9
Chlorpropham	7.325	0.5	1,000	Quadratic	1/x	7.5
Trifluralin	7.462	1	1,000	Quadratic	$1/x^2$	6.6
Benfluralin	7.496	0.1	1,000	Quadratic	$1/x^2$	7.2
BHC-alpha (Benzene Hexachloride)	7.881	1	1,000	Linear	$1/x^2$	1.0
2,6-Diisopropylnaphthalene	8.02	5	1,000	Quadratic	1/x	5.5
Hexachlorobenzene	8.024	0.5	1,000	Quadratic	1/x	4.5
Ethoxyquin	8.034	0.1	1,000	Quadratic	$1/x^2$	25.3
Dichloran	8.04	1	250	Linear	$1/x^2$	7.7
Simazine	8.043	5	1,000	Linear	$1/x^2$	7.0
Pentachloroanisole	8.073	0.1	1,000	Linear	$1/x^2$	3.3
Atrazine	8.124	1	1,000	Linear	$1/x^2$	5.5
Beta-BHC	8.278	1	1,000	Linear	$1/x^2$	4.9
Terbuthylazine	8.363	1	1,000	Linear	$1/x^2$	14.6
BHC-gamma (Lindane, Gamma HCH)	8.398	1	1,000	Linear	$1/x^2$	4.5
Pentachloronitrobenzene	8.478	0.5	1,000	Quadratic	$1/x^2$	33.7
Pentachlorobenzonitrile	8.515	0.1	1,000	Quadratic	$1/x^2$	3.1
Diazinon	8.526	0.5	1,000	Linear	$1/x^2$	5.8
Pyrimethanil	8.53	0.1	1,000	Linear	$1/x^2$	5.0
BHC-delta	8.763	1	1,000	Quadratic	1/x	11.4
Triallate	8.817	1	1,000	Quadratic	$1/x^2$	4.4
Iprobenfos	8.942	0.5	1,000	Quadratic	$1/x^2$	17.3
Pirimicarb	8.976	1	1,000	Linear	$1/x^2$	8.9
Pentachloroaniline	9.178	0.1	1,000	Linear	$1/x^2$	4.6
Propanil	9.193	0.5	1,000	Quadratic	$1/x^2$	11.4
Metribuzin	9.256	0.5	1,000	Quadratic	$1/x^2$	5.8
Dimethachlor	9.255	0.5	1,000	Linear	$1/x^2$	6.0
Vinclozolin	9.372	0.1	1,000	Linear	$1/x^2$	9.5
Chlorpyrifos-methyl	9.404	0.1	1,000	Quadratic	$1/x^2$	8.4
Parathion-methyl	9.403	5	1,000	Quadratic	1/x	9.3
Ametryn	9.495	0.5	1,000	Quadratic	$1/x^2$	6.5
Tolclofos-methyl	9.496	5	1,000	Linear	$1/x^2$	5.8
Prometryn	9.541	0.1	1,000	Quadratic	$1/x^2$	6.9
Pirimiphos-methyl	9.85	0.5	1,000	Quadratic	$1/x^2$	8.9
Fenitrothion	9.855	0.1	1,000	Quadratic	$1/x^2$	10.2

Name	Retention Time (minutes)	Min Cal (ppb)	Max Cal (ppb)	Curve Fit	Curve Weight	%RSD
Ethofumesate	9.877	0.5	1,000	Quadratic	$1/x^2$	6.5
Malathion	9.995	10	1,000	Quadratic	1/x	20.0
Pentachlorothioanisole	10.032	0.1	1,000	Linear	$1/x^2$	3.5
Metolachlor	10.166	5	1,000	Linear	$1/x^2$	6.2
Fenthion	10.187	0.5	1,000	Linear	$1/x^2$	2.4
Chlorpyrifos	10.224	0.5	1,000	Linear	$1/x^2$	4.5
Parathion	10.242	1	1,000	Linear	$1/x^2$	2.7
Triadimefon	10.275	0.5	1,000	Quadratic	1/x	11.6
Tetraconazole	10.319	0.5	1,000	Quadratic	$1/x^2$	7.5
DCPA (Dacthal, Chlorthal-dimethyl)	10.328	0.1	1,000	Quadratic	1/x	7.3
Isocarbophos	10.346	0.1	1,000	Linear	$1/x^2$	4.7
Butralin	10.496	0.5	1,000	Linear	$1/x^2$	5.7
Cyprodinil	10.672	0.5	1,000	Linear	$1/x^2$	7.8
MGK-264	10.709	0.1	1,000	Linear	$1/x^2$	7.3
Pendimethalin	10.797	0.5	1,000	Linear	$1/x^2$	4.6
Penconazole	10.826	0.5	1,000	Quadratic	$1/x^2$	9.8
Heptachlor Exo-epoxide	10.904	10	1,000	Quadratic	1/x	14.1
Fipronil	10.915	0.5	1,000	Quadratic	1/x	10.7
Triadimenol	11.008	0.5	1,000	Quadratic	$1/x^2$	7.1
Quinalphos	11.01	1	1,000	Linear	$1/x^2$	3.3
Chlordane-*trans*	11.326	100	1,000	Quadratic	1/x	13.6
DDE-o,p'	11.363	0.1	500	Quadratic	$1/x^2$	11.8
Mepanipyrim	11.458	0.5	1,000	Linear	$1/x^2$	7.1
Flutriafol	11.596	0.5	1,000	Quadratic	1/x	6.6
Flutolanil	11.664	0.5	1,000	Quadratic	$1/x^2$	4.6
Napropamide	11.697	0.5	1,000	Linear	$1/x^2$	8.5
Hexaconazole	11.73	0.5	1,000	Linear	$1/x^2$	8.6
Isoprothiolane	11.776	0.1	1,000	Linear	$1/x^2$	4.8
Prothiofos	11.782	0.5	1,000	Linear	$1/x^2$	5.2
Fludioxonil	11.819	0.5	1,000	Quadratic	$1/x^2$	6.9
DEF	11.871	0.5	1,000	Linear	$1/x^2$	7.4
DDE-p,p'	11.91	0.1	500	Quadratic	$1/x^2$	9.8
Oxyfluorfen	11.988	0.5	1,000	Linear	$1/x^2$	5.2
Myclobutanil	12.013	0.5	1,000	Quadratic	$1/x^2$	8.2
Buprofezin	12.066	1	1,000	Quadratic	$1/x^2$	6.2
Bupirimate	12.089	0.5	1,000	Linear	$1/x^2$	6.7
Kresoxim-methyl	12.093	0.5	1,000	Quadratic	1/x	5.9
Chlorfenapyr	12.326	0.5	1,000	Quadratic	$1/x^2$	4.8
Endrin	12.425	5	1,000	Quadratic	1/x	10.3
Ethion	12.718	5	1,000	Linear	$1/x^2$	7.5
Benalaxyl	13.167	0.5	1,000	Quadratic	$1/x^2$	7.7
Trifloxystrobin	13.223	0.5	1,000	Linear	$1/x^2$	5.3
Quinoxyfen	13.222	0.1	1,000	Linear	$1/x^2$	7.7
Endosulfan Sulfate	13.328	1	1,000	Quadratic	$1/x^2$	13.2
Tebuconazole	13.565	0.5	1,000	Quadratic	$1/x^2$	6.6
Nuarimol	13.595	0.5	1,000	Quadratic	$1/x^2$	6.4
Triphenyl Phosphate	13.659	0.5	1,000	Quadratic	$1/x^2$	6.1

Name	Retention Time (minutes)	Min Cal (ppb)	Max Cal (ppb)	Curve Fit	Curve Weight	%RSD
Piperonyl butoxide	13.662	0.5	1,000	Quadratic	$1/x^2$	5.9
Epoxiconazole	13.876	0.5	1,000	Quadratic	$1/x^2$	8.5
Spiromesifen	14.014	1	1,000	Quadratic	1/x	15.5
Tetramethrin I	14.207	0.5	1,000	Quadratic	1/x	7.4
Bifenthrin	14.179	0.5	1,000	Quadratic	$1/x^2$	4.4
EPN	14.226	10	1,000	Quadratic	$1/x^2$	5.8
Bromopropylate	14.221	0.5	1,000	Quadratic	$1/x^2$	5.2
Etoxazole	14.375	0.5	1,000	Quadratic	$1/x^2$	6.3
Tebufenpyrad	14.398	0.5	1,000	Quadratic	$1/x^2$	6.9
Fenamidone	14.449	0.5	1,000	Quadratic	$1/x^2$	7.4
Tetradifon	14.72	5	1,000	Quadratic	$1/x^2$	9.2
Metrafenone	15.648	0.5	1,000	Quadratic	$1/x^2$	6.4
Bitertanol I	15.857	0.5	1,000	Quadratic	$1/x^2$	9.7
Spirodiclofen	15.976	5	1,000	Quadratic	$1/x^2$	49.3
Pyridaben	16.081	0.5	1,000	Quadratic	$1/x^2$	5.8
Cyfluthrin I	16.484	5	1,000	Quadratic	$1/x^2$	22.2
Fenbuconazole	16.535	5	1,000	Quadratic	$1/x^2$	9.5
Cypermethrin I	16.8	5	1,000	Quadratic	1/x	23.8
Boscalid	16.909	0.1	1,000	Quadratic	$1/x^2$	8.4
Ethofenprox	17.096	0.5	1,000	Quadratic	$1/x^2$	5.9
Difenoconazole I	18.148	1	1,000	Quadratic	1/x	12.4
Azoxystrobin	18.787	10	1,000	Quadratic	$1/x^2$	11.2
Dimethomorph I	19.175	5	1,000	Quadratic	1/x	15.3

Conclusion

The analytical performance of the Agilent 7010 Series triple quadrupole mass spectrometer (GC/TQ) upgraded with the HES 2.0 electron ionization (EI) source was demonstrated for multiresidue pesticide analysis. The system demonstrates analytical sensitivity as the same or better than the original HES as well as excellent accuracy and robustness. The 7010 GC/TQ with HES 2.0, coupled with an Agilent 8890 GC with an MMI inlet and 15 m × 15 m mid-column backflush configuration minimizes instrument downtime by allowing for inlet maintenance without requiring the cooling of the heated zones. With no impact to the analytical method or degradation of the instrument performance, this application demonstrates the ability of the instrument to provide robust and reliable analytical results, including for challenging matrices.

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DE34182072

Printed in the USA, May 3, 2024
5994-7385EN

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Brewing Excellence: Quantitating Over 200 Pesticides in Black Tea with Steady Performance and Maximized Uptime by GC/MS/MS

Authors

Anastasia A. Andrianova and
Limian Zhao
Agilent Technologies, Inc.

Abstract

This application note presents results for the sensitive and robust quantitation of 246 pesticides in black tea extract with the Agilent 7010D Triple Quadrupole Mass Spectrometer (GC/TQ) featuring a second-generation High Efficiency Source 2.0 (HES 2.0), that addresses the challenges posed by residual pesticide analysis in complex matrices. By optimizing sample preparation and using state-of-the-art GC/MS hardware, including ion source technology and midcolumn backflushing, excellent calibration performance and sensitivity at low-ppb levels were achieved. The method demonstrated exceptional ruggedness and robustness over 800 consecutive injections of a black tea extract spiked with pesticides at 2 ppb, with high precision and low RSDs, ensuring prolonged instrument uptime and maximum throughput. The demonstrated limits of quantitation (LOQs) were as low as 0.01 ppb for over a third of evaluated compounds, and the calibration range spanned up to five orders of magnitude while meeting SANTE 11312/2021 guidelines.

This application note highlights intelligent GC/TQ features, such as early maintenance feedback and an instrument health status dashboard, maintaining confidence in the results for a high-throughput analysis. The updated data acquisition platform provides enhanced user experience, including a new implementation of the retention time locking functionality.

Introduction

Tea is among the most common nonalcoholic beverages consumed worldwide. Like many foods, tea cultivation relies heavily on pesticide application to combat pests, leading to concerns over pesticide residues intensifying.[1]

Assessing pesticide levels in tea is essential for evaluating safety, and is required by many regulatory bodies, including the European Commission and the US Environmental Protection Agency.[2,3] A complete workflow for pesticide testing in tea includes sample extraction via QuEChERS, followed by extract cleanup, and subsequent testing with liquid and gas chromatography coupled with triple quadrupole mass spectrometry (LC/TQ and GC/TQ).[4] Workflow performance should enable sufficient method sensitivity, calibration range, pesticide recovery from the extraction, and precision. Sensitivity requirements are set based on the maximum residue levels (MRLs), which are the highest levels of pesticide residue that are legally allowed in or on food or feed when pesticides are applied correctly. The ability to calibrate over a wide dynamic range allows the varying MRLs for individual compounds monitored in the commodity, which can vary from 10 ppb to 100 ppm. When a specific pesticide lacks an established MRL, a default limit of 10 ppb is commonly applied. Efficiency of extraction and cleanup are characterized in terms of recovery of matrix spikes, and precision is expressed in terms of relative standard deviation (RSD) of repeat analyses.

This application note presents a complete GC/TQ workflow solution for the accurate and reliable analysis of 246 volatile and semivolatile pesticides in black tea. Excellent analytical performance of the workflow was achieved through a combination of cutting-edge technology and optimized methodology that included:

- Sample preparation using QuEChERS extraction, followed by EMR mixed-mode pass-through cleanup using Agilent Captiva EMR–GPD cartridges
- Agilent 8890 GC hardware and GC supplies
- Novel electron ionization (EI) source technology with HES 2.0
- Built-in GC/TQ MS intelligence and new software functionality for method setup, maintenance, and system health evaluation

The presented workflow allowed for quantitating 246 pesticide residues in black tea with LOQs as low as 0.01 ppb for 34% of the targets, at or below 0.1 ppb for 74% of compounds, and below 2 ppb for 96%. Matrix-matched calibrations demonstrated excellent accuracy over a wide dynamic range, spanning up to five orders of magnitude over 0.01 to 1,000 ppb in the complex black tea extract. Method ruggedness was demonstrated through maintaining measurement accuracy with good precision (RSDs < 20% for 176 compounds) for black tea extract spiked at 2 ppb sequentially analyzed over 800 runs spanning 17 days of continuous analysis. The new HES 2.0 ion source is equipped with a novel dipolar radiofrequency (RF) lens that redirects the carrier gas ions and, as a result, enables improved system robustness and maximizes uptime while maintaining unparalleled analytical sensitivity.

Experimental

GC/TQ analysis

The 8890 GC and 7010D GC/TQ systems (Figure 1A) were used and configured to achieve the best sensitivity, maintain a wide calibration range, and provide the most rugged method performance. The GC was configured with the Agilent 7693A automatic liquid sampler (ALS) and 150-position tray. The system used a multimode inlet (MMI) operated in temperature-programmed splitless injection mode (also known as cold splitless). The injection parameters were optimized for maximizing sensitivity while limiting carryover. Midcolumn backflush capability was provided by the Agilent Purged Ultimate Union (PUU) installed between two identical 15 m columns, and the Agilent 8890 pneumatic switching device (PSD) module (Figure 1B). The instrument operating parameters are listed in Table 1.

Data were acquired in dynamic MRM (dMRM) mode, which provides the capability for large multi-analyte assays and the accurate quantitation of narrow peaks by an automatically determined most-efficient dwell time distribution. The dMRM capability enabled successful analysis for a large panel of 246 pesticides, with 749 total MRM transitions with up to 64 concurrent MRMs. Furthermore, dMRM allows the analyst to add and remove additional analytes with ease. The acquisition method was retention time locked to match the retention times in the Agilent MassHunter Pesticides and Environmental Pollutants MRM Database 4.0 (P&EP 4.0)[5],

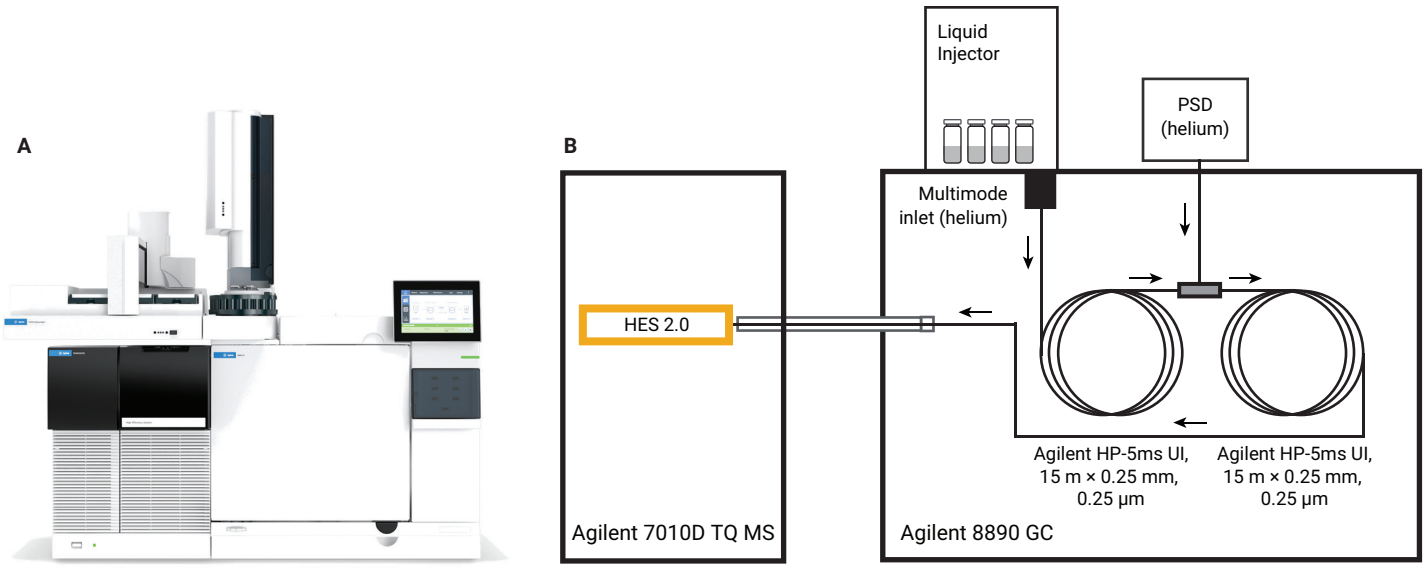


Figure 1. The Agilent 8890 GC system with the Agilent 7010D GC/TQ system (A) and system configuration (B).

which was used to seamlessly create the MS method. The use of P&EP 4.0 increased the ease and speed of setting up a targeted dMRM method. High method selectivity in the presence of coeluting matrix components was achieved by selecting the best MRM transitions from up to nine transitions available for each compound in the P&EP 4.0 database.

Three targets were not available in the P&EP 4.0 database (flonicamid, bioallethrin, and cycloxydim). For these compounds, MRM transitions were developed using Agilent MassHunter Optimizer for GC/TQ, operating in Start from Full Scan mode. The Optimizer is fully integrated into MassHunter Acquisition 13.0 for GC/MS (Figure 2).

The acquisition method was retention time locked to the P&EP database with chlorpyrifos-methyl eluting at 9.143 minutes. The retention time locking functionality, integrated in MassHunter Acquisition 13.0 for GC/MS, has an updated user-friendly and intuitive interface (Figure 3). It allows for semi-automated or manual compound selection, provides a choice to use three or five points for retention time locking calibration, and features both a visual and quantitative assessment of the calibration curve fit, while providing the tools to maintain excellent precision of retention times, even after column trimming.

Full scan data acquisition mode was used for the initial screening of the matrix extract. This screening was used to evaluate in-source loading, and for monitoring the efficiency of the sample cleanup procedure that followed QuEChERS extraction.

Agilent MassHunter Workstation software, including Agilent MassHunter Acquisition 13.0 for GC/MS, MassHunter Quantitative Analysis 12.1, and MassHunter Qualitative Analysis 12.0 packages were used in this work.

Table 1. Agilent 8890 GC system with Agilent 7010D gas chromatograph and mass spectrometer conditions for pesticide analysis.

Parameter	Value
GC	Agilent 8890 with fast oven, auto injector and tray
Inlet	MMI
Mode	Cold splitless
Purge Flow to Split Vent	60 mL/min at 3 min
Septum Purge Flow	3 mL/min
Septum Purge Flow Mode	Switched
Injection Volume	1.0 µL
Injection Type	Reversed 2-layer (L2, L1)
L1 Airgap	0.2 µL
L2 Volume (ISTD)	0.2 µL
L2 Airgap	0.2 µL
Gas Saver	On at 30 mL/min after 5 min
Inlet Temperature	60 °C for 0.1 min, then to 280 °C at 600 °C/min, hold for 5 min, then to 325 °C at 600 °C/min
Postrun Inlet Temperature	310 °C
Postrun Total Flow	25 mL/min
Carrier Gas	Helium
Inlet Liner	Agilent Ultra Inert 2 mm dimpled liner (p/n 5190-2297)
Oven	
Oven Program	60 °C for 1 min; 40 °C/min to 170 °C; Hold 0 min; 10 °C /min to 310 °C; Hold 2.25 min
Total Run Time	20 min
Postrun Time	1.5 min
Equilibration time	0.5 min
Column 1	
Type	Agilent HP-5ms UI, 15 m × 0.25 mm, 0.25 µm (p/n 19091S-431UI-KEY)
Control Mode	Constant flow
Flow	1.0 mL/min (then retention time locked)
Inlet Connection	MMI
Outlet Connection	PSD (PUU)
PSD Purge Flow	5 mL/min
Postrun Flow (Backflushing)	–7.873

Parameter	Value
Column 2	
Type	Agilent HP-5ms UI, 15 m × 0.25 mm, 0.25 µm (p/n 19091S-431UI-KEY)
Control Mode	Constant flow
Flow	1.2 mL/min (then retention time locked)
Inlet Connection	PSD (PUU)
Outlet Connection	MSD
Postrun Flow (Backflushing)	8.202
MSD	
Model	Agilent 7010D
Source	HES 2.0
Vacuum Pump	Performance turbo
Tune File	atunes.eihs2.jtune.xml
Solvent Delay	3.75 min
Quad Temperature (MS1 and MS2)	150 °C
Source Temperature	280 °C
Mode	dMRM or Scan
He Quench Gas	2.25 mL/min
N_2 Collision Gas	1.5 mL/min
MRM Statistics	
Total MRMs (dMRM mode)	749
Minimum Dwell Time	5.42 ms
Minimum Cycle Time	85.01 ms
Maximum Concurrent MRMs	64
EM Voltage Gain Mode	10
Scan Parameters	
Scan Type	MS1 Scan
Scan Range	45 to 450 *m/z*
Scan Time (ms)	220
Step Size	0.1 amu
Threshold	0
EM Voltage Gain Mode	1

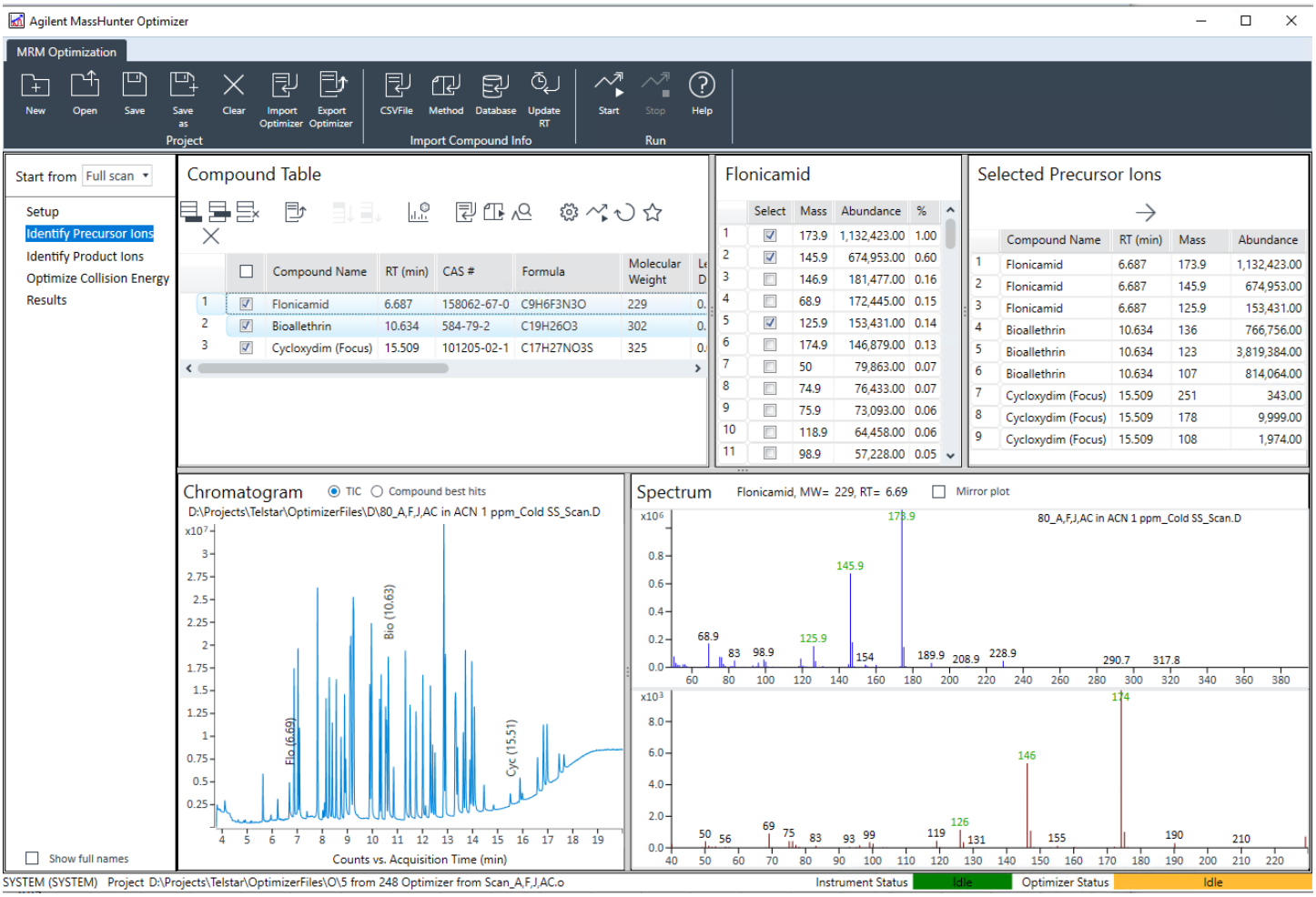


Figure 2. Agilent MassHunter Optimizer software for GC/TQ, used for the automated development of MRM transitions.

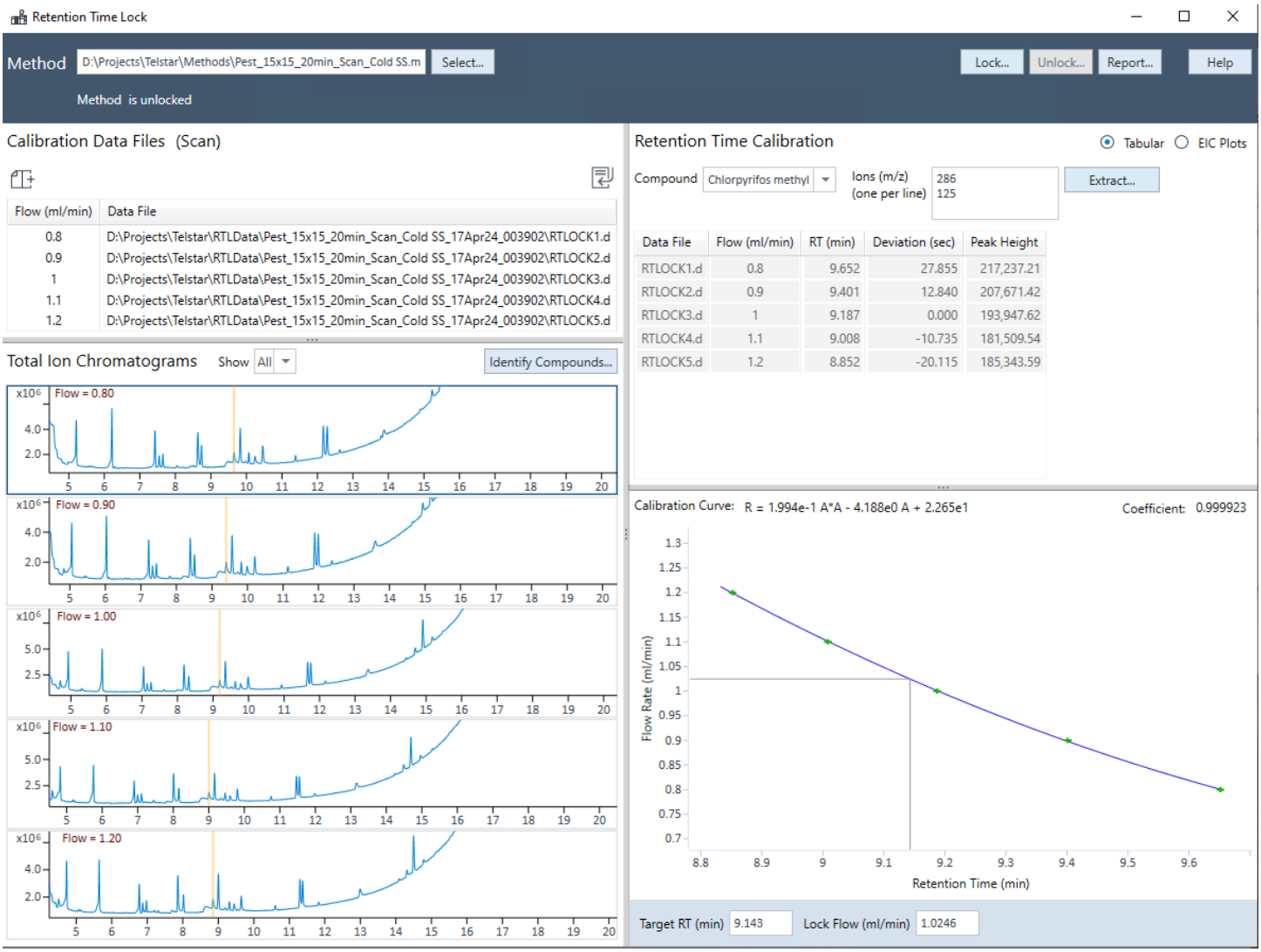


Figure 3. New Agilent retention time locking software in Agilent MassHunter Acquisition 13.0 for GC/MS.

Sample preparation

Black tea powder was obtained from a local grocery store. Black tea powder (2 g) was extracted with a modified QuEChERS extraction using acetonitrile (ACN) with 2% formic acid and EN extraction salt. The crude tea extract then was mixed with 2% of acidic buffer. The sample mixture was cleaned by EMR mixed-mode pass-through cleanup using Agilent Captiva EMR–GPD 6 mL. The sample eluent was dried with anhydrous $MgSO_4$ to remove water residue completely before GC/MS/MS analysis. The sample preparation procedure flowchart is shown in Figure 4, and details will be discussed in a separate application note. The entire sample preparation procedure resulted in a 5x dilution factor.

Pesticide standards

Agilent GC pesticide standards 1 through 12 (part numbers PSM-100-A through -L) and Agilent GC/LC pesticide standards 1, 2, and 3 (part numbers PSM-100-AA, PSM-100-AB, PSM-100-AC) were used for preparing matrix matched calibration standards. A combination of the 15 used standards yielded a mix of 246 pesticides commonly regulated by the FDA, USDA, and other global governmental agencies.

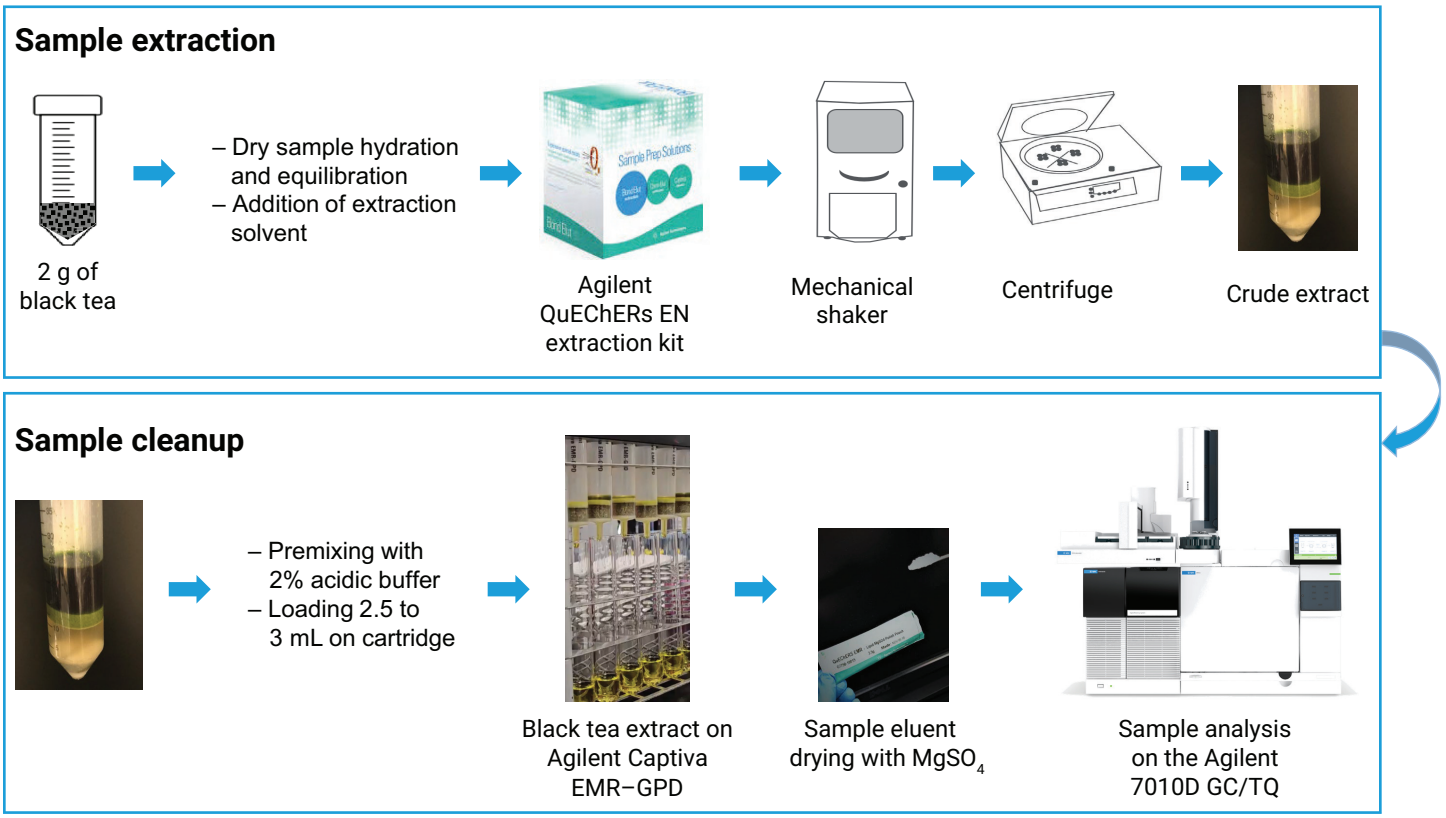


Figure 4. QuEChERS sample preparation and cleanup method for black tea.

Matrix-matched calibration

Calibration performance was evaluated using a series of matrix matched calibration standards, ranging from 0.01 to 1,000 ppb, including 0.01, 0.05, 0.1, 0.5, 1, 2, 5, 10, 50, 100, 200, 500, and 1,000 ppb. The standard parathion-d_{10} (Agilent QuEChERS IS standard number 6, part number PPS-610-1) was used as the internal standard for quantitation of the target pesticides. It was added at 0.2 µL through reversed sandwich injection with the ALS to a final concentration of 10 ppb in the injected sample.

An appropriate calibration function, either linear or quadratic, guided by the lower value of the relative standard error (RSE) was used. A weighting factor of 1/x allowed for maintaining accuracy across the entire calibration range. The deviation of the back-calculated concentrations of the calibration standards from the true concentrations, using the calibration curve in the relevant region, did not exceed ± 20%.

The concentrations expressed in ppb (w:v) correspond to the pesticide concentration in the injected sample. The QuEChERS sample preparation procedure described in the Sample preparation section resulted in a dilution factor of 5. Hence, the concentrations measured in the injected samples were five times lower than the corresponding concentration in the black tea sample, expressed in µg/kg.

Analyte protectants were not used in this work. The preliminary investigation showed that analyte protectants did not have a response enhancement effect on most of the compounds when analyzed in the rich and complex black tea matrix. It is of note that analyte protectants often significantly enhance target analyte response and stability as is described in-depth in the peer-reviewed literature.[6]

Recovery evaluation

Sample preparation efficiency was evaluated by performing recovery studies. The surrogate black tea matrix was spiked at two different levels, 10 and 50 ppb, with six replicates at each level. The samples were extracted and cleaned up. Assuming 100% recovery, pesticide concentrations in the prespiked samples were expected to be 2 and 10 ppb due to a 5x dilution rate. A blank black tea extract was postspiked with the pesticide standard to achieve final concentrations of 2 and 10 ppb. The prespiked and postspiked samples were analyzed, and the response areas were compared. The recovery was measured as the ratio of the pesticide peak area in the prespiked sample to the area in the postspiked samples.

Results and discussion

As contemporary GC/MS technology continues to advance, so do the expectations for high sample throughput, intuitive user-friendly system setup and configuration, and streamlined maintenance. The demands for enhanced analytical performance are driven by the evolving regulations in pesticide residue analysis and food safety.

Several best practices to achieve the best GC/TQ performance in pesticide residue analyses were described in Agilent application note 5994-4965EN.[7] This work presents a complete workflow for analyzing 246 pesticides in black tea, while implementing the previously described best practices and offering further method and technology enhancements. The innovative HES 2.0 yielded enhanced GC/TQ performance stability, as evidenced by precise results at the low concentration of 2 ppb over 800 consecutive injections of complex black tea extract.

The technology and method enhancements that enable unparalleled GC/TQ performance while ensuring stable and reliable results in a high-throughput setting are outlined in this application note and grouped into four categories: sample preparation, GC instrumentation and supplies, MS electron ionization technology advancements, and instrument intelligence and software functionality.

Effective matrix cleanup

Sample preparation is a key component of performing successful pesticide analysis. Performing analysis of samples prepared by QuEChERS extraction, particularly when analyzing complex pigmented samples such as black tea without adequate cleanup, can lead to increased system maintenance. The parts of the system that are affected without adequate sample cleanup include liner replacement, GC column trimming, and inlet and MS source cleaning. As a result, throughput is decreased. Further, the presence of large amounts of matrix can affect the accuracy of results, often most pronounced with difficult to analyze pesticides. The EMR mixed-mode pass-through cleanup using Captiva EMR with Carbon S cartridges is a simplified procedure that demonstrates an improvement on both sample matrix removal, and overall recovery and reproducibility of targets. As shown in Figure 5, the abundance of the TIC signal in full scan data acquisition mode was noticeably reduced for black tea extract after cleanup when comparing the crude extracts before cleanup.

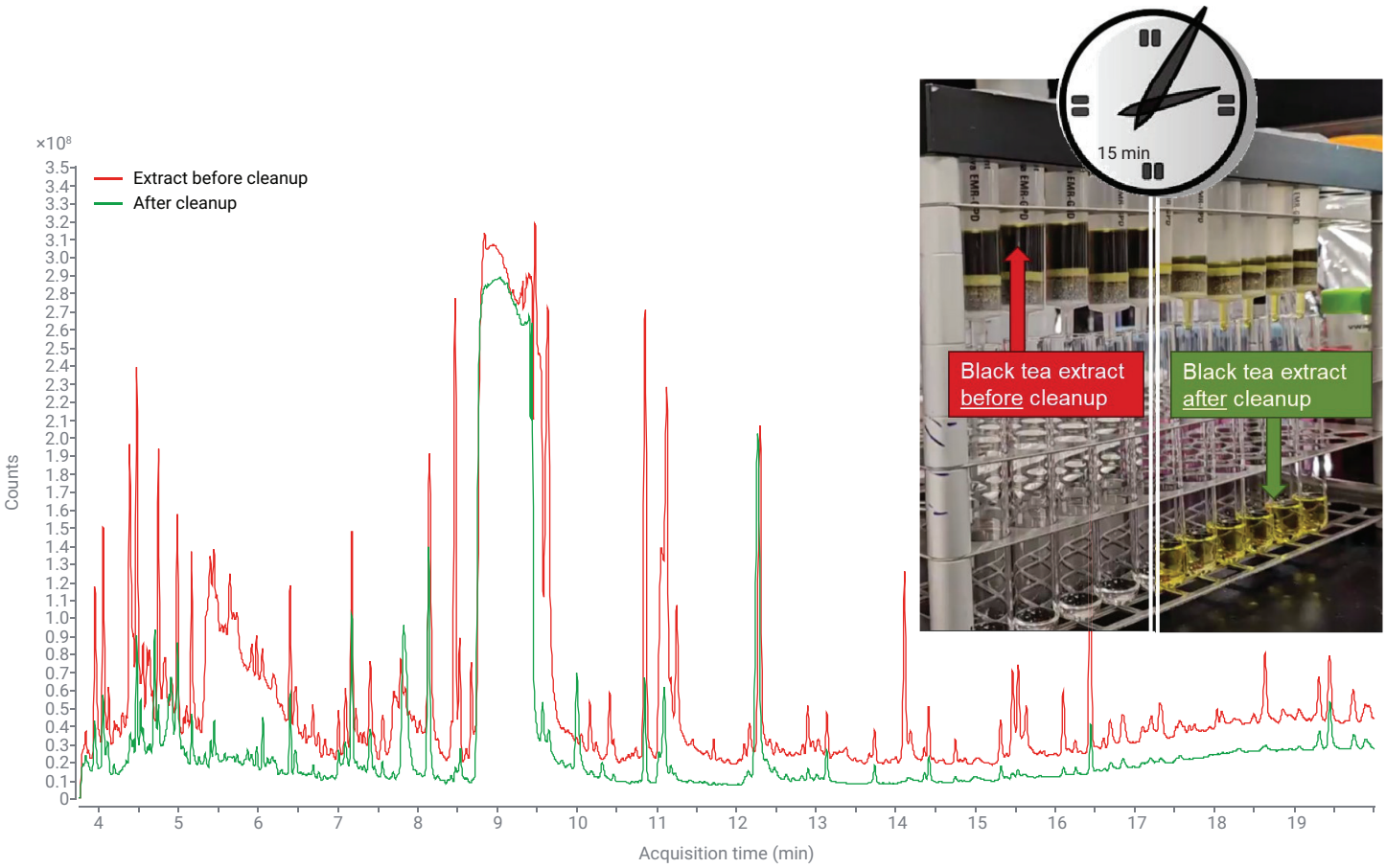


Figure 5. Scan TIC of black tea extract. The green trace corresponds to matrix sample with Agilent Captiva-EMR cleanup, and the red trace corresponds to matrix sample without cleanup.

Performing matrix screening in full scan data acquisition mode, as shown in Figure 5, facilitates the evaluation of in-source matrix loading, as discussed in 5994-4965EN.[7] Every MS source has a limitation on the amount of material present in the source, at any point in time, to maintain optimal performance. Quantitation accuracy of the analysis can be significantly compromised if the source is overloaded with matrix.

Hence, it is essential to analyze the matrix in full scan mode to evaluate the TIC and maintain optimal GC/TQ performance. For best performance with the HES 2.0 source, it is recommended to have a TIC full scan abundance below 7×10^7 counts when analyzing with an EM gain set to 1. As shown in Figure 5, black tea extract is complex, featuring abundant matrix components. Cleaning up the extract is key to lowering the matrix background that leads to adequate in-source loading, enhancing selectivity and sensitivity, widening the dynamic range, and allowing for less frequent system maintenance, increasing productive uptime.

GC instrumentation and supplies

Midcolumn backflushing: The Agilent 8890 GC provides an easy-to-use midcolumn backflushing functionality that results in increased sample throughput through shorter analysis times and less frequent column maintenance.

Midcolumn backflush allows for the elution of the high boiling point matrix components from the column in a shorter time and without eluting high-boiling matrix into the MS. Midcolumn backflushing is a technique in which the carrier gas flow is reversed after the last analyte has exited the column and all MS data are collected. The oven is then held at the final temperature in postrun mode, with the reversed carrier gas flow through the first column. The high boilers are eluted back out the head of the column and into the split vent trap. The ability to reverse the flow is provided by the PUU. The PUU is a tee that is inserted, in this case, between two identical 15 m columns. During the analysis, a small makeup flow of carrier gas from the 8890 PSD is used to sweep the connection. During backflushing, the

makeup flow from the PSD is raised to a much higher value, sweeping high boilers backward out of the first column while simultaneously providing forward flow in the second column. For the configuration in this application, the backflushing time was 1.5 minutes. More details about using PSD for backflushing in the Agilent 8890 GC system can be found in Agilent application note 5994-0550EN.[8]

Figure 6 illustrates the effectiveness of the backflush technique in reducing cycle time without carryover of the black tea matrix. The cycle time was reduced by 50% and the columns did not have to be exposed to the higher bake-out temperatures for an extended time. Using backflushing, excess column bleed and heavy residues are not introduced into the MSD, thereby reducing ion source contamination.

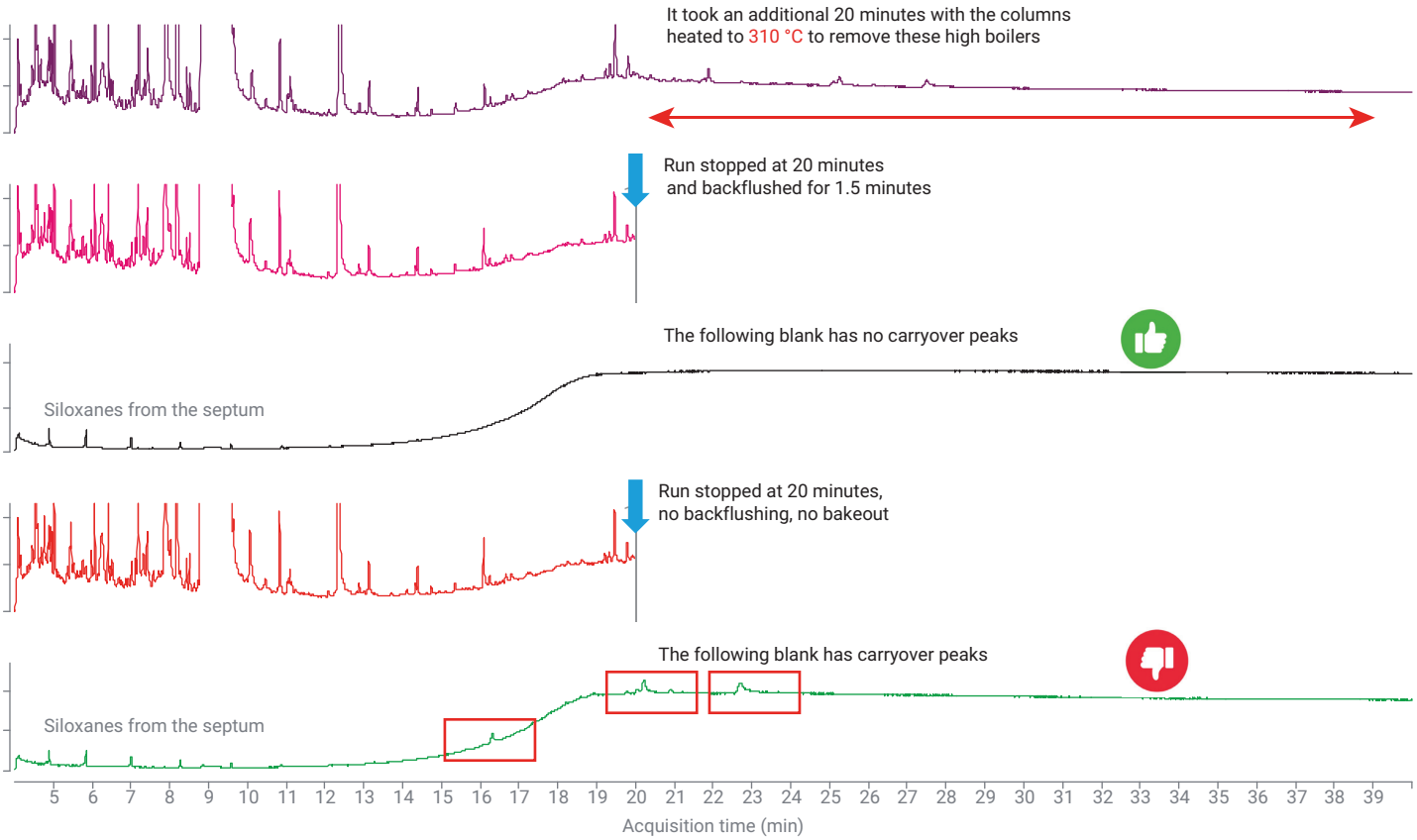


Figure 6. TIC Scan chromatograms of black tea extract, followed by analysis of an instrument blank with: column bake-out, with backflush, and without backflush or bake-out.

The backflush setup process has been simplified with the introduction of new tools that allow for making a capillary flow technology (CFT) connection with ease. These tools include the gold-plated flexible metal ferrules (part number G2855-28501) and the GC column installation preswaging tool for Flexible Metal ferrules into Capillary Flow Technology devices (part number G3440-80227)—shown in Figure 7.

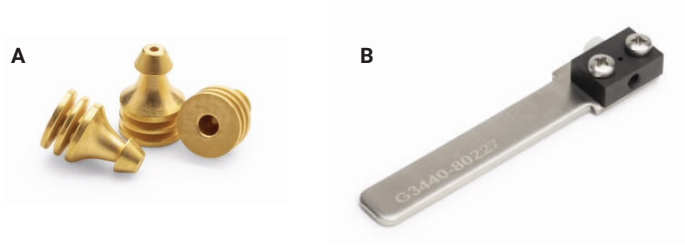


Figure 7. Flexible metal ferrules (part number G2855-28501) (A) and the GC column installation preswaging tool for Flexible Metal ferrules into Capillary Flow Technology devices (part number G3440-80227) (B).

Additionally, MassHunter Acquisition 13.0 for GC/MS provides intuitive guides for backflushing setup and review. Figure 8 shows the backflush overview tab in the GC Method Editor in MassHunter Acquisition 13.0 for GC/MS.

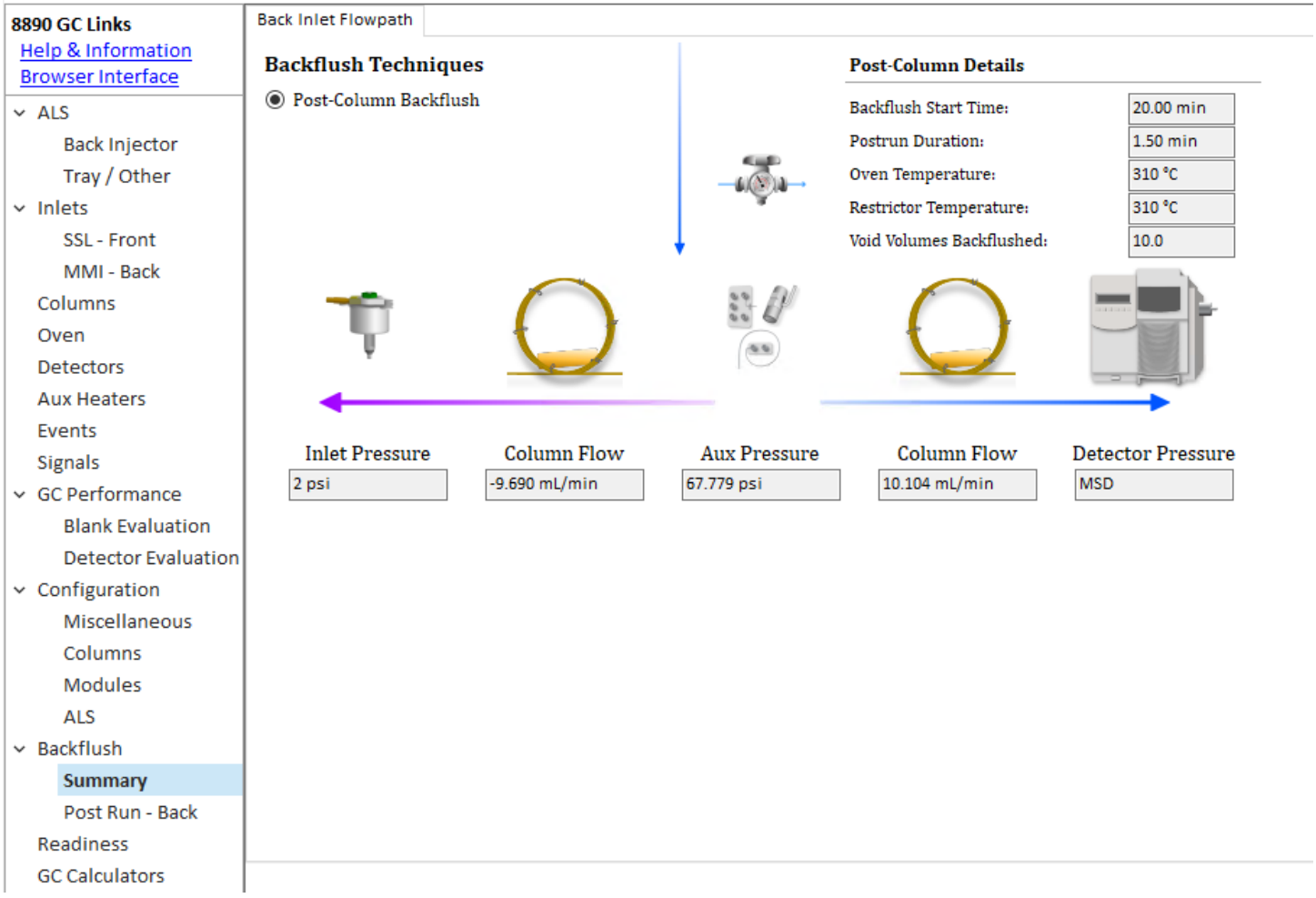


Figure 8. Backflush summary in Agilent MassHunter Acquisition 13.0 for GC/MS.

GC injection optimization: Efficiently volatilizing the sample in the GC inlet is an essential component of successful GC/MS analysis. Various sample introduction techniques are aimed at preserving thermally labile and active compounds. In this work, cold splitless and solvent vent injection modes were evaluated.

As shown on the left in Figure 9, the use of solvent vent mode for analyzing black tea extract resulted in very large caffeine carryover into the subsequent analyses. To reduce caffeine carryover, cold splitless injection mode was used (Figure 9, at right). Increase of the splitless purge time to 3 minutes resulted in enhanced method sensitivity without deteriorating the chromatographic peak shape for the targets.

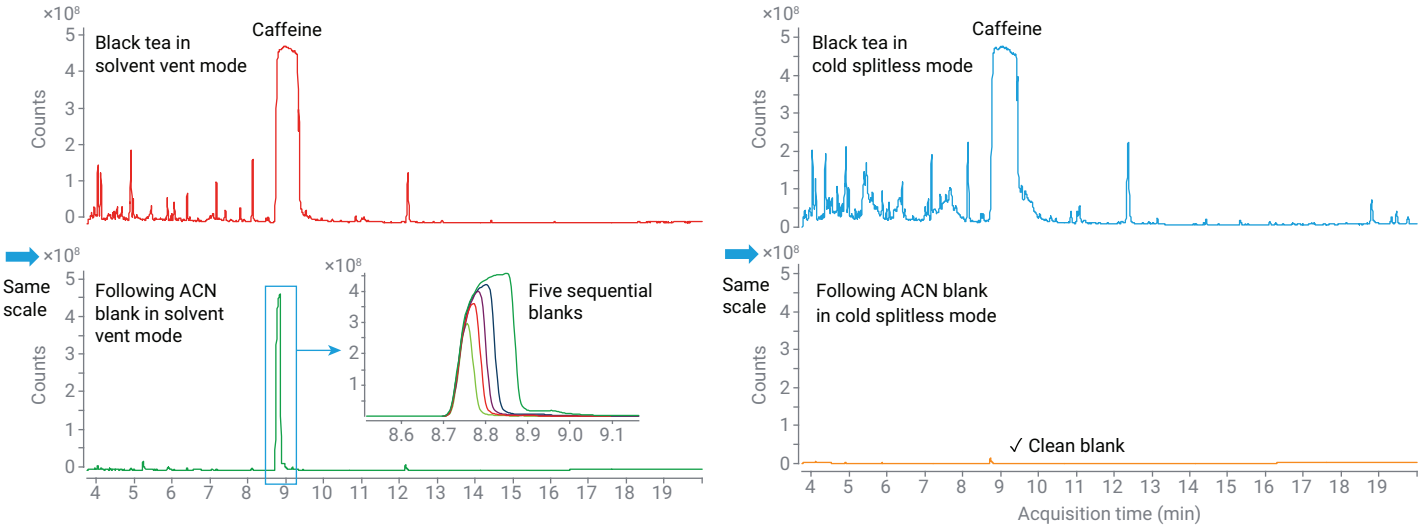


Figure 9. Injection optimization for analyzing black tea: cold splitless (on the right) reduces carryover of caffeine compared to solvent vent mode (on the left).

HES 2.0: Novel electron ionization (EI) source technology

Equipped with the novel HES 2.0 EI source, the 7010D demonstrated sensitivity that allows for ultra-trace level detection when analyzing pesticides. The new HES 2.0 ion source is equipped with a novel dipolar RF lens that redirects the carrier gas ions and, as a result, enables improved system robustness and unparalleled analytical sensitivity.

Figure 10 shows MRM chromatograms for selected pesticides at 0.01 ppb in black tea extract. The overlaid chromatograms show repeatability over seven replicate injections, and the response RSD% as a measure of precision. Appendix Table 1 shows the LOQs for all analyzed pesticides. LOQs as low as 0.01 ppb were observed for 34% of the targets, at or below 0.1 ppb for 74% of compounds, and below 2 ppb for 96%. The number of compounds expressed in percent, with their respective LOQs, are plotted in Figure 11.

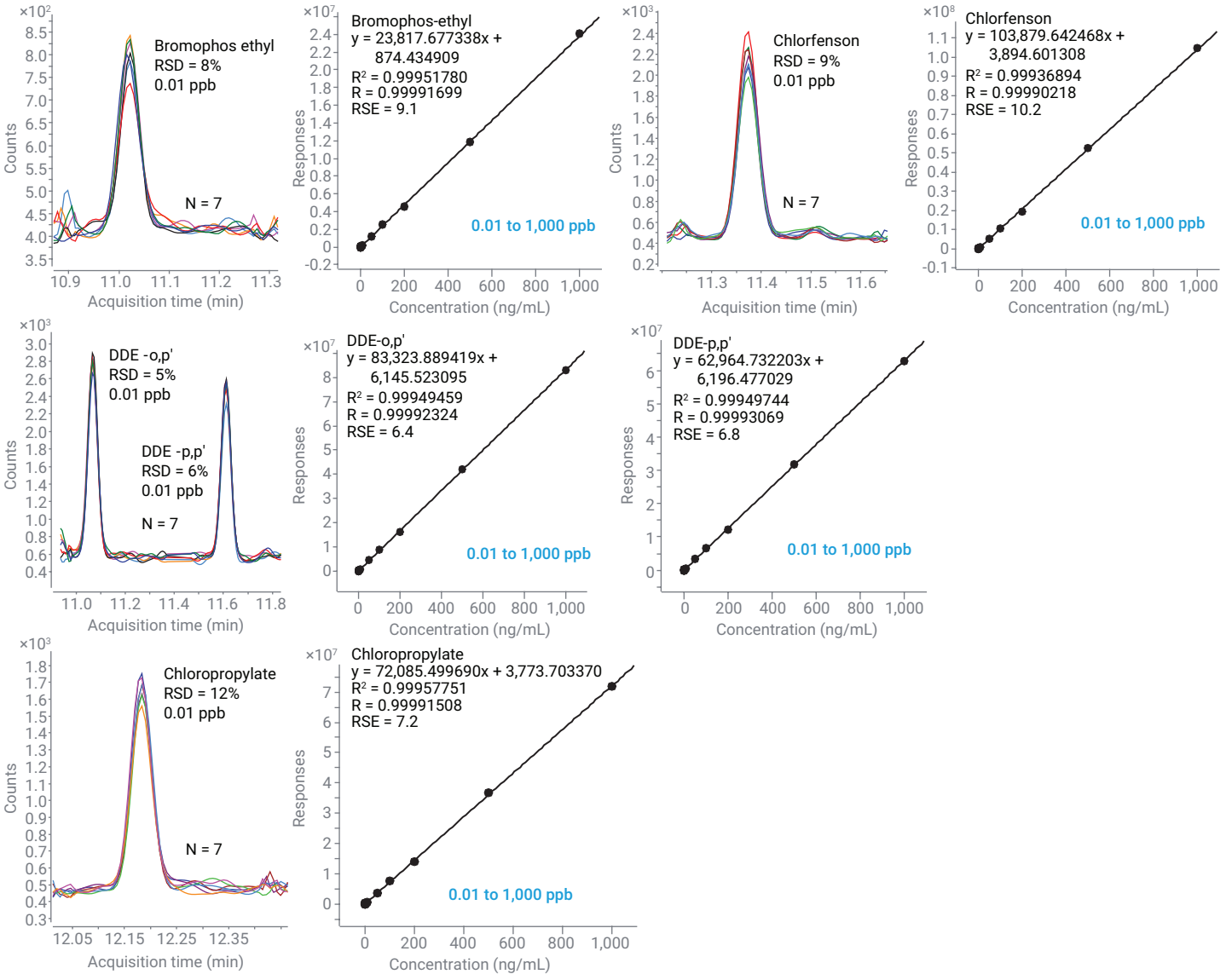


Figure 10. MRM chromatograms with seven replicate injections for the selected pesticides at the LOQ of 0.01 ppb in black tea extract and their calibration curves.

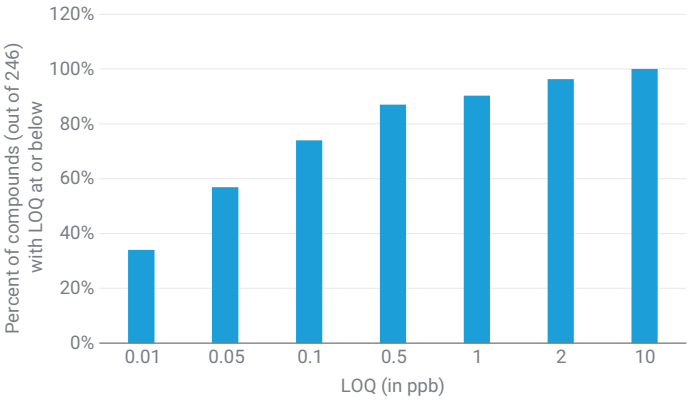


Figure 11. Percentage of compounds with their respective LOQ levels (in ppb) in black tea extract.

Figure 10 also shows the matrix-matched calibration performance in black tea extract with excellent linearity maintained over five orders of magnitude, ranging from 0.01 to 1,000 ppb. All calibration curves were inspected, and if needed, were trimmed to comply with SANTE 11312/2021 guidelines.[2] Appendix Table 1 provides information on calibration ranges and quality of calibration fit for all compounds. The R^2 correlation coefficient for all targets was > 0.99. The RSE was used as an additional criterion for demonstrating the calibration curve quality. The RSE provides an improved criterion for evaluation of calibration curves, as it is consistent for evaluation of all curve fitting types.[9] In this work, the calibration curves for all compounds had RSE values below 20.

For the compounds for which quadratic calibration fit was used, a linear calibration curve fit can be used instead by narrowing the calibration range. For example, oxyfluorfen could be calibrated over five orders of magnitude, from 0.01 to 1,000 ppb using quadratic calibration fit with R^2 = 0.9995 and RSE = 14. Alternatively, a linear calibration fit could be applied over a calibration range of 0.01 to 500 ppb with R^2 = 0.9960 and RSE = 26. The selection of the calibration curve fit was guided by the lower RSE value.

Some pesticides are known to present a particular challenge for analysis. As stated in the EURL Analytical Observation Report[10], captan and folpet are analytically among the most challenging pesticides due to their nonamenability to LC/TQ, and their tendency to degrade both in solution as well as in the GC inlet. Figure 12 demonstrates that captan and folpet could be quantitated with great precision at LOQs as low as 2 and 0.5 ppb, respectively. Freshly diluted standard, acidified sample extracts, and optimized injection conditions with cold splitless injection were key to achieving high recoveries and precision in analyzing captan and folpet. Deltamethrin, a synthetic pyrethroid, elutes at the end of the chromatographic run and is also known to be difficult for GC/MS analysis.[11] As shown in Figure 12, deltamethrin could be reliably quantitated down to 0.5 ppb using the developed method. Other compounds shown in Figure 12 include organochlorine pesticides, aldrin, dieldrin, and endrin, and the two most widely used multipurpose pyrethroids, cypermethrin and cyfluthrin, quantitated with great precision and excellent linearity over a wide dynamic range.

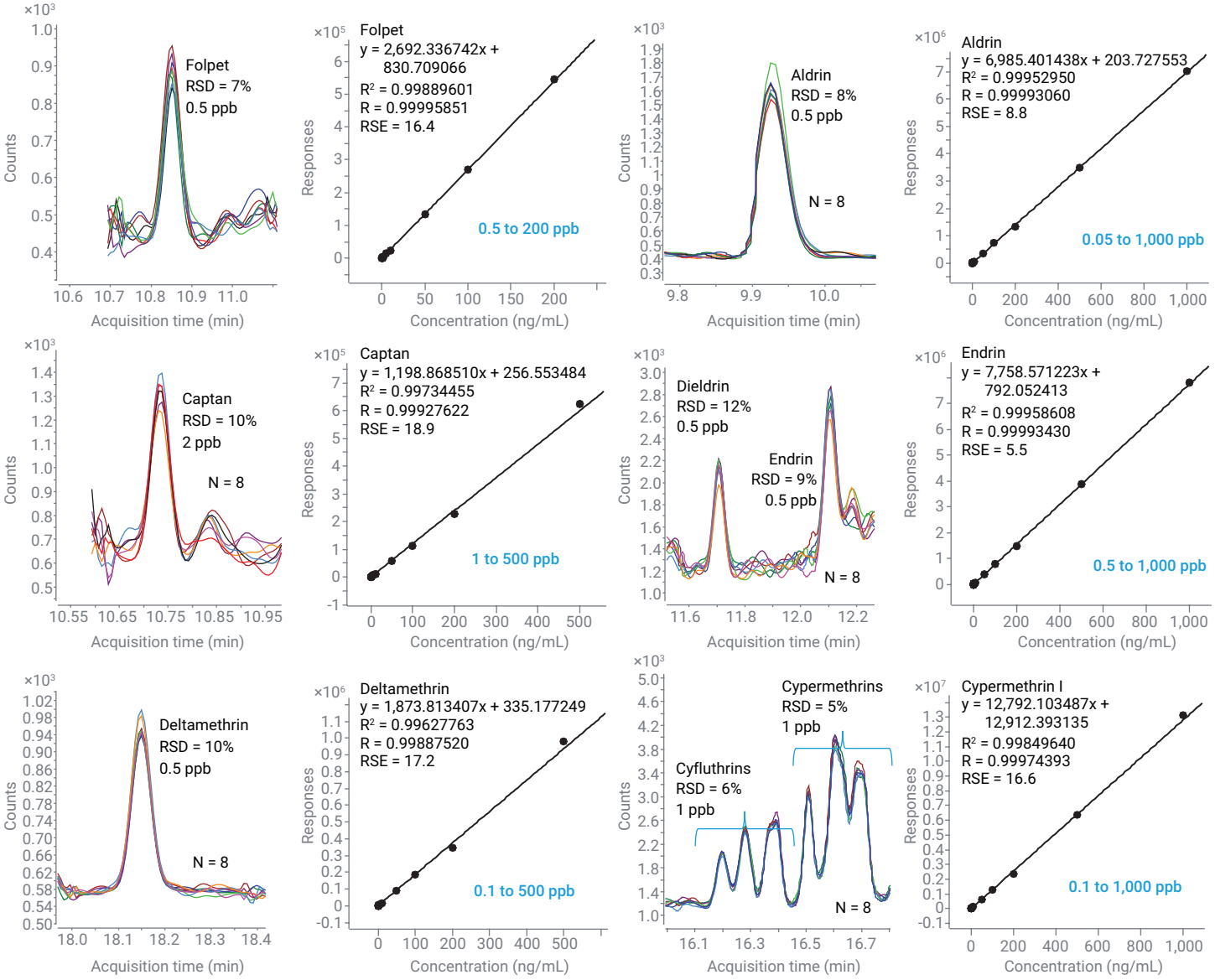


Figure 12. MRM chromatograms with eight replicate injections for the selected challenging pesticides. Included are LOQ levels and their calibration curves.

Recovery and precision

To validate the complete workflow solution and ensure that enhanced matrix cleanup did not have a negative impact on pesticide recovery, a study aimed at recovery and precision evaluation was performed. Two concentrations were selected for the study (10 and 50 ng/g) in dry black tea, resulting in 2 and 10 ppb in the final extract due to the 5x dilution factor. Figure 13 shows target results at 10 and 50 ng/g in black tea, demonstrating the acceptable recoveries achieved for the majority of pesticides, even for the common problematic pesticides such as planar and labile.

Longevity and maximized throughput with confidence

The ruggedness of the analysis was demonstrated by analyzing a challenging black tea extract spiked with pesticides at 2 ppb. The area of the analyte response was monitored over 800 consecutive injections. Analyte response, normalized by the internal standards (ISTD), remained consistent over 800 injections that spanned over 400 hours of continuous running with RSDs < 20% for 176 compounds. Figure 14 shows the response for 60 compounds, normalized by the ISTD and by the average response for each analyte.

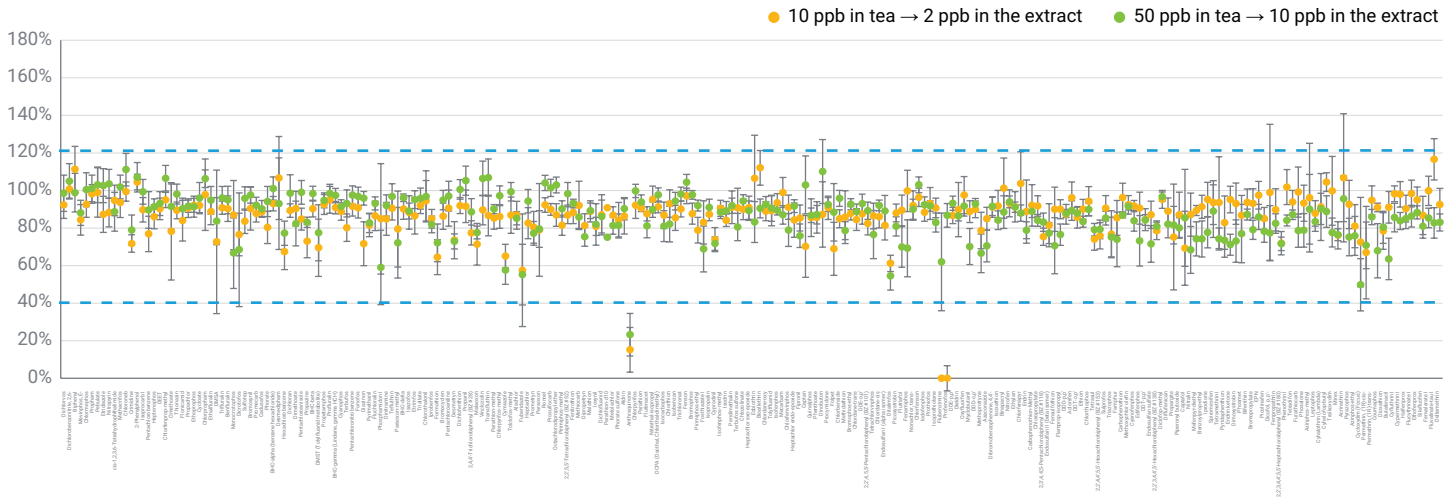


Figure 13. Pesticide recoveries in black tea at 10 and 50 ppb shown for all 244 pesticides.

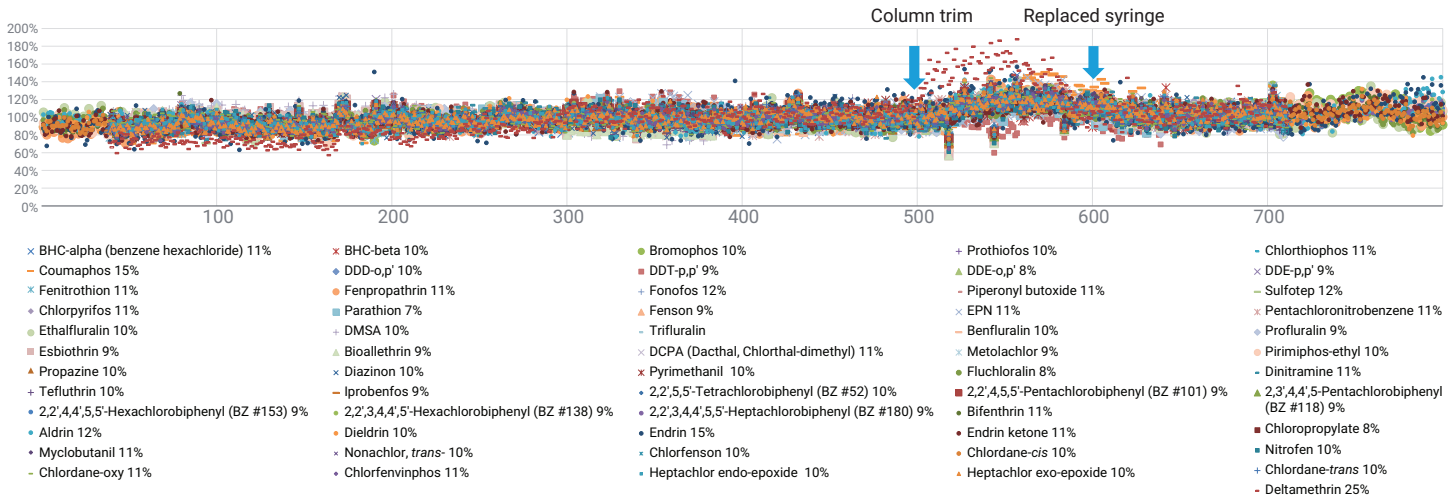


Figure 14. Stability of the peak area for pesticides spiked at 2 ppb into black tea extract, normalized by the ISTD and the average response, over 800 consecutive injections with the Agilent 8890 GC and 7010D GC/TQ systems.

The graph shows that analyte responses were stable and within 80 to 120% throughout the entire study that lasted over 17 days of continuous analysis. The RSDs for each of the target responses are shown in the legend in Figure 14, with most of them below 12%. The absolute responses in terms of peak areas also remained consistent throughout the longevity study. For example, the RSDs on the peak areas over 800 injections for early-eluting BHC-beta, mid-range eluting fenson, and late-eluting coumaphos were 9%, 10%, and 16%, respectively.

The maintenance performed during the robustness testing involved septum and liner replacement every 100 injections. With the midcolumn backflush configuration and the use of the temperature-programmed MMI inlet, inlet liner and septum replacement could be performed in under four minutes, providing a productivity boost to the workflow.

Two inches of the GC column head were trimmed after 500 injections. The use of the backflushing allowed for a substantial increase in the number of injections before column head maintenance was needed when analyzing a complex black tea extract. Similar to GC inlet maintenance, column trimming could be performed efficiently in a short amount of time (5 to 10 minutes) and did not require MS cool-down and venting due to the midcolumn configuration coupled with the temperature-programmed MMI.

The autoinjector syringe was replaced after 600 injections in the longevity study, as noted in Figure 14, with a total of 1,000 injections made with the syringe. The decision to replace the syringe was driven by the decreased measurement precision resulting from a higher variability in the target and ISTD responses. The replacement procedure was performed as guided by the user manual for the 7693A ALS.[12] The precision of measurements was restored after the syringe replacement. As a result, the graph in Figure 14 shows an increased response variability between 500 and 600 injections. This effect was particularly pronounced for deltamethrin, which can present a challenge for achieving good precision at low concentrations. Washing the syringe needle support foot is an additional autoinjector maintenance procedure to consider when analyzing challenging samples to minimize carryover and ensure precision.

It is of note that there was no need to perform GC inlet or MS source cleaning during the entire study, which spanned over 1,000 injections, including the calibration assessment and precision and recovery studies.

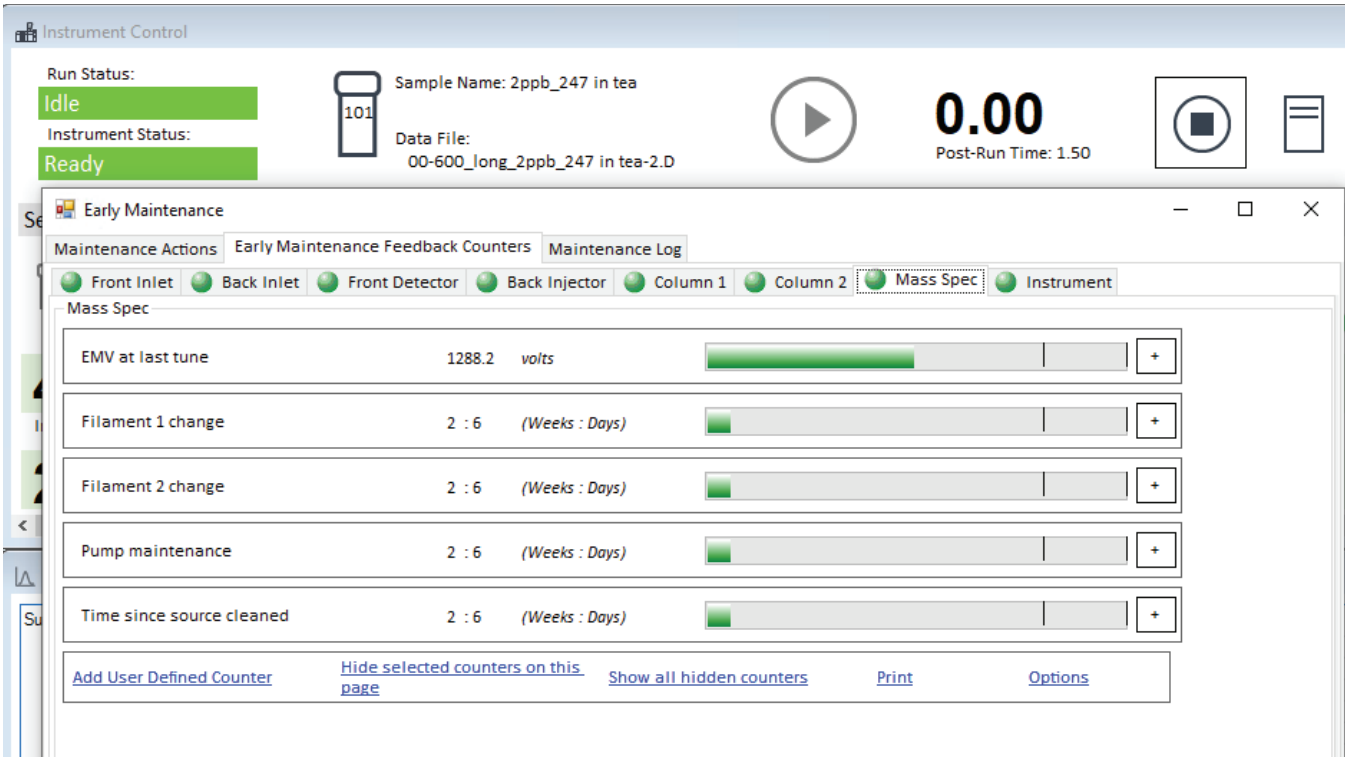
The exceptional method ruggedness shown in this work was achieved by:

- Following the key practices to successful pesticide analysis outlined in this application note and in another application note[7]
- Performing effective sample preparation and cleanup
- Using the state-of-the art GC and MS technology in the 8890 and 7010D GC/TQ systems

GC/TQ intelligence and new software functionality

The health and status of the GC/TQ system was continuously monitored though the longevity study by using the Early Maintenance Feedback functionality in MassHunter Acquisition 13.0 for GC/MS. Figure 15A shows a screenshot of the MS health status, featuring the electron multiplier (EM) voltage at last tune, filament age, pump maintenance schedule, and time since the source was cleaned. The dashboard allows for daily tracking for essential maintenance procedures and reminds the user to perform maintenance on time. In addition to the dashboard view, the built-in intelligent functionality in the 7010D GC/TQ system allows for plotting tune-related parameters over time to track the EI source health and performance. The plot for the EM voltage is shown in Figure 15B. The routine tune check procedures, which could be built into the sequence table via keywords, are helpful in guiding when the EM gain curve needs to be updated. This procedure allows for adjusting the EM voltage to maintain a stable response while not altering tune parameters and ion ratio, maintaining MS tune and method calibration validity.

A



B

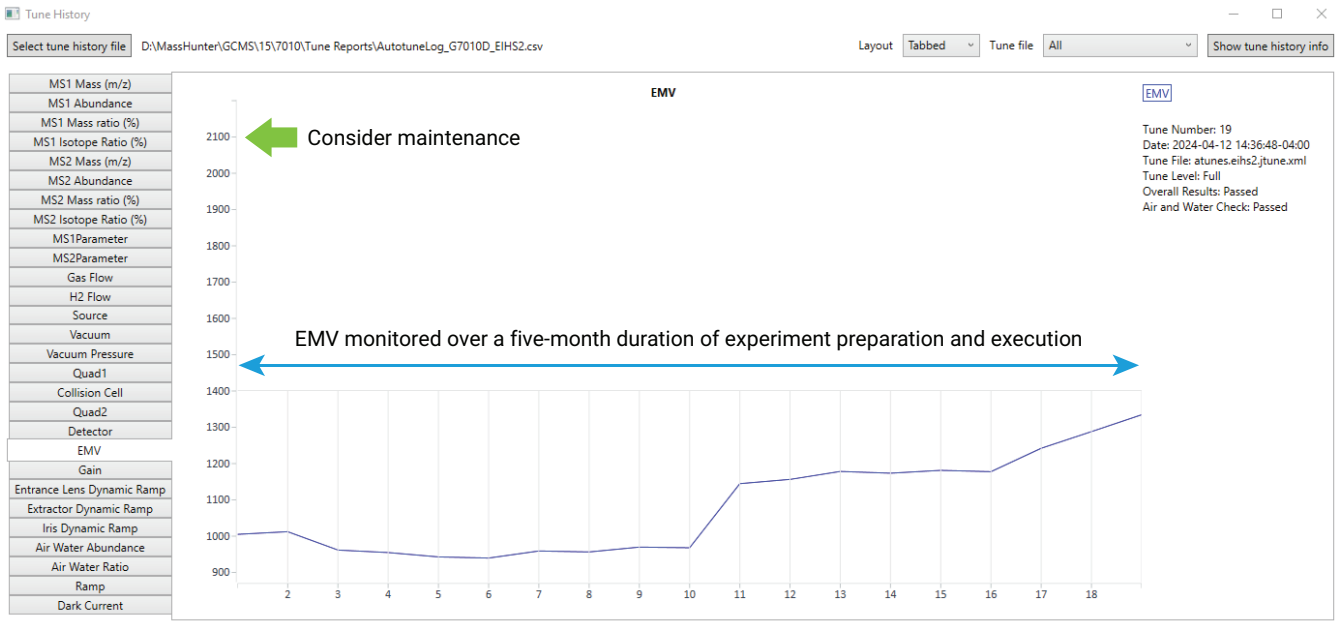


Figure 15. The early maintenance dashboard for GC/TQ (A) and the EM voltage plot for the tune history (B) shown in Agilent MassHunter Acquisition 13.0 for GC/MS.

Conclusion

This application note presents a workflow solution for analyzing pesticides in black tea with the new 7010D GC/TQ, allowing for the quantitation of 246 pesticide residues at trace levels with LOQs as low as 0.01 ppb for 34% of the targets, at or below 0.1 ppb for 74% of compounds, and below 2 ppb for 96%. Matrix-matched calibration allowed for excellent accuracy over a wide dynamic range, spanning up to five orders of magnitude over the 0.01 to 1,000 ppb range in a complex black tea extract. Method ruggedness was demonstrated through maintaining measurement accuracy with good precision (RSDs < 20% for 176 compounds) for black tea extract spiked at 2 ppb sequentially analyzed over 800 runs and spanning over 17 days of continuous analysis. The key components for a robust workflow included a combination of efficient sample preparation and cleanup, the Agilent 8890 GC hardware, functionality, and GC supplies, the novel EI source technology with HES 2.0, and lastly, the built-in GC/TQ intelligence and new software functionality.

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Appendix Table 1. Calibration performance for 246 pesticides in black tea using the Agilent 7010D GC/TQ equipped with the Agilent High-Efficiency Source (HES) 2.0.

Name	RT	Transition	Calibration Range (ppb)			CF	CF R^2	Relative Standard Error
Methamidophos	4.520	141.0 → 64.0	0.1	–	1,000	Linear	0.9994	7.5
Dichlorvos	4.643	184.9 → 93.0	0.05	–	1,000	Linear	0.9988	11.2
Dichlorobenzonitrile, 2,6-	5.210	171.0 → 100.0	0.01	–	1,000	Linear	0.9990	10.4
Biphenyl	5.390	154.1 → 153.1	0.5	–	1,000	Quadratic	0.9991	11.8
Mevinphos, E-	5.578	127.0 → 94.9	0.5	–	1,000	Linear	0.9994	7.9
Acephate	5.679	136.0 → 94.0	5	–	1,000	Quadratic	0.9990	8.1
Chlormephos	5.687	153.9 → 121.1	0.5	–	1,000	Linear	0.9979	5.0
Propham	5.740	178.9 → 137.1	1	–	1,000	Linear	0.9986	9.6
Pebulate	5.774	128.0 → 57.1	0.5	–	1,000	Linear	0.9983	6.6
Etridiazole	5.798	213.1 → 185.0	0.1	–	500	Linear	0.9986	10.7
Nitrapyrin	5.804	194.0 → 158.0	0.05	–	1,000	Quadratic	0.9994	8.7
cis-1,2,3,6-Tetrahydrophthalimide	5.956	151.1 → 80.0	0.1	–	1,000	Linear	0.9994	6.1
Methacrifos	6.027	207.9 → 180.1	0.05	–	1,000	Quadratic	0.9996	11.4
Chloroneb	6.110	191.0 → 113.0	0.01	–	1,000	Linear	0.9995	8.2
Crimidine	6.212	170.9 → 142.1	0.1	–	1,000	Linear	0.9994	10.4
2-Phenylphenol	6.213	169.1 → 115.1	0.5	–	1,000	Linear	0.9995	5.6
Isoprocarb I	6.295	136.0 → 121.1	0.5	–	1,000	Linear	0.9994	9.3
Pentachlorobenzene	6.311	251.9 → 217.0	0.01	–	1,000	Linear	0.9984	11.4
Heptenophos	6.585	124.0 → 89.0	0.01	–	500	Linear	0.9990	12.5
DEET	6.600	191.0 → 190.0	0.5	–	1,000	Linear	0.9981	11.5
Chlorfenprop-methyl	6.696	165.0 → 102.0	0.01	–	1,000	Linear	0.9989	14.0
Omethoate	6.773	110.0 → 47.0	0.1	–	1,000	Quadratic	0.9997	8.8
Thionazin	6.781	143.0 → 79.0	0.1	–	1,000	Quadratic	0.9998	9.5
Flonicamid	6.859	173.9 → 68.9	0.01	–	1,000	Linear	0.9994	11.5
Propachlor	6.865	176.1 → 57.1	0.05	–	1,000	Quadratic	0.9997	8.5
Ethoprophos	6.996	157.9 → 97.0	0.1	–	1,000	Quadratic	0.9997	8.2
Cycloate	7.017	154.1 → 83.1	0.05	–	1,000	Linear	0.9991	16.2
Chlorpropham	7.080	171.0 → 127.1	0.05	–	500	Linear	0.9993	13.7
Ethalfluralin	7.109	275.9 → 202.1	0.05	–	1,000	Quadratic	0.9997	11.4
DMSA	7.169	200.0 → 108.0	2	–	1,000	Quadratic	0.9963	17.5
Trifluralin	7.217	306.1 → 264.0	0.05	–	1,000	Quadratic	0.9997	11.8
Benfluralin	7.251	292.0 → 264.0	0.1	–	1,000	Quadratic	0.9996	12.0
Monocrotophos	7.258	192.0 → 127.0	0.1	–	1,000	Quadratic	0.9998	7.4
Dicrotofos	7.264	193.0 → 127.1	0.5	–	1,000	Quadratic	0.9998	10.1
Sulfotep	7.349	321.8 → 201.9	0.05	–	1,000	Quadratic	0.9997	10.4
Bromoxynil	7.395	276.8 → 88.0	0.05	–	1,000	Quadratic	0.9997	7.1
Promecarb	7.399	135.1 → 115.1	2	–	1,000	Linear	0.9967	16.1
Cadusafos	7.405	158.8 → 97.0	0.01	–	1,000	Quadratic	0.9997	13.1
Phorate	7.475	121.0 → 47.0	0.5	–	1,000	Linear	0.9983	11.1
BHC-alpha (Benzene Hexachloride)	7.609	218.9 → 183.0	0.01	–	1,000	Quadratic	0.9998	9.9
Desmedipham	7.690	181.0 → 122.0	2	–	1,000	Linear	0.9985	11.8
Hexachlorobenzene	7.741	283.8 → 213.9	0.01	–	1,000	Quadratic	0.9996	10.7
Dichloran	7.771	160.1 → 124.1	0.01	–	1,000	Linear	0.9996	6.7
Dimethoate	7.781	87.0 → 46.0	0.01	–	1,000	Linear	0.9997	11.8
Pentachloroanisole	7.797	279.9 → 236.8	0.05	–	1,000	Linear	0.9993	8.0

Name	RT	Transition	Calibration Range (ppb)			CF	CF R^2	Relative Standard Error
Propazine	7.933	229.1 → 58.1	0.01	–	1,000	Linear	0.9988	13.5
BHC-beta	8.010	218.9 → 183.1	0.01	–	1,000	Linear	0.9994	12.2
DMST (Tolylfluanid Metabolite)	8.032	214.0 → 106.0	2	–	1,000	Quadratic	0.9954	17.7
Propetamphos	8.079	138.0 → 64.0	0.1	–	1,000	Linear	0.9988	6.9
Profluralin	8.087	318.1 → 199.1	0.05	–	1,000	Quadratic	0.9997	12.7
BHC-gamma (Lindane, Gamma HCH)	8.119	216.9 → 181.0	0.01	–	1,000	Linear	0.9981	15.6
Cyanophos	8.135	242.9 → 109.0	0.05	–	1,000	Linear	0.9993	12.8
Terbufos	8.137	230.9 → 129.0	0.1	–	1,000	Linear	0.9994	12.5
Pentachloronitrobenzene	8.195	141.9 → 106.9	0.01	–	1,000	Quadratic	0.9996	9.4
Fonofos	8.223	246.1 → 109.0	0.01	–	500	Linear	0.9981	11.9
Diazinon	8.264	137.1 → 84.0	0.05	–	1,000	Quadratic	0.9997	17.0
Pyrimethanil	8.269	198.0 → 118.1	0.01	–	500	Linear	0.9990	12.6
Fluchloralin	8.299	325.8 → 62.9	0.05	–	500	Quadratic	0.9995	13.5
Phosphamidon I	8.339	127.0 → 95.0	0.5	–	500	Linear	0.9981	17.4
Dinitramine	8.382	260.7 → 241.0	0.05	–	1,000	Quadratic	0.9997	11.6
Tefluthrin	8.400	177.1 → 127.1	0.01	–	1,000	Linear	0.9986	16.0
Paraoxon-methyl	8.411	229.9 → 106.1	0.05	–	500	Linear	0.9947	18.3
BHC-delta	8.489	219.0 → 183.1	0.5	–	1,000	Quadratic	0.9998	16.5
Isazofos	8.504	256.9 → 162.0	0.01	–	1,000	Quadratic	0.9997	13.8
Etrimfos	8.523	292.0 → 153.1	0.01	–	500	Linear	0.9985	16.7
Triallate	8.540	268.0 → 184.1	0.05	–	1,000	Linear	0.9995	10.8
Chlorothalonil	8.568	265.9 → 168.0	0.1	–	500	Quadratic	0.9971	10.7
Iprobenfos	8.673	203.9 → 91.0	0.01	–	1,000	Linear	0.9986	13.9
Formothion	8.763	124.9 → 47.0	0.1	–	1,000	Quadratic	0.9997	16.0
Bromocyclen	8.764	271.8 → 236.9	0.1	–	1,000	Linear	0.9996	8.6
Pentachloroaniline	8.897	158.0 → 123.0	0.5	–	1,000	Linear	0.9986	14.7
Desmetryn	8.916	213.0 → 58.1	0.05	–	1,000	Linear	0.9972	11.7
Dichlofenthion	8.961	279.0 → 223.0	0.01	–	500	Linear	0.9975	11.4
Propanil	8.980	161.0 → 99.0	0.01	–	1,000	Linear	0.9983	16.8
2,4,4'-Trichlorobiphenyl (BZ #28)	9.030	256.0 → 186.0	0.05	–	1,000	Linear	0.9981	11.7
Malaoxon	9.103	126.9 → 99.0	2	–	1,000	Quadratic	0.9989	15.0
Vinclozolin	9.128	187.0 → 124.0	0.05	–	1,000	Linear	0.9962	15.8
Transfluthrin	9.129	163.1 → 143.1	0.1	–	1,000	Linear	0.9972	14.3
Parathion-methyl	9.151	262.9 → 109.0	0.5	–	500	Quadratic	0.9991	18.2
Chlorpyrifos-methyl	9.151	288.0 → 93.0	0.05	–	1,000	Quadratic	0.9995	12.0
Cymiazole	9.213	218.0 → 144.1	2	–	1,000	Linear	0.9986	12.3
Tolclofos-methyl	9.242	267.0 → 93.0	0.5	–	1,000	Linear	0.9987	12.8
Alachlor	9.280	237.0 → 160.1	1	–	1,000	Linear	0.9969	12.2
Fuberidazole	9.306	184.0 → 156.2	5	–	500	Linear	0.9921	19.5
Heptachlor	9.330	271.7 → 236.9	0.1	–	200	Linear	0.9990	15.4
Prometryn	9.339	241.0 → 58.2	5	–	200	Quadratic	0.9967	19.4
Paraoxon	9.383	148.9 → 119.0	50	–	1,000	Quadratic	0.9982	11.3
Ronnel	9.411	286.9 → 272.0	1	–	1,000	Linear	0.9989	11.7
Prosulfocarb	9.424	251.0 → 128.2	0.1	–	1,000	Quadratic	0.9995	14.9
Octachlorodipropyl Ether	9.431	129.9 → 94.9	0.5	–	1,000	Quadratic	0.9996	10.0
Pirimiphos-methyl	9.610	290.0 → 125.0	0.01	–	1,000	Quadratic	0.9996	9.3

Name	RT	Transition	Calibration Range (ppb)			CF	CF R^2	Relative Standard Error
2,2',5,5'-Tetrachlorobiphenyl (BZ #52)	9.617	289.9 → 219.9	0.01	–	1,000	Linear	0.9997	11.9
Fenitrothion	9.622	125.1 → 47.0	0.01	–	1,000	Linear	0.9994	11.3
Methiocarb	9.628	168.0 → 109.1	2	–	1,000	Quadratic	0.9998	11.3
Dipropetryn	9.748	255.1 → 222.1	0.01	–	500	Linear	0.9982	13.5
Malathion	9.759	172.9 → 99.0	0.01	–	1,000	Quadratic	0.9997	12.1
Ioxynil	9.780	370.8 → 117.0	0.05	–	1,000	Linear	0.9982	12.2
Dichlofluanid	9.785	167.0 → 97.0	1	–	500	Quadratic	0.9991	13.8
Metolachlor	9.913	238.0 → 162.2	0.01	–	1,000	Linear	0.9993	12.5
Phorate Sulfone	9.914	199.0 → 97.0	0.1	–	500	Linear	0.9975	11.7
Aldrin	9.932	254.9 → 220.0	0.05	–	1,000	Linear	0.9995	8.8
Anthraquinone	9.941	208.0 → 152.2	0.05	–	1,000	Linear	0.9990	19.1
Chlorpyrifos	9.968	313.8 → 257.8	0.05	–	1,000	Linear	0.9994	11.7
Parathion	9.983	291.0 → 109.0	0.01	–	1,000	Linear	0.9985	15.2
Flufenacet	10.004	151.0 → 95.0	0.5	–	1,000	Linear	0.9994	11.9
Nitrothal-isopropyl	10.057	254.0 → 212.0	0.5	–	500	Linear	0.9967	16.3
DCPA (Dacthal, Chlorthal-dimethyl)	10.068	298.9 → 221.0	0.01	–	1,000	Linear	0.9993	14.1
Isocarbophos	10.114	136.0 → 69.0	0.5	–	1,000	Linear	0.9985	13.1
Chlorthion	10.156	125.1 → 47.1	0.05	–	1,000	Quadratic	0.9995	14.7
Isobenzan	10.186	274.7 → 240.0	0.1	–	1,000	Linear	0.9992	11.2
Trichloronat	10.199	296.8 → 268.9	0.01	–	1,000	Linear	0.9987	14.5
Fenson	10.210	141.0 → 77.1	0.01	–	1,000	Linear	0.9996	6.7
Bromophos	10.294	330.9 → 315.9	0.01	–	1,000	Linear	0.9986	15.8
Pirimiphos-ethyl	10.294	318.1 → 166.1	0.05	–	1,000	Linear	0.9977	14.8
Fosthiazate I	10.299	195.0 → 103.0	0.05	–	1,000	Quadratic	0.9997	12.1
Isopropalin	10.350	280.1 → 238.1	0.01	–	500	Linear	0.9975	16.4
Cyprodinil	10.413	225.2 → 224.3	0.05	–	1,000	Linear	0.9990	14.4
Isofenphos-methyl	10.420	199.0 → 121.0	0.01	–	1,000	Quadratic	0.9995	9.9
Isodrin	10.442	193.0 → 123.0	0.05	–	1,000	Linear	0.9985	10.9
Pendimethalin	10.522	251.8 → 162.2	0.05	–	1,000	Quadratic	0.9995	11.5
Terbufos Sulfone	10.573	264.0 → 199.0	0.05	–	1,000	Quadratic	0.9996	8.7
Chlozolinate	10.586	186.0 → 109.0	0.1	–	1,000	Quadratic	0.9995	8.6
Heptachlor Exo-epoxide	10.616	354.8 → 264.9	0.01	–	1,000	Linear	0.9993	15.3
Esbiothrin	10.622	123.0 → 93.0	2	–	1,000	Linear	0.9975	15.2
Bioallethrin	10.629	123.0 → 81.0	2	–	1,000	Linear	0.9924	12.2
Chlordane-oxy	10.629	114.9 → 51.1	0.5	–	1,000	Linear	0.9996	3.8
Tolylfluanid	10.639	238.0 → 137.0	0.05	–	1,000	Quadratic	0.9996	14.6
Isofenphos	10.674	212.9 → 121.1	0.01	–	1,000	Quadratic	0.9994	10.7
Mecarbam	10.675	130.9 → 86.0	0.5	–	1,000	Quadratic	0.9992	13.0
Chlorfenvinphos	10.679	266.9 → 159.0	0.01	–	200	Linear	0.9986	11.0
Heptachlor Endo-epoxide	10.683	135.0 → 99.0	0.5	–	1,000	Linear	0.9993	6.6
Fipronil	10.698	366.8 → 212.8	0.05	–	1,000	Quadratic	0.9997	8.8
Captan	10.738	149.0 → 70.0	1	–	500	Linear	0.9973	18.9
Quinalphos	10.738	298.0 → 156.0	0.01	–	500	Quadratic	0.9992	17.6
Phenthoate	10.741	274.0 → 125.0	0.01	–	1,000	Quadratic	0.9988	8.1
Dinobuton	10.743	211.0 → 163.0	0.5	–	1,000	Quadratic	0.9999	9.0
Procymidone	10.850	282.8 → 96.0	0.01	–	1,000	Linear	0.9996	8.3

Name	RT	Transition	Calibration Range (ppb)			CF	CF R^2	Relative Standard Error
Folpet	10.851	259.8 → 130.1	0.5	–	200	Linear	0.9989	16.4
Chlorbenside	10.904	125.0 → 89.0	0.01	–	1,000	Linear	0.9993	8.2
Methidathion	11.007	125.0 → 47.0	0.5	–	1,000	Linear	0.9993	16.2
Bromophos-ethyl	11.022	358.7 → 302.8	0.01	–	1,000	Linear	0.9995	9.1
Chlordane-*trans*	11.024	271.7 → 236.9	0.05	–	1,000	Linear	0.9995	8.4
DDE-o,p'	11.073	246.0 → 176.2	0.01	–	1,000	Linear	0.9995	6.4
2,2',4,5,5'-Pentachlorobiphenyl (BZ #101)	11.111	325.9 → 255.9	0.01	–	1,000	Linear	0.9995	7.7
Tetrachlorvinphos	11.166	329.0 → 108.9	0.01	–	1,000	Linear	0.9972	16.9
Chlordane-*cis*	11.288	372.8 → 265.9	0.01	–	1,000	Linear	0.9994	15.4
Endosulfan I (Alpha Isomer)	11.290	194.9 → 160.0	0.1	–	1,000	Linear	0.9993	14.8
Ditalimfos	11.299	242.9 → 148.1	0.05	–	500	Linear	0.9977	14.8
Picoxystrobin	11.307	145.0 → 102.1	0.05	–	1,000	Linear	0.9994	14.1
Flutriafol	11.335	123.1 → 75.1	0.05	–	1,000	Linear	0.9995	11.9
Fenamiphos	11.360	303.0 → 154.0	0.1	–	500	Linear	0.9959	17.1
Nonachlor, *trans*-	11.369	406.8 → 299.8	0.05	–	1,000	Linear	0.9993	11.9
Chlorfenson	11.374	175.0 → 111.0	0.01	–	1,000	Linear	0.9994	10.2
Iodofenphos	11.466	376.8 → 361.8	0.05	–	1,000	Quadratic	0.9998	12.4
Prothiofos	11.488	308.9 → 238.9	0.05	–	500	Linear	0.9975	16.8
Isoprothiolane	11.498	162.1 → 85.0	0.01	–	1,000	Linear	0.9986	13.4
Flubenzimine	11.538	186.0 → 69.0	2	–	500	Quadratic	0.9974	10.5
Profenofos	11.544	207.9 → 63.0	0.1	–	200	Linear	0.9984	12.4
DDE-p,p'	11.613	246.1 → 176.2	0.01	–	1,000	Linear	0.9995	6.8
Dieldrin	11.713	262.9 → 193.0	0.5	–	1,000	Linear	0.9990	13.8
Oxyfluorfen	11.721	252.0 → 146.0	0.01	–	1,000	Quadratic	0.9995	14.0
Myclobutanil	11.750	179.0 → 125.1	0.01	–	1,000	Quadratic	0.9995	12.6
DDD-o,p'	11.783	235.0 → 165.1	0.01	–	1,000	Linear	0.9993	12.7
Methoprotryne	11.788	256.0 → 212.1	0.01	–	1,000	Quadratic	0.9996	12.6
Azaconazole	11.865	217.0 → 173.1	0.01	–	1,000	Linear	0.9994	11.4
Dibromobenzophenone, 4,4'-	11.913	340.0 → 183.0	0.05	–	1,000	Quadratic	0.9997	7.6
Isoxathion	11.941	313.0 → 177.0	0.05	–	1,000	Quadratic	0.9993	14.9
Binapacryl	12.003	100.0 → 82.0	5	–	1,000	Quadratic	0.9988	18.9
Nitrofen	12.011	282.9 → 253.0	0.01	–	500	Linear	0.9963	14.5
Ethylan	12.041	223.1 → 193.1	0.05	–	500	Linear	0.9976	14.7
Chlorfenapyr	12.051	328.0 → 247.0	0.5	–	1,000	Linear	0.9983	14.8
Endrin	12.108	262.8 → 193.0	0.5	–	1,000	Linear	0.9996	5.5
Carbophenothion-methyl	12.167	125.0 → 47.0	0.1	–	1,000	Linear	0.9986	19.2
Chloropropylate	12.187	139.0 → 75.0	0.01	–	1,000	Linear	0.9996	7.2
2,3',4,4',5-Pentachlorobiphenyl (BZ #118)	12.222	325.9 → 255.9	0.01	–	1,000	Linear	0.9996	7.6
Endosulfan II (Beta Isomer)	12.274	206.9 → 172.0	0.1	–	1,000	Quadratic	0.9989	13.0
Fensulfothion	12.284	293.0 → 97.0	0.01	–	1,000	Linear	0.9978	14.8
Flamprop-isopropyl	12.305	276.0 → 105.1	0.05	–	1,000	Linear	0.9991	10.4
DDD-p,p'	12.369	237.0 → 165.1	0.01	–	1,000	Linear	0.9993	13.7
Aclonifen	12.397	264.1 → 194.2	0.1	–	1,000	Quadratic	0.9995	10.3
DDT-o,p'	12.430	235.0 → 199.1	0.01	–	1,000	Linear	0.9974	19.4
Ethion	12.431	231.0 → 129.0	0.01	–	1,000	Linear	0.9962	19.9

Name	RT	Transition	Calibration Range (ppb)			CF	CF R^2	Relative Standard Error
Chlorthiophos	12.484	268.9 → 205.1	0.05	–	1,000	Linear	0.9983	13.9
Tetrasul	12.572	321.7 → 252.0	0.01	–	1,000	Linear	0.9987	11.5
2,2',4,4',5,5'-Hexachlorobiphenyl (BZ #153)	12.610	359.9 → 289.9	0.01	–	1,000	Linear	0.9992	10.9
Sulprofos	12.650	322.0 → 156.0	0.01	–	500	Linear	0.9975	12.5
Triazophos	12.662	161.2 → 134.2	1	–	500	Quadratic	0.9995	15.0
Famphur	12.810	218.0 → 109.0	2	–	1,000	Linear	0.9982	14.6
Carbophenothion	12.826	342.0 → 157.0	0.05	–	500	Linear	0.9974	14.5
Methoxychlor Olefin	12.837	308.0 → 238.0	0.01	–	1,000	Linear	0.9984	13.5
Cyanofenphos	12.906	169.0 → 77.1	0.1	–	1,000	Linear	0.9988	10.7
Edifenphos	12.940	309.9 → 172.9	0.5	–	1,000	Quadratic	0.9998	13.3
DDT-p,p'	13.027	235.0 → 165.2	0.01	–	1,000	Linear	0.9976	19.9
Endosulfan Sulfate	13.032	271.9 → 237.0	0.5	–	1,000	Quadratic	0.9997	18.2
2,2',3,4,4',5'-Hexachlorobiphenyl (BZ #138)	13.118	359.9 → 289.9	0.01	–	1,000	Linear	0.9996	6.5
Diclofop-methyl	13.284	339.9 → 252.9	0.01	–	500	Linear	0.9973	13.1
Diflufenican	13.310	266.0 → 246.1	0.01	–	1,000	Linear	0.9971	18.7
Propargite	13.327	231.0 → 135.0	0.5	–	1,000	Linear	0.9993	13.7
Piperonyl Butoxide	13.380	176.1 → 103.1	0.1	–	1,000	Quadratic	0.9995	9.0
Captafol	13.440	310.8 → 78.8	10	–	1,000	Quadratic	0.9987	19.3
Nitralin	13.551	315.9 → 274.0	0.1	–	500	Quadratic	0.9993	13.9
Mefenpyr-diethyl	13.608	253.0 → 189.0	0.01	–	500	Quadratic	0.9989	15.8
Benzoylprop-ethyl	13.699	292.0 → 105.0	0.1	–	1,000	Linear	0.9991	11.8
Iprodione	13.721	313.8 → 55.9	0.1	–	500	Linear	0.9984	12.4
Spiromesifen	13.722	272.0 → 254.2	0.1	–	500	Quadratic	0.9994	15.7
Tetramethrin I	13.814	164.0 → 77.1	5	–	1,000	Quadratic	0.9990	17.1
Pyridaphenthion	13.822	340.0 → 199.0	0.05	–	1,000	Quadratic	0.9996	12.9
Endrin Ketone	13.876	316.9 → 101.0	0.01	–	500	Quadratic	0.9993	13.6
Dimoxystrobin	13.880	205.0 → 58.0	0.1	–	1,000	Quadratic	0.9996	8.4
Phosmet	13.917	160.0 → 77.1	2	–	1,000	Quadratic	0.9993	10.6
Bifenthrin	13.922	181.2 → 165.2	0.1	–	500	Quadratic	0.9990	13.8
Bromopropylate	13.928	338.8 → 182.9	0.05	–	1,000	Quadratic	0.9993	10.7
EPN	13.935	169.0 → 77.1	0.05	–	1,000	Quadratic	0.9991	13.3
Picolinafen	13.958	376.0 → 238.1	0.01	–	200	Linear	0.9978	17.1
Bifenazate	13.975	168.1 → 61.9	10	–	1,000	Quadratic	0.9990	6.6
Dicofol, p, p'-	13.976	183.9 → 141.2	1	–	1,000	Linear	0.9972	18.8
Fenpropathrin	14.056	265.0 → 89.0	0.01	–	1,000	Quadratic	0.9996	13.5
2,2',3,4,4',5,5'-Heptachlorobiphenyl (BZ #180)	14.299	393.8 → 323.8	0.01	–	1,000	Linear	0.9992	12.2
Phenothrin I	14.399	122.9 → 81.1	0.1	–	1,000	Linear	0.9987	11.5
Tetradifon	14.424	158.9 → 111.0	0.01	–	1,000	Linear	0.9992	7.4
Furathiocarb	14.437	163.1 → 135.1	2	–	1,000	Linear	0.9992	6.1
Phosalone	14.590	182.0 → 75.0	0.05	–	500	Linear	0.9958	19.2
Azinphos-methyl	14.626	160.0 → 77.0	2	–	1,000	Quadratic	0.9993	11.4
Leptophos	14.638	171.0 → 51.0	0.05	–	1,000	Quadratic	0.9995	12.4
Cyhalothrin (Lambda)	14.698	181.1 → 152.1	5	–	1,000	Quadratic	0.9979	12.9
Cyhalofop-butyl	14.703	357.1 → 229.1	0.01	–	500	Linear	0.9958	16.1

Name	RT	Transition	Calibration Range (ppb)			CF	CF R^2	Relative Standard Error
Tralkoxydim	14.830	137.0 → 57.0	0.05	–	1,000	Linear	0.9990	7.9
Mirex	14.865	271.8 → 236.8	0.01	–	1,000	Linear	0.9994	13.5
Acrinathrin	15.045	247.0 → 68.0	1	–	1,000	Quadratic	0.9996	12.8
Pyrazophos	15.144	221.0 → 149.0	0.01	–	1,000	Quadratic	0.9994	14.1
Azinphos-ethyl	15.228	160.0 → 77.1	0.5	–	1,000	Quadratic	0.9997	12.4
Cycloxydim (Focus)	15.500	178.0 → 80.9	0.1	–	1,000	Quadratic	0.9997	8.0
Permethrin, (1R)-*cis*-	15.622	163.0 → 91.0	2	–	1,000	Quadratic	0.9978	18.4
Permethrin, (1R)-*trans*-	15.744	163.0 → 127.0	0.01	–	1,000	Linear	0.9990	12.0
Coumaphos	15.880	361.9 → 109.0	0.05	–	500	Linear	0.9972	16.0
Dioxathion	15.963	271.0 → 96.9	0.1	–	500	Linear	0.9969	19.8
Butafenacil	15.988	331.0 → 180.0	0.01	–	500	Linear	0.9973	14.4
Cyfluthrin I	16.202	163.0 → 127.0	0.5	–	1,000	Linear	0.9980	15.8
Cypermethrin I	16.510	163.0 → 127.0	0.1	–	1,000	Linear	0.9985	16.6
Halfenprox	16.565	262.9 → 169.0	0.05	–	1,000	Quadratic	0.9994	11.9
Flucythrinate I	16.725	156.9 → 107.1	0.01	–	1,000	Linear	0.9992	13.7
Ethofenprox	16.798	163.0 → 107.1	0.1	–	1,000	Linear	0.9989	14.3
Silafluofen	16.944	286.0 → 207.0	0.1	–	1,000	Quadratic	0.9995	9.7
Fenvalerate I	17.428	167.0 → 125.1	0.05	–	1,000	Linear	0.9988	12.6
Fluvalinate-tau I	17.601	250.0 → 200.0	0.1	–	1,000	Quadratic	0.9996	15.2
Deltamethrin	18.152	252.9 → 174.0	0.1	–	500	Linear	0.9963	17.2

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DE28615044

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Printed in the USA, May 16, 2024
5994-7436EN

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Application Note

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Qualitative Analysis of Essential Oils Using GC/MS with Hydrogen Carrier Gas and the Agilent HydroInert Source

Author

Bruce D. Quimby
Agilent Technologies, Inc.

Abstract

Due to ongoing concerns with the price and availability of helium (He), many laboratories are looking for alternative carrier gases for their gas chromatography/mass spectrometry (GC/MS) methods. This application note describes the conversion of a typical GC/MS method for the qualitative analysis of flavor and fragrance compounds in essential oils from helium to hydrogen (H_2). The Agilent 8890 GC coupled with the Agilent 5977C GC/MSD system were used with hydrogen carrier gas and a new source that has been optimized for hydrogen operation—the Agilent HydroInert source. Unlike most conventional electron ionization (EI) sources, the HydroInert source provides excellent mass spectral fidelity for flavor compounds when using hydrogen. To further increase confidence in compound identification, deconvoluted mass spectra and linear retention indexes (RI) from Agilent MassHunter Unknowns Analysis software were searched against the NIST23 mass spectral library. Using the Agilent Method Translator tool, a column and chromatographic conditions for hydrogen were chosen that allowed reduction of the analysis time by a factor of 2.5 compared to the typical helium method. By proper selection of instrument configuration and operating conditions, the system with hydrogen carrier gas can generate results comparable to those with helium, but with significantly reduced run time.

Introduction

Essential oils are widely used as a source of flavors and fragrances in both food and nonfood consumer products. Quality control analysis of essential oils has long been challenging due to the hundreds of terpenes and terpenoid compounds that can be present in the oils. To address this, methods using high-resolution capillary GC combined with MS have typically been employed. Searching the acquired mass spectra against libraries of flavor and fragrance compounds can be performed for identification, but is usually insufficient because many compounds, especially isomers, give similar spectra. For this reason, RIs are often used as a complement to spectral matching for more dependable identifications. The measured RI of an unknown is used in conjunction with the results of the spectral library search to determine the best candidate for identification.

This application note describes the conversion of a typical GC/MS method for the qualitative analysis of flavor and fragrance compounds in essential oils from helium to hydrogen. The two methods are then applied to the analysis of two common essential oils—orange oil, of Brazilian origin, and neroli oil—for comparison. The hydrogen method was further evaluated using different EI source components to determine the optimal source for maintaining spectral fidelity.

The conversion of a method from helium to hydrogen carrier gas requires consideration of the chromatographic parameters such as column choice, column flow, amount injected, and temperature program rates.[1] In addition, it is important to consider the MS parameters of column flow rate and MS source configuration. All these considerations and parameters are included in the Agilent EI GC/MS Instrument Helium to Hydrogen Carrier Gas Conversion User Guide.[1]

When determining the chromatographic parameters for the hydrogen method, it is desirable to have a method that has:

- Column dimensions that result in a high enough inlet pressure for accurate flow control
- Similar chromatographic resolution to the original helium method
- Maintenance of the same analyte elution order as the original helium method
- Use of a column flow near the optimum for the column diameter used
- Use of a column flow near the optimum for MS source sensitivity

The Agilent Method Translator tool[2-4] is a calculator designed to greatly simplify this process. It was used in this application note.

For the MS parameters, the column flow rate should be kept within or near the optimum range of approximately 0.8 to 1.4 mL/min to maximize the MS response. Another important consideration is the choice of MS EI source hardware. The concern is that some analytes undergo reactions with hydrogen in the source, changing their ion ratios and spectra, thus reducing their library match scores (LMS), and possibly resulting in misidentification. With the Agilent inert extractor source used in the Agilent 5977 series GC/MSD systems, this effect has been reduced in the past using an extractor lens with a larger diameter, such as 9 mm. However, this only partially addressed the problem, as many compounds such as nitro compounds and some terpenes and terpenoids still exhibited reactions. For this reason, Agilent developed the HydroInert source[5,6], which greatly reduces or eliminates these reactions. To evaluate the effectiveness of the HydroInert source, the qualitative analysis of the two oils was carried out with the helium method using the standard 3 mm inert extractor source and the hydrogen method using the standard HydroInert source, which is equipped by default with the 9 mm lens. In addition, the oils were analyzed with the hydrogen method and a conventional inert extractor source using both the 3 and 9 mm extractor lenses for comparison.

The next consideration is how to process the data files to obtain the mass spectrum of each oil component and search it against a library. In the past, this was done largely by obtaining the apex or average spectrum over the peak then subtracting a baseline spectrum taken next to the peak. The resulting spectrum was then searched against the spectral library. While this process is effective for handling a few peaks that were relatively well resolved, it becomes overwhelming with large numbers of peaks and/or overlapping peaks.

Fortunately, there is now a powerful solution for mass spectral identification called Agilent MassHunter Unknowns Analysis (MHUA), which is part of the Agilent MassHunter Quantitative Analysis software suite. MHUA uses spectral deconvolution to extract clean analyte spectra from the complex overlapping peaks. The deconvolution and library search processes are automated and take approximately 1 to 8 minutes per data file depending on the file size, library size, computer hardware, and so on. The result is cleaner spectra than with the previous approach, which therefore results in higher LMS and greater confidence in peak identifications.[7]

A second major feature of MHUA is the ability to calculate the RI for each peak. If the searched library has the appropriate reference RI values associated with spectral entries, the measured RI value for an unknown spectrum can be used to filter the spectral search results. This is especially important in the identification of essential oil components because of spectral similarities. RI values were used in this application note for this reason.

There are multiple mass spectral libraries available with RIs for flavor and fragrance compounds. For example, the Adams library[8] has been used widely for many years for this analysis. Recently, NIST released a newer version of their mass spectral library (NIST23), which has numerous enhancements. Among them are the incorporation of the entire Adams library and the expansion of semistandard, nonpolar RI entries to cover all EI spectra. "Semistandard, nonpolar" refers to phases such as HP-5, DB-5, HP-5ms, and other 5% phenylmethyl silicone phases.[9] The new RI values are either experimental values, if available, or artificial intelligence (AI)-generated values. Note that the AI-generated values have better accuracy than the previous "estimated" values. The new semistandard, nonpolar values are of specific interest here because this type of stationary phase is commonly used in essential oil analysis with GC/MS. Also, these are the NIST23 values currently usable with the RI function in MHUA. When the RI function of MHUA is used, the experimental semistandard, nonpolar RI values are used if available; if not, the AI-generated values are used. Therefore, NIST23 is the library used here.

Experimental

Column selection

For the reference helium method, the column and conditions chosen are like those frequently used in the past.[8,10]
A 30 m × 0.25 mm id, 0.25 µm Agilent J&W HP-5ms Ultra Inert (UI) column (part number 19091S-433UI) was used with an oven temperature program from 60 to 240 °C at 3 °C/min. Although some of the older methods[10] used constant pressure control mode for column flow, constant flow mode is far better in terms of MS performance. A constant column flow rate of 1.0 mL/min of helium was used.

For the hydrogen method, a 20 m × 0.18 mm id, 0.18 µm Agilent J&W HP-5ms UI column (part number 19091S-577UI) was chosen. This column makes an excellent choice for several reasons:

- The column dimensions provide an inlet pressure with hydrogen that is high enough for accurate flow control.
- The column provides similar or better chromatographic resolution compared to the helium method.
- The phase ratio is the same, helping maintain the same analyte elution order as the original method.
- A column flow of hydrogen near the optimum for both chromatographic separation and MS source sensitivity can be used.

Method translation

The Method Translator tool is included as part of the Agilent MassHunter acquisition software or can be downloaded for standalone use from Agilent.com: https://www.agilent.com/en/support/gas-chromatography/gccalculators. The free download includes the Method Translator, Vapor Volume Calculator, Pressure Flow Calculator, and Solvent Vent Calculator tools, which are all useful when developing GC methods.

After installation, the Method Translator is opened from an icon on the desktop. The opened Method Translator is shown in Figure 1. First, the chromatographic parameters of the original helium method are entered in the left column labeled Original Method Parameters. The carrier gas type (He), column dimensions, column outlet pressure, and oven temperature program ramp should be entered first. The column outlet flow is then entered. As shown in Figure 1, the other values such as phase ratio, inlet pressure, and so on, are calculated automatically.

Next, the carrier gas type (H_2), column dimensions, and column outlet pressure for the hydrogen method are entered in the right side of the calculator under the Calculated Method Parameters column.

After entry, the calculated hydrogen parameters are displayed. In the upper-left corner, the calculated speed gain is shown as 2.5877, meaning that the predicted retention times (RTs) with the hydrogen method would be a factor of approximately 2.6 smaller than the helium method. The calculated oven

ramp rate for hydrogen would be 7.763 °C/min. Note that it would be easier to have the oven ramp rate and the speed gain closer to 7.5 and 2.5, respectively. This can be done by selecting **Speed gain** and entering 2.5 into the field. The parameters are recalculated, resulting in the desired parameters. Figure 1 shows the results. Note that the calculated flow for the hydrogen method shown in Figure 1 is 0.84004 mL/min. Before using the method, retention time locking (RTL) was used to make the RT of n-pentadecane in the hydrogen method precisely 2.5 times faster than that with the helium method. This made comparison of RTs easier. The resulting flow for the hydrogen method after RTL was 0.958 mL/min. For library searching, this step is not necessary as the RI calibration accounts for differences in flow.

Figure 1. Agilent Method Translator tool, used to determine method parameters for conversion of the helium method to hydrogen.

MS source hardware

The standard inert extractor source with the 3 mm extractor lens is an excellent choice for the helium method when analyzing flavor and fragrance compounds, and was used in this application note. For the hydrogen method, the HydroInert source with the 9 mm extractor lens was used, as it reduces in-source reactions with hydrogen and provides improved peak shape. The hydrogen method was also run with a conventional inert extractor source using both the 3 and 9 mm extractor lenses. These data were compared to that from the HydroInert and helium results to identify components of the oils that were most susceptible to in-source reactions by comparing their spectra and LMS values. Figure 2 shows the system configurations for the helium and hydrogen methods.

Chemicals and standards

In-house hydrogen with 99.9999% purity specification and low individual specifications on water and oxygen was used as the carrier gas for the hydrogen method. In-house helium with similar specifications was used as the carrier gas for the helium method.

Cold-pressed orange oil (Brazil origin) and neroli oil (Morocco origin) were purchased from Sigma-Aldrich (Milwaukee, WI, USA). The oils were diluted to 20:1 (v:v) in ethanol.

A custom RI calibration standard consisting of all the n-alkanes from n-C_5 to n-C_{40} plus n-C_{44} was purchased from Ultra Scientific (now Agilent). All alkanes were at a concentration of 500 ng/µL in n-hexane except n-C_{13}, n-C_{18}, n-C_{22}, n-C_{28}, n-C_{31}, and n-C_{39}, which were at 1,000 ng/µL. The standard was then diluted to 50:1 (v:v) in isooctane.

Table 1. GC and MS conditions for helium and hydrogen methods.

Method Parameters		
	Helium Method	**Hydrogen Method**
Inlet	EPC split/splitless	
Mode	Split 25:1	
Column Flow	1.0 mL/min helium	0.958 mL/min hydrogen
Injection Volume	1.0 µL	
Inlet Temperature	250 °C	
Inlet Liner	Agilent universal low pressure drop UI liner with wool (p/n 5190-2295)	
Column	Agilent J&W HP-5ms UI, 30 m × 0.25 mm, 0.25 µm (p/n 19091S-433UI)	Agilent J&W HP-5ms UI, 20 m × 0.18 mm, 0.18 µm (p/n 19091S-577UI)
Column Temperature Program	60 °C (no hold) 3 °C/min to 240 °C (no hold)	60 °C (no hold) 7.5 °C/min to 240 °C (no hold)
Run Time	60 min	24 min
MSD Source	Agilent inert extractor (3 mm lens)	Agilent HydroInert source (9 mm lens)
Transfer Line Temperature	300 °C	
Ion Source Temperature	300 °C	
Quadrupole Temperature	150 °C	
EM, Gain Mode	0.1	
Mode	Scan 40 to 400 *m/z*	
TID, A/D Samples	TID ON, 8	TID ON, 4
Solvent Delay	2.2 min	0.88 min
Tune	etune.u	

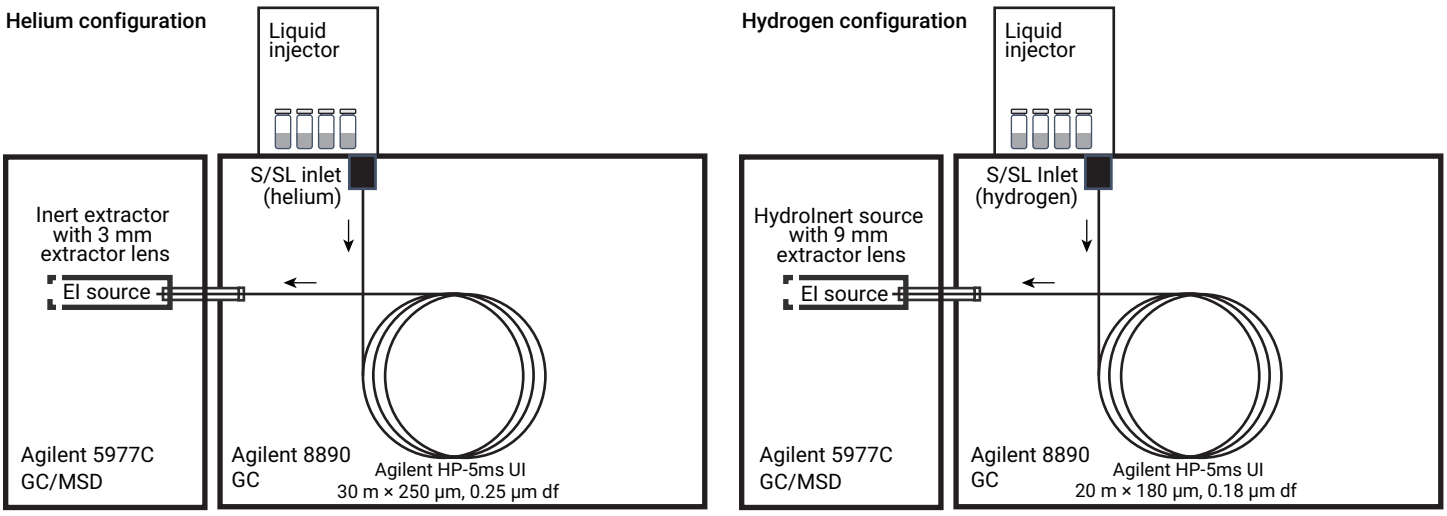

Figure 2. System configurations for the helium and hydrogen methods.

Results and discussion

Retention index calibration

The diluted RI calibration standard was run with both methods. The chromatograms are shown in Figure 3. Although the oil sample analysis is finished before n-C_{26}, the standard contains n-alkanes up to n-C_{40}. Therefore, the temperature ramps for both RI calibration methods were extended to 300 °C and held until n-C_{40} eluted to prevent carryover peaks in subsequent chromatograms. The red arrow in each chromatogram indicates the normal end of run for the oil methods. For determining the RT of the RI calibration compounds, integrating the EIC for *m/z* 57 is preferred over the total ion chromatogram (TIC), as it has a better signal-to-noise ratio.

To use RI values in MHUA, a calibration file needs to be created for each method. The file can be created as a .csv file in Microsoft Excel, or as a text file in Microsoft Windows Notepad. Figure 4 shows the calibration .rtc files created in Notepad. The blue headers are not included in the files; they are included here to indicate the entry format. Each entry consists of the format name, CAS number, RI, and RT, and the associated text file is then saved with a .rtc extension in the filename. The text files are usually saved in either the library directory or the directory containing the data files.

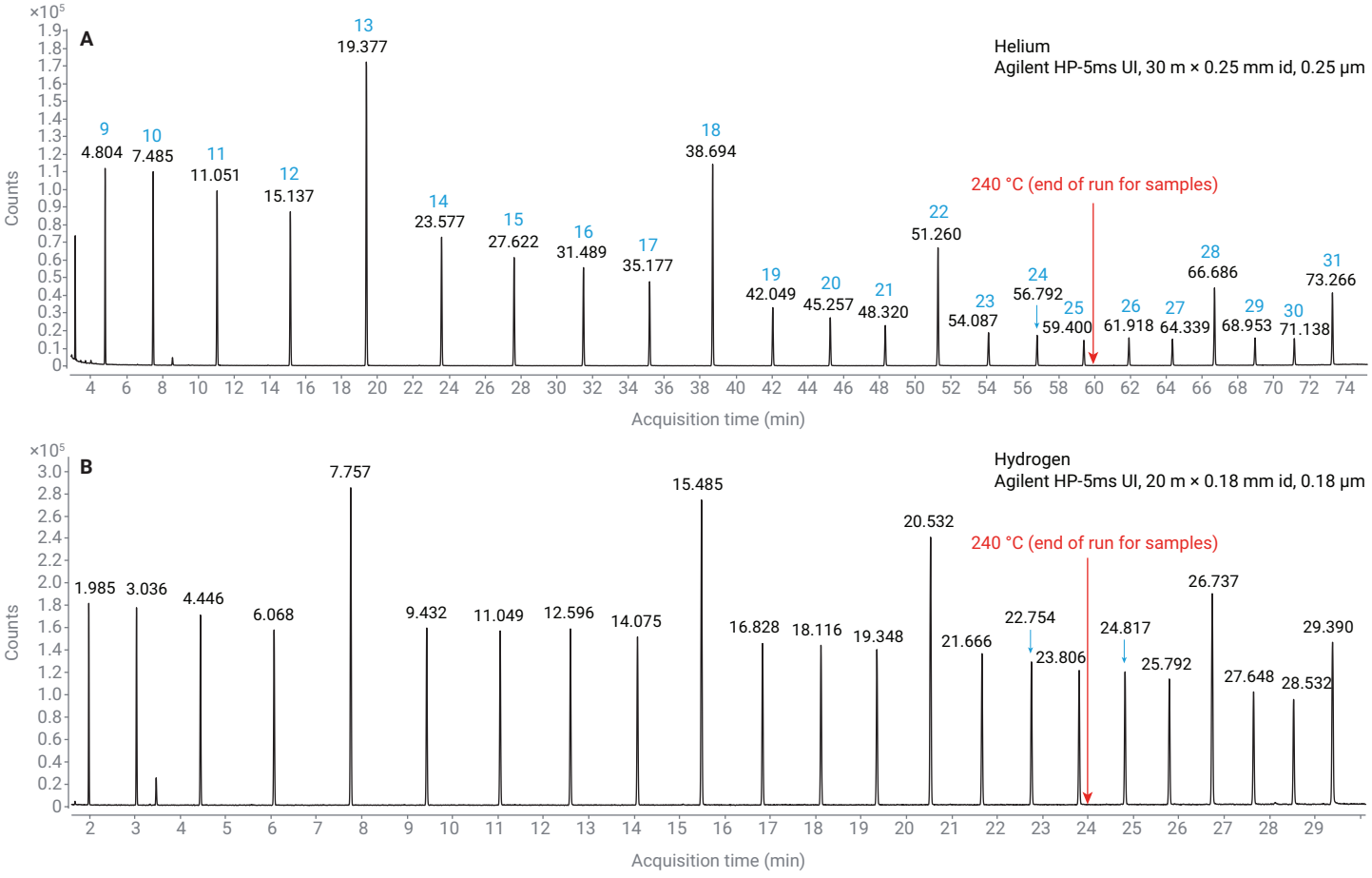

Figure 3. EICs at *m/z* 57 for the RI calibration standard. (A) Helium method; (B) hydrogen method.

Helium method

```
Name, CAS,          RI,   RT
n-C5,109-66-0 ,500,1.602
n-C6,110-54-3 ,600,1.801
n-C7,142-82-5 ,700,2.236
n-C8,111-65-9,800,3.139
n-C9,111-84-2,900,4.804
n-C10,124-18-5,1000,7.485
n-C11,1120-21-4,1100,11.050
n-C12,112-40-3,1200,15.137
n-C13,629-50-5,1300,19.377
n-C14,629-59-4,1400,23.577
n-C15,629-62-9,1500,27.622
n-C16,544-76-3,1600,31.489
n-C17,629-78-7,1700,35.117
n-C18,593-45-3,1800,38.694
n-C19,629-92-5,1900,42.049
n-C20,112-95-8,2000,45.257
n-C21,629-94-7,2100,48.320
n-C22,629-97-0,2200,51.260
n-C23,638-67-5,2300,54.087
n-C24,646-31-1,2400,56.792
n-C25,629-99-2,2500,59.400
n-C26,630-01-3,2600,61.918
n-C27,593-49-7,2700,64.339
n-C28,630-02-4,2800,66.686
n-C29,630-03-5,2900,68.953
n-C30,638-68-6,3000,71.138
```

Hydrogen method

```
Name, CAS,          RI,   RT
n-C5,109-66-0 ,500,0.694
n-C6,110-54-3 ,600,0.774
n-C7,142-82-5 ,700,0.946
n-C8,111-65-9,800,1.299
n-C9,111-84-2,900,1.985
n-C10,124-18-5,1000,3.036
n-C11,1120-21-4,1100,4.446
n-C12,112-40-3,1200,6.068
n-C13,629-50-5,1300,7.757
n-C14,629-59-4,1400,9.432
n-C15,629-62-9,1500,11.049
n-C16,544-76-3,1600,12.596
n-C17,629-78-7,1700,14.075
n-C18,593-45-3,1800,15.485
n-C19,629-92-5,1900,16.828
n-C20,112-95-8,2000,18.116
n-C21,629-94-7,2100,19.348
n-C22,629-97-0,2200,20.532
n-C23,638-67-5,2300,21.666
n-C24,646-31-1,2400,22.754
n-C25,629-99-2,2500,23.806
n-C26,630-01-3,2600,24.817
n-C27,593-49-7,2700,25.792
n-C28,630-02-4,2800,26.737
n-C29,630-03-5,2900,27.648
n-C30,638-68-6,3000,28.532
```

Figure 4. RI calibration text files (.rtc) used in Agilent MassHunter Unknowns Analysis.

Brazilian orange oil

Figure 5 compares the TICs obtained with the helium and hydrogen methods for the Brazilian orange oil sample. Figure 5A shows the complete time range of elution, and 5B is an expanded view to better compare the peak shapes and chromatographic resolution. As can be seen, by using the Method Translator technique, the relative elution order of peaks is maintained, as is the resolution. Many of the larger peaks in the hydrogen chromatogram exhibited some fronting. This was predicted by the Method Translator tool, which indicated that the hydrogen setup has 36% of the original column capacity of the helium method. However, the chromatographic resolution is still about the same as the helium method.

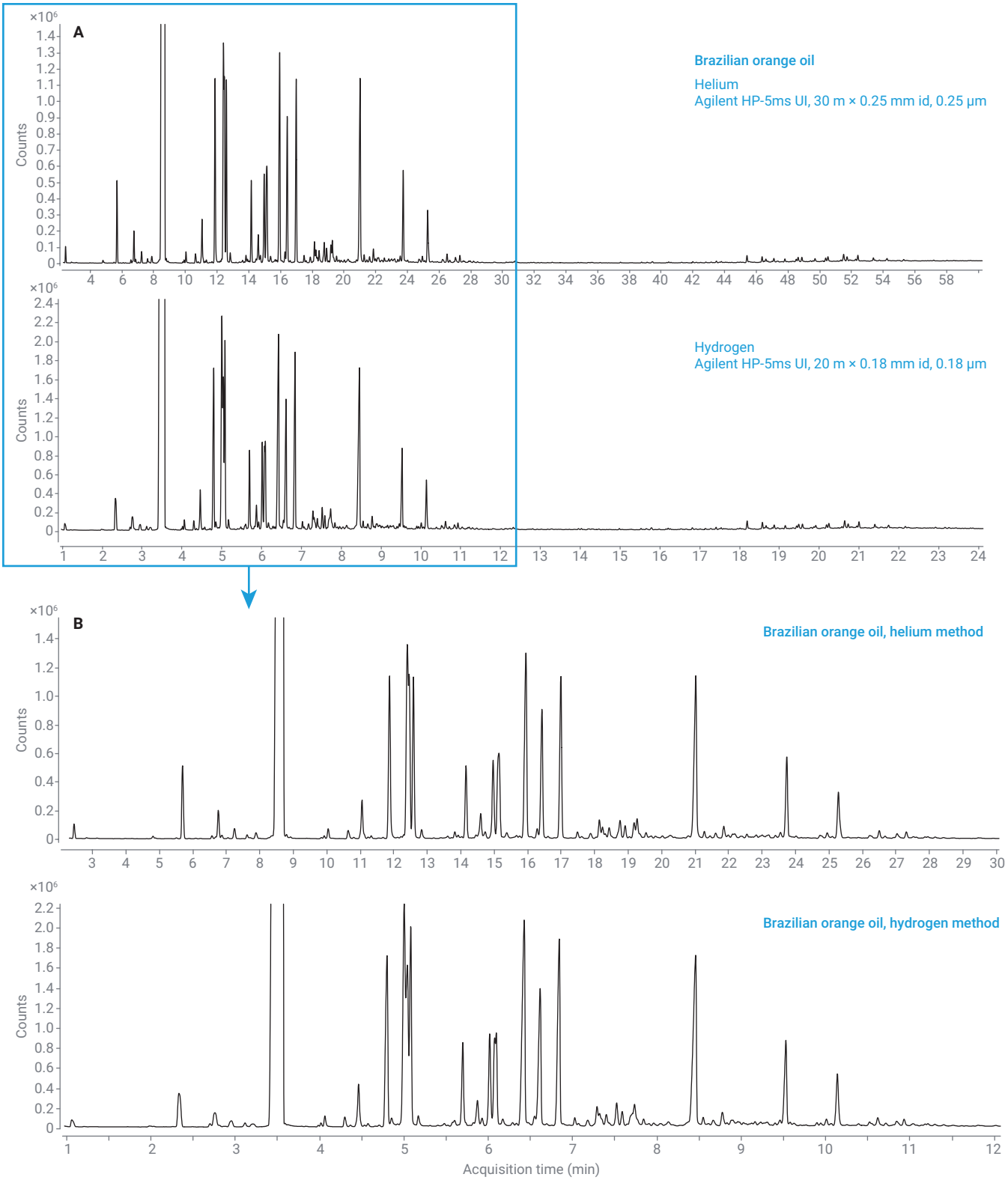

Figure 5. (A) Brazilian orange oil with helium and hydrogen methods showing full range of compound elution. (B) Expanded view of earlier elution time region.

Neroli oil

Figure 6 shows the TICs for the neroli oil run with the helium and hydrogen methods. As with the orange oil, the relative elution order of peaks is maintained, as is the resolution. The reduced column capacity of the hydrogen method is again evident in the increased fronting of the large peak at 4.6 minutes in the hydrogen chromatogram.

Peak identification with MassHunter Unknowns Analysis

The parameters used with MHUA are listed in Table 2. If the library has appropriate RI values for the spectrum entries, RIs can be used as a filter for library hits. The program uses RI if an RI calibration filename is entered in the RT calibration file box. A more detailed description for setting up and running an analysis is shown in the Appendix. Also, an excellent source of information about MHUA is available in a video on the Agilent YouTube channel.[7]

With the settings listed in Table 2 and a data file analyzed, the program will deconvolute the entire scan file and determine where each detectable peak (component) is. It will then take the deconvoluted (cleaned) spectrum of each component and search it against the library (NIST23). The library entry that best matches the spectrum of the component is checked to see if it exceeds the minimum match factor parameter of 70. If it does, it is next checked to see if the measured RI of the component falls within ± the penalty free range, in this case ± 10 seconds. With the RT mismatch penalty set to Additive and the maximum RT penalty set to 100, if the difference between the measured RT and the library RI (converted to RT) is greater than 10 seconds, the entry is completely discarded. If the difference is less than 10 seconds and the LMS is greater than 70 and higher than the other possible hits, the hit is included in the results table.

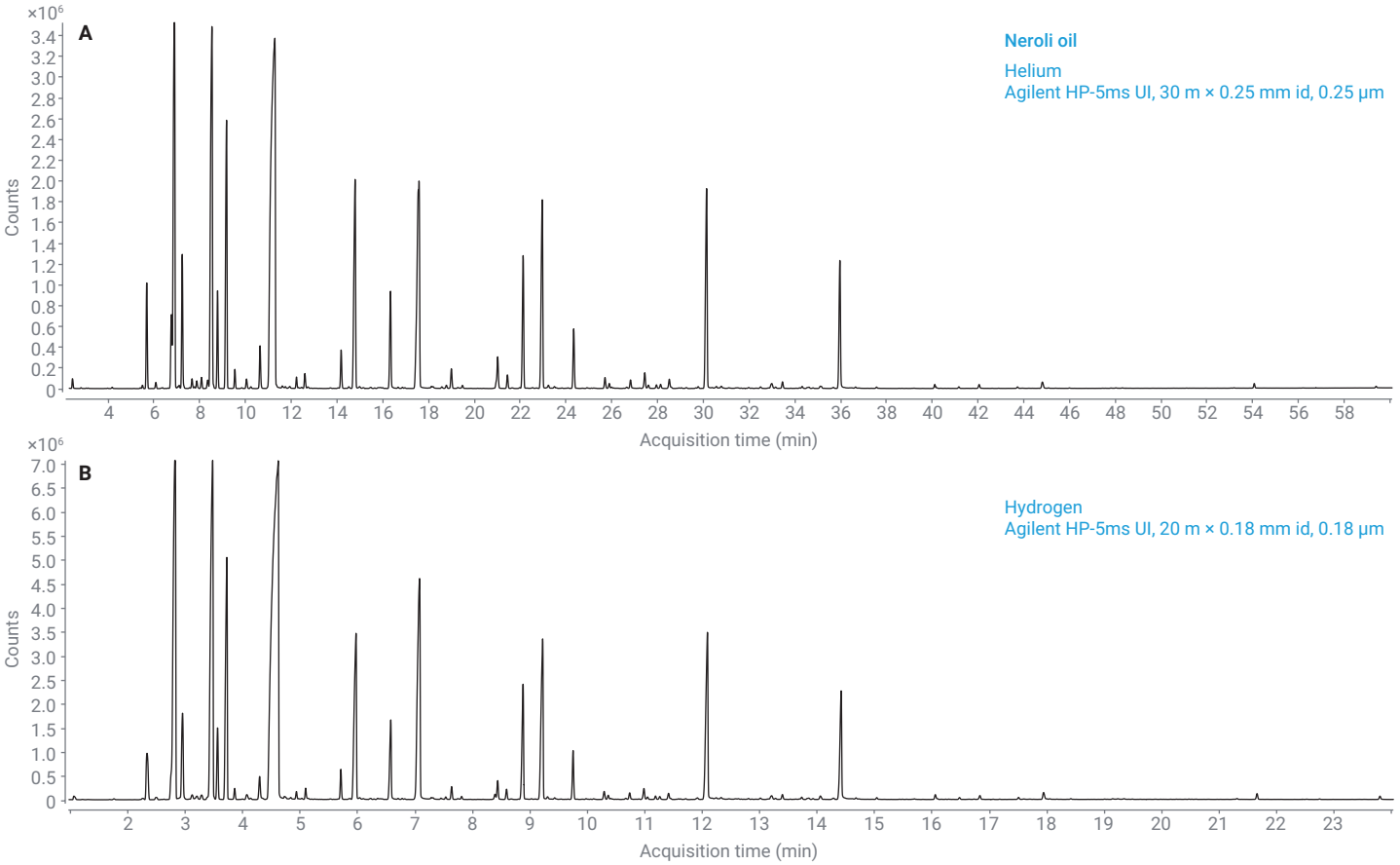

Figure 6. Neroli oil analyzed with (A) helium and (B) hydrogen methods.

Table 2. Agilent MassHunter Unknowns Analysis method parameters.

Parameter	Setting
RT Window Size Factor	10, 25, 50, 100, 200, 400, 600, 800
Library	NIST23.L
RT Penalty function	Trapezoidal
RT Range	10 s
Penalty Free Range	10 s
RT Mismatch Penalty	Additive
Maximum RT Penalty	100
Minimum Match Factor	70

Once the entire data file has been processed, the results can be reviewed. Figure 7 shows the results for the Brazilian orange oil with the helium method. Left-clicking on the compound name for one of the listed results displays the deconvoluted component spectrum head to tail with the library spectrum.

The selected component peak at 16.989 minutes is highlighted in red in the TIC chromatogram, which can be zoomed in for closer inspection. The five most abundant ions are extracted and overlaid in the box to the left of the spectrum display. This is to allow inspection of the peak shapes and apex retention times. If the apex RT or shape of one of the extracted ions is substantially different from the others, this suggests that there might be an interference, which should be considered when interpreting the identification.

In practice, reviewing the results consists of going down the list of hits and looking at the Match Factor (Library Match Score), Delta RI, and Base Peak Area. Using the peak at 16.989 as an example, the spectrum has a high-quality match for D-carvone of 98.2 listed, and the head-tail component and library spectra visually match well. The overlay of EICs of the principal ions all have the same shape and apex RT. The delta RI value of 2, which is the difference between the measured RI for the peak and that from the library, is small at 2. Finally, the base peak area and observed peak size in the TIC chromatogram indicate that the response is large enough to produce good-quality spectra. From these observations, the identification of D-carvone is confirmed with high confidence.

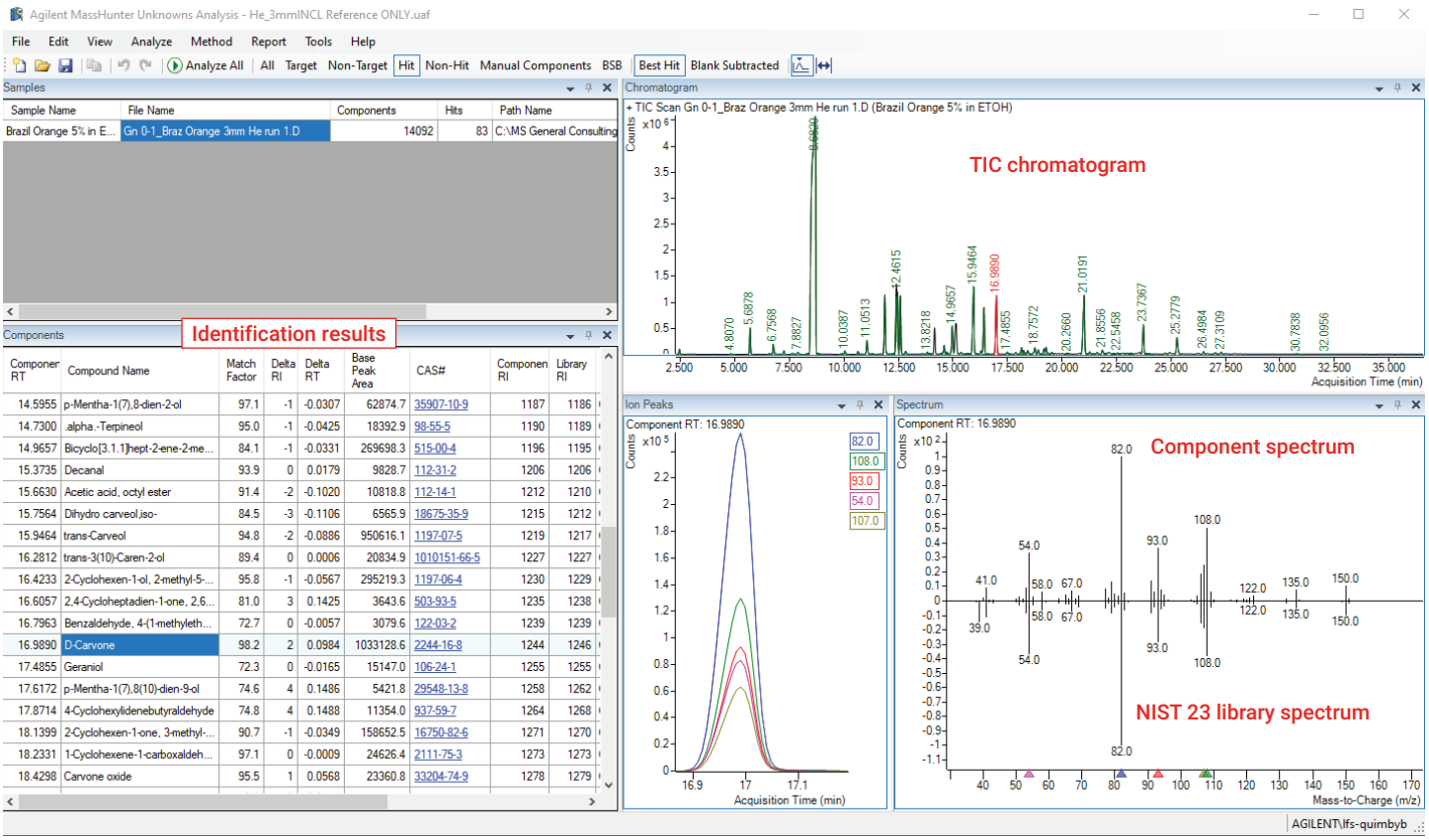

Figure 7. Agilent MassHunter Unknowns Analysis results for Brazilian orange oil run with the helium method.

In contrast, the peak at 17.6172, identified as p-mentha-1(7),8(10)-dien-9-ol, has a low LMS of 74.6, a larger delta RI of 4, one of the EICs has a noticeably different apex RT, and the base peak area is small—approximately 190 times smaller than the D-carvone peak. This would be a low-confidence identification. If the reviewer decides it should not be reported, the hit can be removed from the results by right-clicking the name and selecting **Delete Components/Hits**.

If an identification is questioned based on other information, the reviewer can right-click the name in the results table and select **Show Alternate Hits**. This will display a list of the other spectra in the library that also met the LMS and RI criteria, but with an LMS less than the listed Best Hit. This is useful as sometimes the LMS of the lesser hits is only a fraction of a point smaller. If desired, the reviewer can select one of the alternate hits and set it as the identification.

This review process is used to evaluate all the hits. Once completed, the reviewed analysis can be saved and a report can be generated if desired.

Evaluating in-source reactions with hydrogen

While the inert extractor source with the 3 mm lens is standard for use with helium, it is not the source of choice for use with hydrogen carrier. The metal surfaces inside the source tend to catalyze reactions between hydrogen and some analyte molecules in the source, resulting in peak tailing and spectral changes for some compounds. In the past, substituting the 9 mm extractor lens for the 3 mm lens was used as it reduced the tailing and spectral changes to some degree, but did not eliminate them. For this reason, the HydroInert source was developed.

In this section, the spectra of carvone oxide (CAS number 33204-74-9) obtained with hydrogen using the HydroInert source, the inert extractor source with the 3 and 9 mm lenses, and with helium are compared to illustrate the effects of source reactivity. Several other examples are presented in the Appendix.

Figure 8 shows the chromatograms and spectra of the carvone oxide peak with the helium and hydrogen methods under the optimized conditions. The library reference spectrum from NIST23 is shown upside down for comparison. With both methods, the deconvoluted spectra have high LMS values of > 95, demonstrating the excellent spectral fidelity provided by the HydroInert source with hydrogen carrier gas.

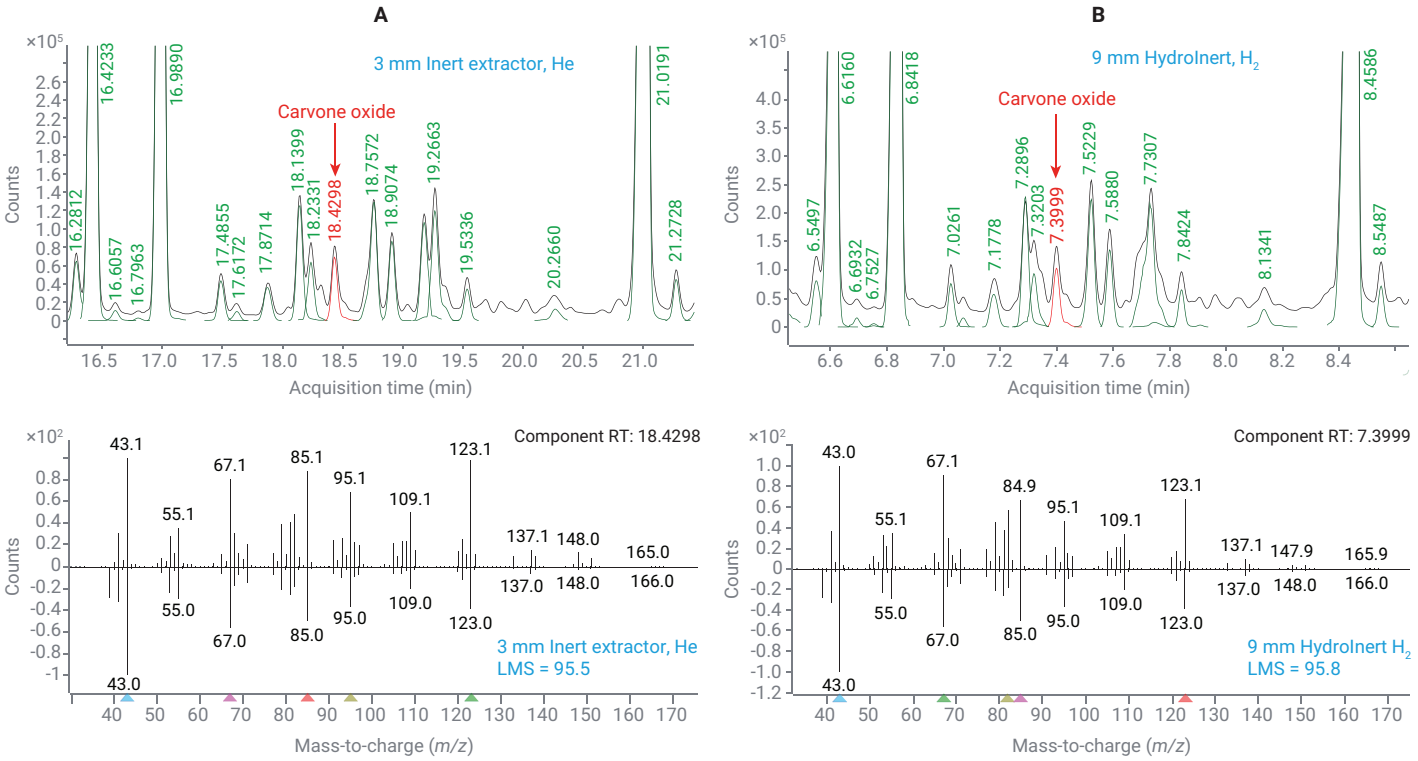

Figure 8. (A) Chromatogram and spectrum of the carvone oxide peak with the helium method. (B) Chromatogram and spectrum of the carvone oxide peak with the hydrogen method and Agilent HydroInert source.

For comparison, Figure 9 shows the spectra of the carvone oxide peak with hydrogen carrier using the HydroInert source and the inert extractor source with both the 9 and 3 mm extractor lenses.

With the 9 mm inert extractor lens shown in the spectrum in Figure 9B, the LMS value is still a respectable 91.2. However, there is clear evidence of some spectral changes. Most notably, the ions 82 and 108 have increased in abundance relative to the rest of the ions in the spectrum. While the degree of spectral fidelity is still useful, it demonstrates that in-source reactions, albeit limited, are occurring.

In contrast, the spectrum with the 3 mm inert extractor lens and hydrogen carrier is significantly changed. The spectrum is changed to the extent that the LMS for matching carvone oxide is below the 70 cutoff and thus is not listed, even in the alternate hits list. The search identified the peak as (2,6,6-trimethylbicyclo[3.1.1]heptan-3-yl)methanamine (CAS 61299-72-7), also known as pinane-3-(methylamine). Note that with an LMS value of 84.8 and a close RI match with a delta RI of only –1, this identification looks plausible but is incorrect.

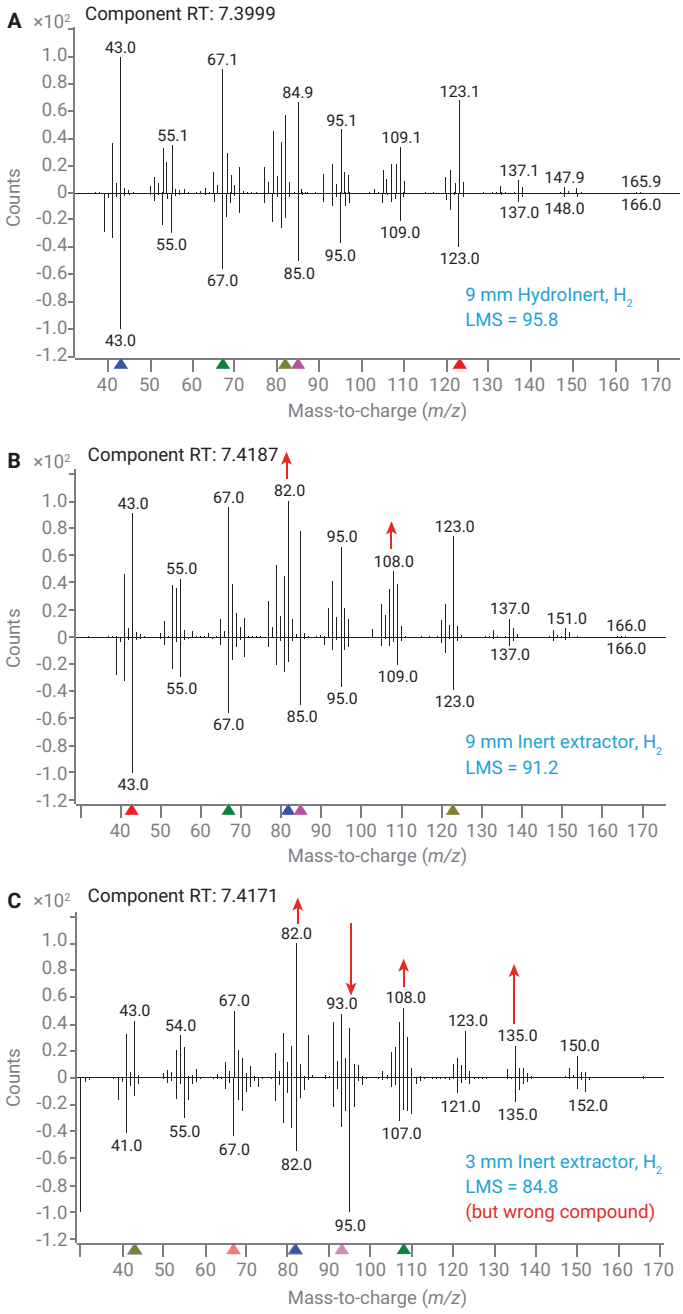

Figure 9. (A) Spectrum of the carvone oxide with hydrogen carrier and the Agilent HydroInert source. The reference spectrum is carvone oxide from NIST23. (B) Spectrum with an Agilent inert extractor source and a 9 mm lens. The reference spectrum is carvone oxide from NIST23. (C) Spectrum with an Agilent inert extractor source and a 3 mm lens. The reference spectrum is (2,6,6-trimethylbicyclo[3.1.1]heptan-3-yl)methanamine from NIST23.

To further investigate the nature of the in-source reaction with the 3 mm inert extractor source, the data file was reanalyzed with MHUA using the same parameters, except not using the RI match criteria. This would list the best hits solely on LMS. If the carvone oxide is reacting with hydrogen in the source to produce a reaction product, the spectral search may reveal what it is. Figure 10 shows the spectrum obtained with (A) the 3 mm lens at the carvone oxide RT compared with (B) that of the best match and (C) the carvone oxide library spectrum.

Note that the spectrum obtained with the 3 mm inert extractor source looks very much like a combination of that of carvone oxide and 3-hydroxy-2-methyl-5-(prop-1-en-2-yl) cyclohexanone. Examining the structures in Figure 10, it appears that the epoxide structure of carvone oxide reacts with hydrogen to form the OH group.

This example clearly illustrates the perils of using a GC/MS source that allows reactions between hydrogen and analytes, and why the HydroInert source is the best choice when using hydrogen carrier gas. Several other examples are shown in the Appendix.

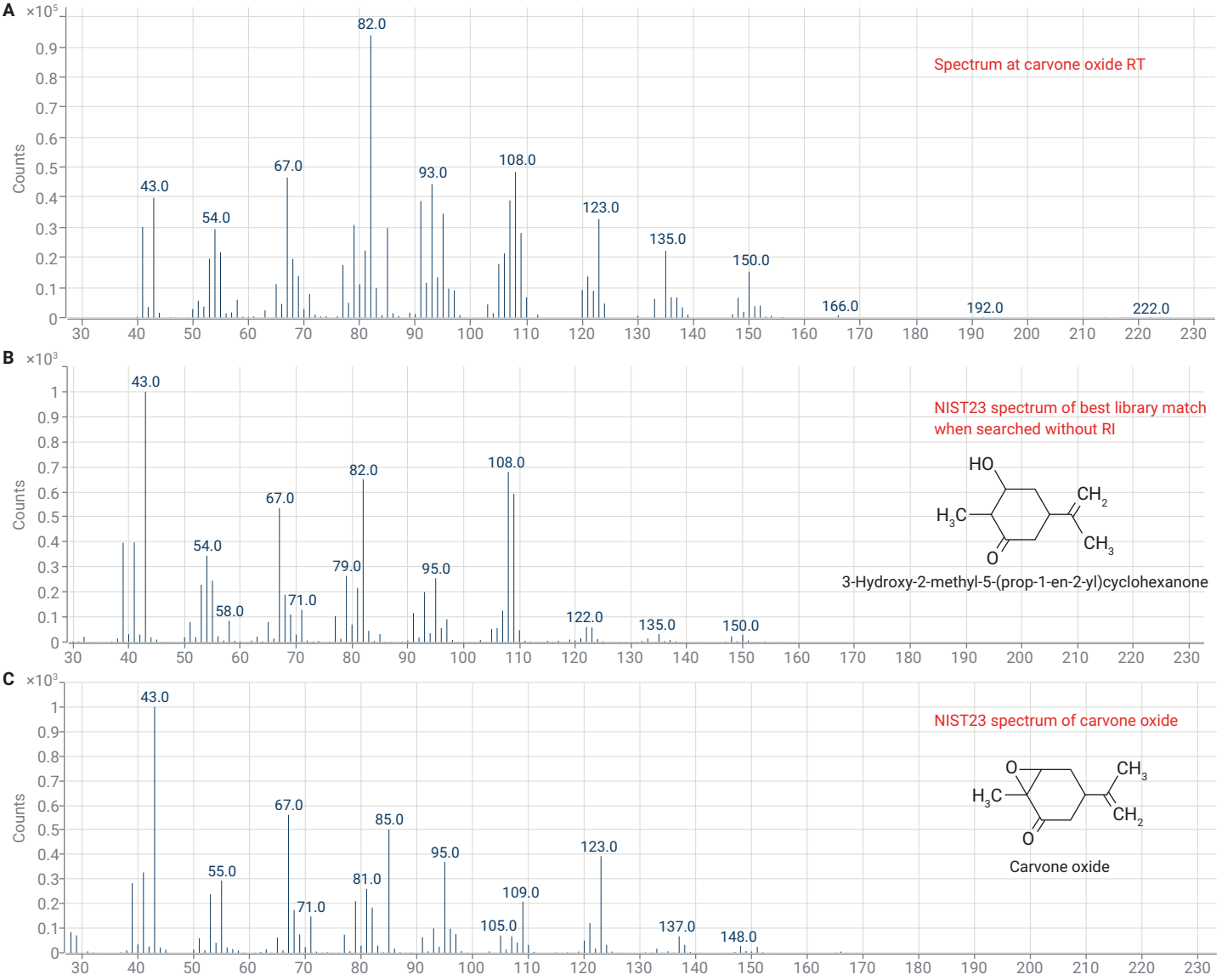

Figure 10. (A) Spectrum obtained with the 3 mm lens at the carvone oxide RT. (B) NIST23 library reference spectrum for the best match when searched without RI filtering, 3-hydroxy-2-methyl-5-(prop-1-en-2-yl)cyclohexanone. (C) NIST23 library reference spectrum for carvone oxide.

Analysis results for Brazilian orange and neroli oils

Table 3 presents the results of the analysis of the Brazilian orange oil with both the helium method using the 3 mm inert extractor source and the hydrogen method using the 9 mm HydroInert source. Table 4 presents the results for neroli oil with the same methods.

The results were reviewed to address questions relevant to converting the method helium to hydrogen carrier gas:

- **RIs compared to NIST23:** The RIs measured with both methods closely matched those in the NIST23 library for most compounds. However, it should be recognized that the RI recognition window used in MassHunter Unknowns Analysis limits the maximum delta RI when listing hits. The second consideration is that if the NIST23 RI value is an AI-predicted value instead of a true experimental value, the errors, and thus the delta RIs, can be larger.
- **Comparing RIs between helium and hydrogen methods:** As seen in the columns listing the difference between the RI measured with helium and that with hydrogen (labeled RI He-RI H_2), the agreement of the RIs measured with both methods is good. The only exceptions are the earliest peaks and those such as linalool and D-limonene that are chromatographically overloaded, thus shifting their RT. This is one of the benefits of using the Method Translator tool to choose the chromatographic parameters for the hydrogen method, because it maintains the same relative elution order for analytes and RI calibrators between the two methods.
- **LMS versus NIST23:** The deconvolution process in general yields cleaner spectra, which results in improved LMS scores when compared to previous approaches. Looking at the column of LMS scores for the helium results, most of them are > 85. The smaller values can result from smaller responding compounds, overlapping peaks causing spectral interferences, or search results where the analyte is not in the library and an incorrect hit is listed.
- **Comparing LMS between helium and hydrogen methods:** The columns listing the difference between the LMS measured with helium and that with hydrogen (labeled LMS He-LMS H_2) shows that, in general, there is good agreement between the two methods. The exceptions where the hydrogen method values are significantly lower are either due to lower signal response or spectral interference from overlapping peaks. In general, the signal-to-noise ratios obtained with hydrogen are two to five times less than with helium, and this is reflected in lower LMS scores for the smallest peaks.

Table 3. Analysis results for the Brazilian orange oil with both the helium and hydrogen methods. (LR = low response; Int = interference.)

Compound Name	CAS	Lib RI	Helium				Hydrogen with Hydroinert				RI He-RI H_2	LMS He-LMS H_2	
			RT	RI	Delta RI	LMS	RT	RI	Delta RI	LMS			
Ethane, 1,1-diethoxy-	105-57-7	726	2.445	723	3	98	1.061	732	-6	96	-9	2	
Nonane	111-84-2	900	4.807	900	0	95	1.983	900	0	91	0	4	
(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	7785-70-8	932	5.688	933	-1	99	2.332	933	-1	97	0	2	
1-Heptanol	111-70-6	970	6.566	966	4	98	2.699	968	2	95	-2	2	
Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	3387-41-5	974	6.757	973	1	98	2.759	974	0	98	-1	0	
beta-Myrcene	123-35-3	991	7.240	991	0	97	2.953	992	-1	95	-1	2	
Octanal	124-13-0	1,003	7.616	1,004	-1	98	3.116	1,006	-3	97	-2	1	
3-Carene	13466-78-9	1,011	7.883	1,011	0	98	3.211	1,012	-1	89	-1	9	
D-Limonene	5989-27-5	1,031	8.682	1,034	-3	99	3.562	1,037	-6	99	-3	0	
1-Methylbicyclo[2.2.1]heptan-exo-2-ol	766-25-6	1,039	8.796	1,037	2	76	3.593	1,039	0	74	-2	2	
trans-Sabinene hydrate	17699-16-0	1,070	9.837	1,066	4	74	3.985	1,067	3	77	-1	-3	
1-Octanol	111-87-5	1,070	9.919	1,068	2	98	4.016	1,070	0	97	-2	1	
cis-Linalool oxide	5989-33-3	1,074	10.039	1,072	2	94	4.063	1,073	1	94	-1	0	
trans-Linalool oxide (furanoid)	34995-77-2	1,086	10.630	1,088	-2	95	4.297	1,089	-3	94	-1	0	
Benzene, 1-methyl-4-(1-methylethenyl)-	1195-32-0	1,090	10.665	1,089	1	91	4.306	1,090	0	92	-1	-1	
Epoxymyrcene,6,7-	29414-55-9	1,090	10.804	1,093	-3	73	4.365	1,094	-4	79	-1	-6	
Linalool	78-70-6	1,099	11.051	1,100	-1	97	4.462	1,101	-2	95	-1	1	
Nonanal	124-19-6	1,104	11.232	1,104	0	96	4.533	1,105	-1	86	-1	10	LR
cis-Pinen-3-ol	1010292-85-2	1,108	11.324	1,107	1	81	4.574	1,108	0	73	-1	8	
2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethenyl)-, *trans*-	7212-40-0	1,123	11.872	1,120	3	95	4.799	1,122	1	96	-2	-1	
5-Undecene, 4-methyl-	143185-91-5	1,132	12.217	1,129	3	74	4.925	1,130	2	71	-1	4	
7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(1-methylethenyl)-	1195-92-2	1,133	12.401	1,133	0	86	5.003	1,134	-1	90	-1	-4	
cis-p-Mentha-2,8-dien-1-ol	3886-78-0	1,133	12.462	1,135	-2	79	5.042	1,137	-4	89	-2	-10	
(+)-(E)-Limonene oxide	6909-30-4	1,139	12.586	1,138	1	97	5.082	1,139	0	98	-1	-1	
Cyclohexanol, 1-methyl-4-(1-methylethenyl)-, *cis*-	7299-41-4	1,144	12.826	1,143	1	95	5.171	1,145	-1	93	-2	1	
Cyclohexanol, 1-methyl-4-(1-methylethenyl)-, *trans*-	7299-40-3	1,161	13.610	1,163	-2	88	5.480	1,164	-3	83	-1	4	
Bicyclo[3.3.0]octan-2-one, 7-methylene-	1000151-92-1	1,166	13.822	1,168	-2	84	5.578	1,170	-4	64	-2	19	Int
1-Nonanol	143-08-8	1,173	13.921	1,170	3	98	5.603	1,171	2	96	-1	2	
Ethanone, 1-(4-methylphenyl)-	122-00-9	1,183	14.452	1,183	0	93	5.822	1,185	-2	95	-2	-2	
p-Mentha-1(7),8-dien-2-ol	35907-10-9	1,186	14.596	1,187	-1	97	5.873	1,188	-2	96	-1	1	
alpha-Terpineol	98-55-5	1,189	14.730	1,190	-1	95	5.928	1,191	-2	94	-1	1	
Bicyclo[3.1.1]hept-2-ene-2-methanol, 6,6-dimethyl-	515-00-4	1,195	14.966	1,196	-1	84	6.018	1,197	-2	86	-1	-2	
Decanal	112-31-2	1,206	15.374	1,206	0	94	6.173	1,206	0	93	0	1	
Acetic acid, octyl ester	112-14-1	1,210	15.663	1,212	-2	91	6.287	1,213	-3	79	-1	12	LR, Int
Dihydro carveol, iso-	18675-35-9	1,212	15.756	1,215	-3	85	6.334	1,216	-4	78	-1	7	
trans-Carveol	1197-07-5	1,217	15.946	1,219	-2	95	6.427	1,221	-4	96	-2	-1	
trans-3(10)-Caren-2-ol	1010151-66-5	1,227	16.281	1,227	0	89	6.550	1,229	-2	85	-2	4	
2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, *cis*-	1197-06-4	1,229	16.423	1,230	-1	96	6.616	1,232	-3	97	-2	-1	
2,4-Cycloheptadien-1-one, 2,6,6-trimethyl-	503-93-5	1,238	16.606	1,235	3	81	6.693	1,237	1	73	-2	8	
Benzaldehyde, 4-(1-methylethyl)-	122-03-2	1,239	16.796	1,239	0	73	6.753	1,241	-2	58	-2	14	LR
D-Carvone	2244-16-8	1,246	16.989	1,244	2	98	6.842	1,246	0	98	-2	0	
Geraniol	106-24-1	1,255	17.486	1,255	0	72	7.026	1,257	-2	77	-2	-5	
p-Mentha-1(7),8(10)-dien-9-ol	29548-13-8	1,262	17.617	1,258	4	75	7.070	1,259	3	73	-1	2	

Compound Name	CAS	Lib RI	Helium				Hydrogen with Hydroinert				RI He-RI H_2	LMS He-LMS H_2	
			RT	RI	Delta RI	LMS	RT	RI	Delta RI	LMS			
4-Cyclohexylidenebutyraldehyde	937-59-7	1,268	17.871	1,264	4	75	7.178	1,266	2	76	-2	-2	
2-Cyclohexen-1-one, 3-methyl-6-(1-methylethenyl)-, (S)-	16750-82-6	1,270	18.140	1,271	-1	91	7.290	1,272	-2	83	-1	7	
1-Cyclohexene-1-carboxaldehyde, 4-(1-methylethenyl)-	2111-75-3	1,273	18.233	1,273	0	97	7.320	1,274	-1	84	-1	14	LR
Carvone oxide	33204-74-9	1,279	18.430	1,278	1	96	7.400	1,279	0	96	-1	0	
Pinocarvyl acetate, *cis*-	73366-18-4	1,285	18.757	1,285	0	80	7.523	1,286	-1	82	-1	-2	
Verbenyl acetate, *trans*-	1203-21-0	1,291	18.907	1,289	2	79	7.588	1,290	1	80	-1	0	
p-Mentha-1,8-dien-7-ol	536-59-4	1,297	19.266	1,297	0	95	7.731	1,298	-1	93	-1	2	
2-Propanol, 1-[(1-ethynylcyclohexyl)oxy]-	54644-17-6	1,303	19.534	1,304	-1	77	7.842	1,305	-2	79	-1	-1	
1,2-Cyclohexanediol, 1-methyl-4-(1-methylethenyl)-	1946-00-5	1,321	20.266	1,321	0	80	8.134	1,323	-2	84	-2	-4	
(1S,4R,5R)-1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octan-5-yl acetate	81781-24-0	1,343	21.019	1,339	4	83	8.459	1,342	1	82	-3	1	
exo-2-Hydroxycineole acetate	57709-95-2	1,344	21.273	1,345	-1	86	8.549	1,347	-3	89	-2	-2	
1-Cyclohexene-1-methanol, 4-(1-methylethenyl)-, formate	29621-55-4	1,356	21.612	1,353	3	87	8.664	1,354	2	90	-1	-4	
2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, acetate, (1R-*cis*)-	7111-29-7	1,358	21.856	1,359	-1	80	8.775	1,361	-3	79	-2	1	
Copaene	3856-25-5	1,376	22.546	1,375	1	91	9.024	1,376	0	84	-1	6	
2-Methyl-4-(2,6,6-trimethylcyclohex-2-enyl)but-3-en-2-ol	56763-65-6	1,406	23.737	1,404	2	83	9.528	1,406	0	85	-2	-2	
cis-β-Copaene	18252-44-3	1,432	24.728	1,428	4	93	9.897	1,429	3	89	-1	3	
Sesquicineole, 7-epi-1,2-dehydro-	149067-90-3	1,471	26.498	1,472	-1	78	10.618	1,473	-2	79	-1	-1	
3-Tetradecen-5-yne, (E)-	74744-44-8	1,488	27.032	1,485	3	72	10.842	1,487	1	73	-2	-1	
Valencene	4630-07-3	1,492	27.311	1,492	0	97	10.930	1,493	-1	96	-1	0	
Caryophyllene oxide	1139-30-6	1,581	30.784	1,582	-1	73	12.320	1,582	-1	81	0	-7	
1,5,9-Cyclododecanetriol	2938-55-8	2,007	45.413	2,005	2	72	18.190	2,006	1	76	-1	-4	
3-Eicosyne	61886-66-6	2,032	46.366	2,036	-4	75	18.573	2,037	-5	76	-1	-2	
Uvidin C, diacetate	1000501-90-0	2,107	48.516	2,107	0	75	19.432	2,107	0	73	0	2	
(9E,11E)-Octadecadienoic acid	544-71-8	2,237	52.406	2,241	-4	74	20.997	2,241	-4	73	0	2	
Incensole oxide, acetate	1000513-23-1	2,270	53.383	2,275	-5	72	21.395	2,276	-6	69	-1	3	

Table 4. Analysis results for neroli oil with both the helium and hydrogen methods. (LR = low response; Int = interference.)

Compound Name	CAS	Lib RI	Helium				Hydrogen with Hydroinert				RI He-RI H_2	LMS He-LMS H_2	
			RT	RI	Delta RI	LMS	RT	RI	Delta RI	LMS			
Ethane, 1,1-diethoxy-	105-57-7	726	2.445	723	3	98	1.060	732	-6	97	-9	1	
3-Hexen-1-ol	544-12-7	856	3.982	851	5	93	1.685	856	0	80	-5	13	LR
1-Hexanol	111-27-3	868	4.184	863	5	95	1.762	867	1	92	-4	3	
Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	2867-05-2	929	5.501	926	3	98	2.258	926	3	92	0	6	
(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	7785-70-8	932	5.688	933	-1	98	2.334	933	-1	98	0	1	
Camphene	79-92-5	952	6.087	948	4	98	2.496	949	3	97	-1	1	
Benzaldehyde	100-52-7	962	6.380	959	3	98	2.628	961	1	96	-2	2	
Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	18172-67-3	978	6.899	978	0	97	2.824	980	-2	98	-2	0	
m-Mentha-4,8-diene, (1S,3S)-(+)-	5208-51-5	983	7.047	984	-1	82	2.875	985	-2	77	-1	5	
beta-Myrcene	123-35-3	991	7.240	991	0	97	2.952	992	-1	97	-1	0	
Cyclohexane, 1-methylene-4-(1-methylethenyl)-	499-97-8	1,004	7.667	1,005	-1	98	3.121	1,006	-2	97	-1	1	
cis-Anhydrolinalool oxide	54750-69-5	1,007	7.775	1,008	-1	93	3.166	1,009	-2	79	-1	14	LR, Int
3-Carene	13466-78-9	1,011	7.874	1,011	0	97	3.203	1,012	-1	97	-1	1	
1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	99-86-5	1,017	8.082	1,017	0	97	3.285	1,018	-1	97	-1	0	
D-Limonene	5989-27-5	1,031	8.536	1,029	2	99	3.476	1,031	0	99	-2	0	
1,3,6-Octatriene, 3,7-dimethyl-, (Z)-	3338-55-4	1,038	8.782	1,036	2	97	3.562	1,037	1	97	-1	0	
trans-beta-Ocimene	3779-61-1	1,049	9.174	1,047	2	97	3.724	1,049	0	97	-2	0	
gamma-Terpinene	99-85-4	1,060	9.540	1,058	2	99	3.860	1,058	2	99	0	0	
trans-Sabinene hydrate	17699-16-0	1,070	9.843	1,066	4	90	3.994	1,068	2	81	-2	9	
cis-Linalool oxide	5989-33-3	1,074	10.047	1,072	2	98	4.071	1,073	1	96	-1	2	
1,4-Undecadiene, (Z)-	55976-14-2	1,080	10.241	1,077	3	81	4.138	1,078	2	80	-1	1	
Cyclohexene, 1-methyl-4-(1-methylethylidene)-	586-62-9	1,088	10.638	1,088	0	98	4.294	1,089	-1	98	-1	0	
Linalool	78-70-6	1,099	11.285	1,106	-7	98	4.618	1,111	-12	98	-5	0	
Phenylethyl alcohol	60-12-8	1,116	11.611	1,114	2	98	4.725	1,117	-1	96	-3	1	
2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, cis-	29803-82-5	1,122	11.938	1,122	0	93	4.837	1,124	-2	93	-2	0	
2,4,6-Octatriene, 2,6-dimethyl-, (E,Z)-	7216-56-0	1,131	12.231	1,129	2	99	4.935	1,130	1	99	-1	0	
2-Isopropylimidazole	36947-68-9	1,132	12.318	1,131	1	71	4.973	1,132	0	70	-1	1	
cis-p-Mentha-2,8-dien-1-ol	3886-78-0	1,133	12.460	1,134	-1	83	5.029	1,136	-3	82	-2	1	
Benzyl nitrile	140-29-4	1,144	12.594	1,138	6	98	5.095	1,140	4	94	-2	5	
Myroxide	28977-57-3	1,140	12.747	1,142	-2	92	5.144	1,143	-3	71	-1	20	LR, Int
Terpinen-4-ol	562-74-3	1,177	14.182	1,177	0	96	5.709	1,178	-1	96	-1	1	
Benzenemethanol, alpha,alpha,4-trimethyl-	1197-01-9	1,183	14.515	1,185	-2	91	5.864	1,187	-4	90	-2	1	
alpha-Terpineol	98-55-5	1,189	14.790	1,192	-3	99	5.972	1,194	-5	99	-2	0	
1,3-Cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl-	116-26-7	1,201	15.153	1,200	1	78	6.099	1,202	-1	79	-2	-2	
(3E,5E)-2,6-Dimethylocta-3,5,7-trien-2-ol	206115-88-0	1,202	15.481	1,208	-6	88	6.229	1,210	-8	89	-2	-1	
Acetic acid, octyl ester	112-14-1	1,210	15.648	1,212	-2	92	6.285	1,213	-3	89	-1	3	
Benzofuran, 2-ethenyl-	7522-79-4	1,220	15.994	1,220	0	89	6.428	1,221	-1	86	-1	4	
2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-	106-25-2	1,228	16.337	1,228	0	97	6.577	1,230	-2	98	-2	0	
Neral	106-26-3	1,240	16.860	1,241	-1	90	6.776	1,242	-2	88	-1	2	
Carvone	99-49-0	1,242	16.966	1,243	-1	87	6.821	1,245	-3	86	-2	2	
Linalyl acetate	115-95-7	1,257	17.587	1,258	-1	95	7.079	1,260	-3	92	-2	3	
Citral	5392-40-5	1,273	18.124	1,270	3	93	7.283	1,272	1	89	-2	4	

Compound Name	CAS	Lib RI	Helium				Hydrogen with Hydroinert				RI He-RI H_2	LMS He-LMS H_2	
			RT	RI	Delta RI	LMS	RT	RI	Delta RI	LMS			
2,6-Octadien-1-ol, 3,7-dimethyl-, formate, (Z)-	2142-94-1	1,282	18.575	1,281	1	89	7.452	1,282	0	89	-1	0	
Levo-bornyl acetate	5655-61-8	1,285	18.780	1,286	-1	99	7.534	1,287	-2	95	-1	4	
Indole	120-72-9	1,294	18.998	1,291	3	99	7.637	1,293	1	99	-2	0	
Benzene, (2-nitroethyl)-	6125-24-2	1,302	19.280	1,298	4	89	7.746	1,299	3	86	-1	3	
Geranyl formate	105-86-2	1,300	19.483	1,303	-3	91	7.811	1,303	-3	91	0	1	
δ-Elemene	20307-84-0	1,338	20.958	1,338	0	86	8.392	1,338	0	96	0	-10	
Methyl anthranilate	134-20-3	1,343	21.020	1,339	4	94	8.439	1,341	2	98	-2	-4	
alpha-Terpinyl acetate	80-26-2	1,350	21.445	1,349	1	97	8.593	1,350	0	96	-1	2	
6-Octen-1-ol, 3,7-dimethyl-, acetate	150-84-5	1,354	21.647	1,354	0	84	8.669	1,354	0	81	0	2	
2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	141-12-8	1,364	22.133	1,366	-2	99	8.881	1,367	-3	99	-1	0	
Copaene	3856-25-5	1,376	22.543	1,375	1	82	9.026	1,376	0	80	-1	2	
Geranyl acetate	105-87-3	1,382	22.966	1,385	-3	98	9.222	1,387	-5	98	-2	0	
levo-β-Elemene	515-13-9	1,391	23.236	1,392	-1	96	9.307	1,393	-2	95	-1	1	
Benzoic acid, 2-amino-, ethyl ester	87-25-2	1,414	24.091	1,413	1	86	9.662	1,414	0	93	-1	-7	
Caryophyllene	87-44-5	1,419	24.340	1,419	0	99	9.751	1,420	-1	99	-1	0	
gamma-Elemene	29873-99-2	1,434	24.921	1,433	1	83	9.977	1,434	0	88	-1	-6	
Humulene	6753-98-6	1,454	25.707	1,453	1	92	10.293	1,453	1	91	0	1	
(E)-beta-Farnesene	18794-84-8	1,457	25.894	1,457	0	96	10.363	1,458	-1	96	-1	0	
Alloaromadendrene	25246-27-9	1,461	26.002	1,460	1	92	10.408	1,460	1	84	0	8	
gamma-Muurolene	30021-74-0	1,477	26.657	1,476	1	93	10.669	1,476	1	91	0	2	
Germacrene D	23986-74-5	1,481	26.824	1,480	1	95	10.736	1,481	0	97	-1	-2	
Bicyclogermacrene	24703-35-3	1,496	27.445	1,496	0	98	10.985	1,496	0	97	0	1	
alpha-Muurolene	10208-80-7	1,499	27.605	1,500	-1	96	11.048	1,500	-1	93	0	3	
alpha-Farnesene	502-61-4	1,508	27.955	1,509	-1	94	11.186	1,509	-1	96	0	-1	
γ-Cadinene	39029-41-9	1,513	28.137	1,513	0	97	11.261	1,514	-1	96	-1	1	
δ-Cadinene	483-76-1	1,524	28.519	1,523	1	97	11.413	1,524	0	97	-1	1	
α-Cadinene	24406-05-1	1,538	29.047	1,537	1	80	11.626	1,537	1	81	0	-2	
alpha-Calacorene	21391-99-1	1,542	29.254	1,542	0	94	11.710	1,543	-1	82	-1	13	LR
β-Germacrene	15423-57-1	1,557	29.779	1,556	1	96	11.915	1,556	1	87	0	10	Int
1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-	7212-44-4	1,564	30.146	1,565	-1	97	12.091	1,567	-3	97	-2	0	
Spathulenol	6750-60-3	1,576	30.574	1,576	0	93	12.244	1,577	-1	85	-1	9	
Caryophyllene oxide	1139-30-6	1,581	30.788	1,582	-1	92	12.329	1,583	-2	90	-1	2	
tau-Cadinol	5937-11-1	1,640	32.985	1,641	-1	96	13.198	1,641	-1	95	0	2	
δ-Cadinol	19435-97-3	1,645	33.163	1,646	-1	92	13.276	1,646	-1	88	0	4	
alpha-Cadinol	481-34-5	1,653	33.462	1,654	-1	96	13.396	1,654	-1	94	0	2	
Naphthalene, 1,6-dimethyl-4-(1-methylethyl)-	483-78-3	1,674	34.205	1,675	-1	92	13.695	1,674	0	78	1	14	LR, Int
8-Heptadecene	2579-04-6	1,677	34.320	1,678	-1	95	13.731	1,677	0	94	1	2	
6,10-Dodecadien-1-ol, 3,7,11-trimethyl-	51411-24-6	1,692	34.795	1,691	1	81	13.928	1,690	2	87	1	-6	
2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (Z,E)-	3790-71-4	1,697	35.110	1,700	-3	92	14.058	1,699	-2	89	1	3	
trans-Farnesol	106-28-5	1,722	35.968	1,724	-2	97	14.418	1,724	-2	98	0	-1	
Farnesol, 2E, 6Z-	3879-60-5	1,742	36.652	1,743	-1	79	14.676	1,743	-1	78	0	1	
all-*trans*-Farnesyl acetate	4128-17-0	1,843	40.112	1,842	1	94	16.057	1,843	0	92	-1	3	
Cubitene	66723-19-1	1,878	41.166	1,874	4	82	16.479	1,874	4	78	0	3	
m-Camphorene	20016-73-3	1,960	43.728	1,952	8	95	17.508	1,953	7	93	-1	3	
p-Camphorene	20016-72-2	1,995	44.815	1,986	9	92	17.942	1,986	9	91	0	1	
Hexadecanoic acid, ethyl ester	628-97-7	1,993	45.083	1,995	-2	73	18.054	1,995	-2	75	0	-2	

Conclusion

Using the techniques described in this application note, a typical method for the qualitative analysis of essential oils was successfully converted to a method using hydrogen carrier gas. The resulting hydrogen method retains the same chromatographic resolution and relative elution order of the original method, but with a run time 2.5 times shorter. The column capacity with the new method is reduced, being a calculated 36% of the original method, so the amount injected may need adjustment in some cases.

The new hydrogen method was then applied to the analysis of two essential oils and found to generate results comparable to those from the helium method. The use of spectral deconvolution and retention index search filtering with Agilent MassHunter Unknowns Analysis gave improved search results that were faster than previous identification methods. The NIST23 library, with expanded content for essential oil components and RIs, allowed identification of a significant portion of the compounds present.

The Agilent HydroInert source is a key component in the successful conversion to hydrogen. Without it, in-source reactions were shown to degrade the spectra of some compounds to the point where they were misidentified.

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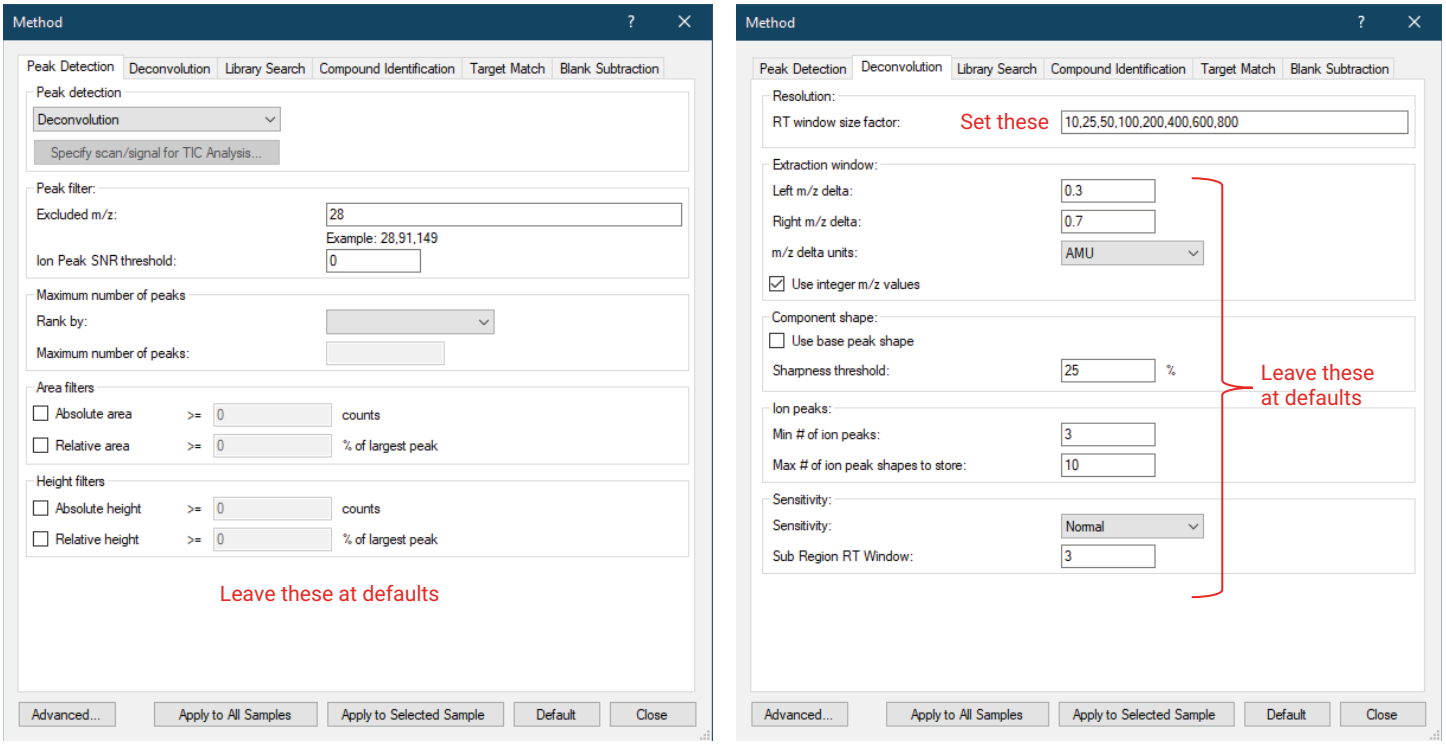
Appendix

Setting up MassHunter Unknowns Analysis

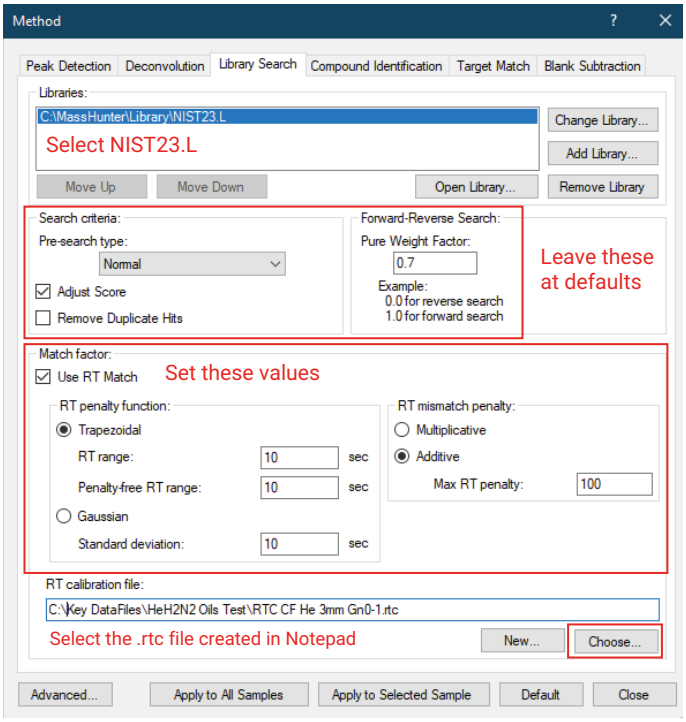
This section shows how to set up Agilent MassHunter Unknowns Analysis. The parameters shown here were used in this specific application. For other applications, different parameters can be used to optimize the process.

Locate the Agilent MassHunter Quantitative Analysis software folder under the Microsoft Windows Start menu and open the MassHunter Unknowns Analysis program.

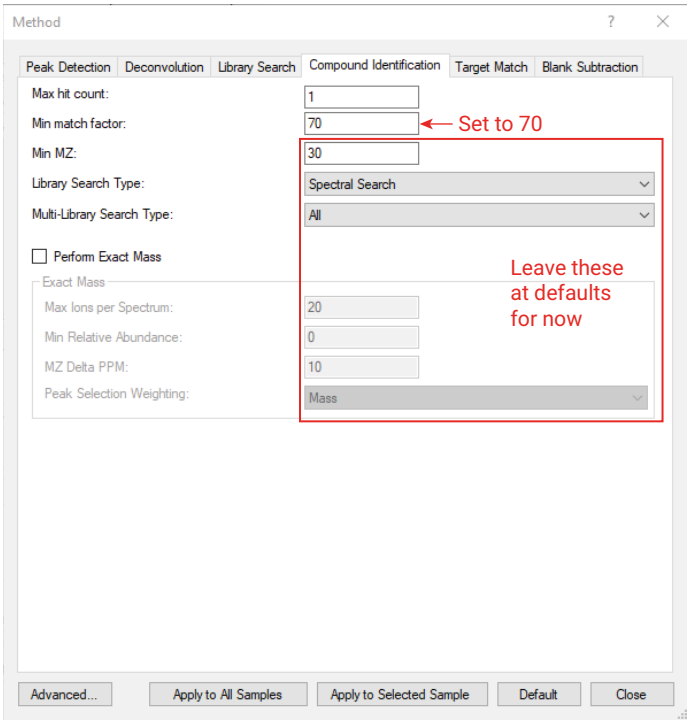
1. Click **File > New Analysis**. Navigate to the directory containing your data files.
2. Enter a file name for the analysis.
3. Click **File > Add Samples**. Select the data files that you want to process. You should see the TIC of your chromatogram appear.
4. Click **Method > Edit**. Set the parameters as shown in Appendix Figures 1 to 5.



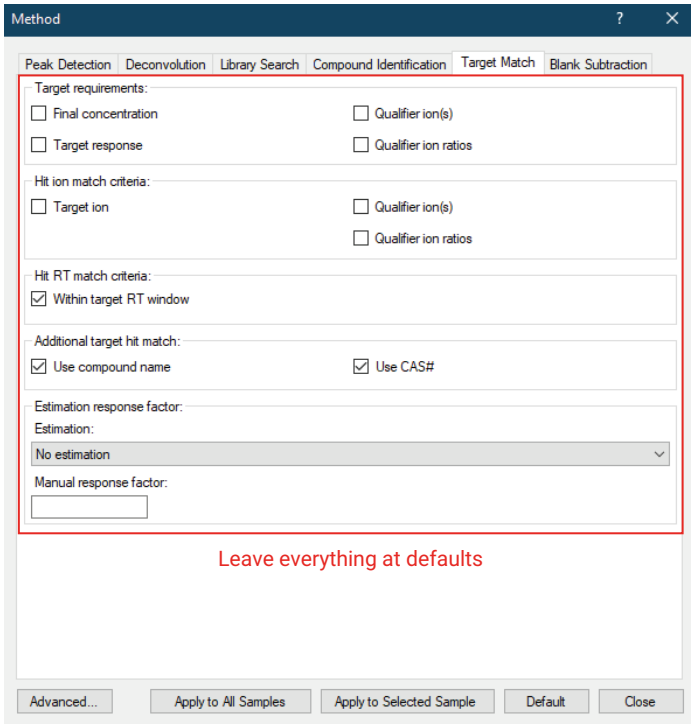
Appendix Figure 1. Setting the parameters for the Peak Detection and Deconvolution tabs in Agilent MassHunter Unknowns Analysis.



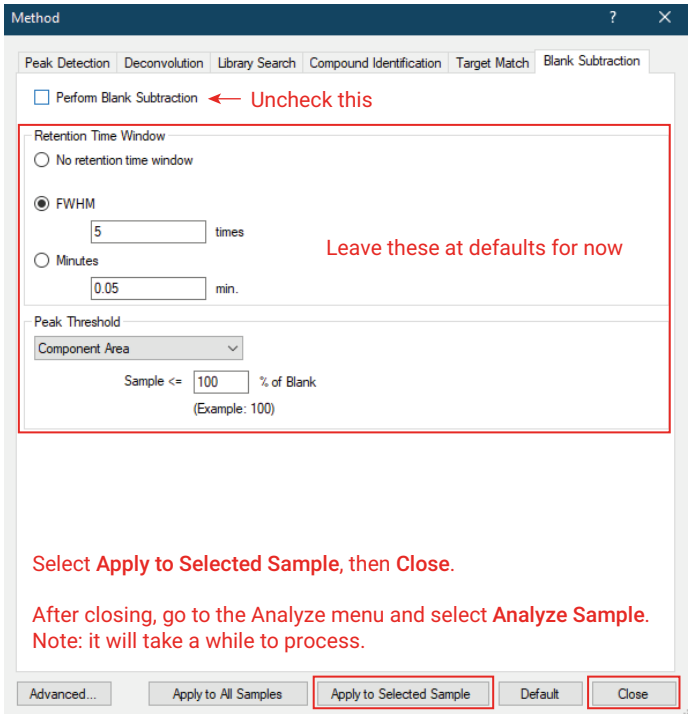
Appendix Figure 2. Setting the parameters for the Library Search tab in Agilent MassHunter Unknowns Analysis.



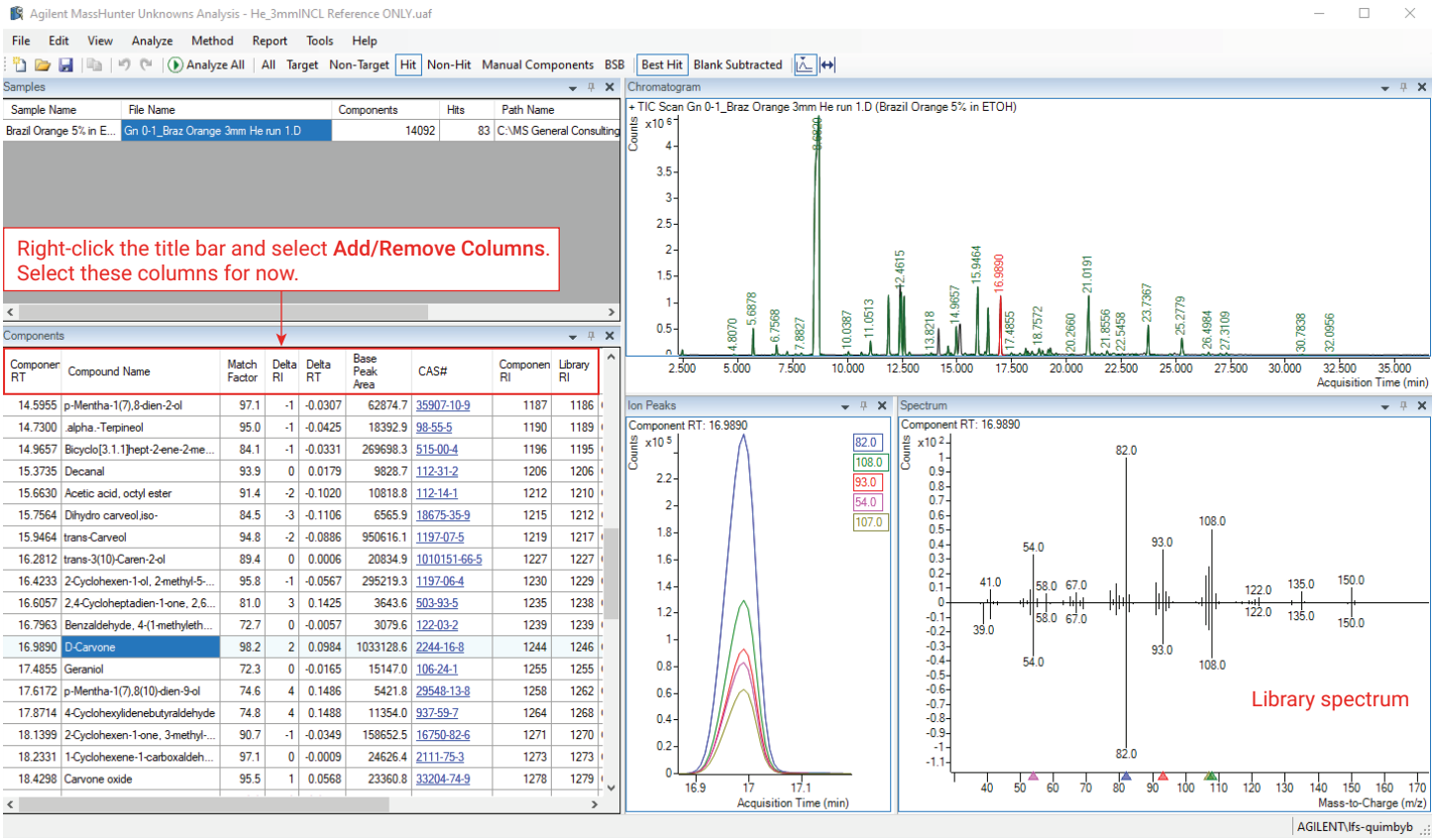
Appendix Figure 3. Setting the parameters for the Compound Identification tab in Agilent MassHunter Unknowns Analysis.



Appendix Figure 4. Setting the parameters for the Target Match tab in Agilent MassHunter Unknowns Analysis.



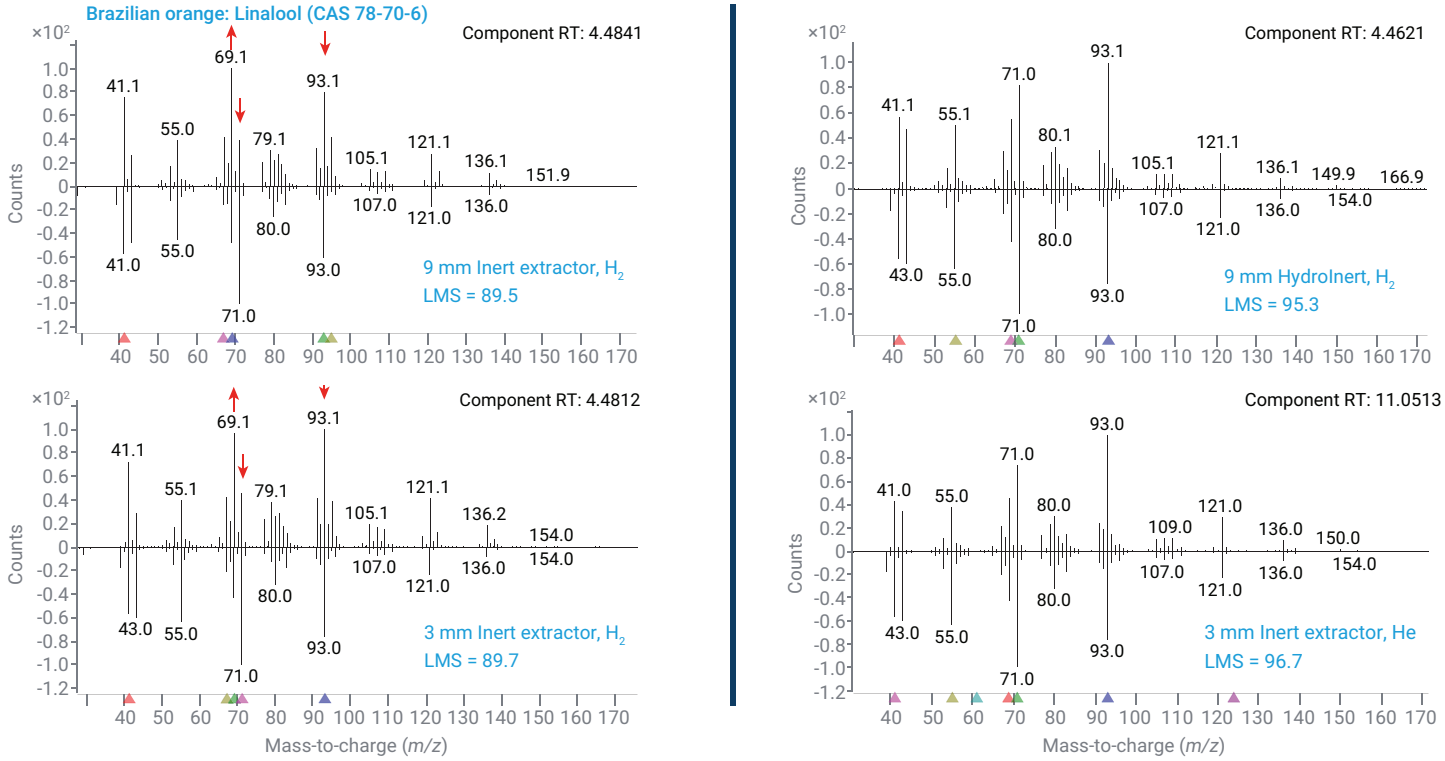
Appendix Figure 5. Setting the parameters for the Blank Subtraction tab in Agilent MassHunter Unknowns Analysis.



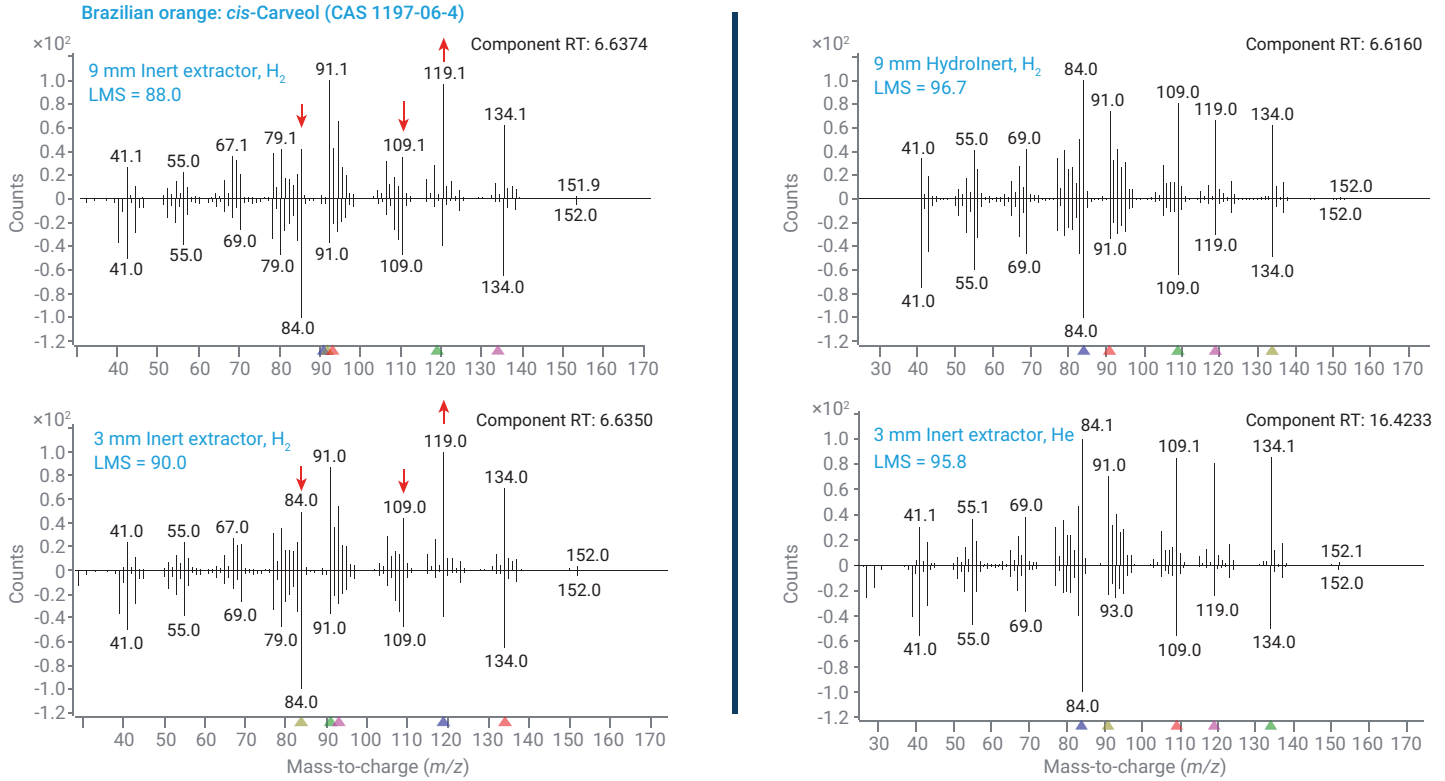
Appendix Figure 6. Example result of a deconvoluted/searched data file in Agilent MassHunter Unknowns Analysis.

More examples of in-source reactions eliminated with the HydroInert source

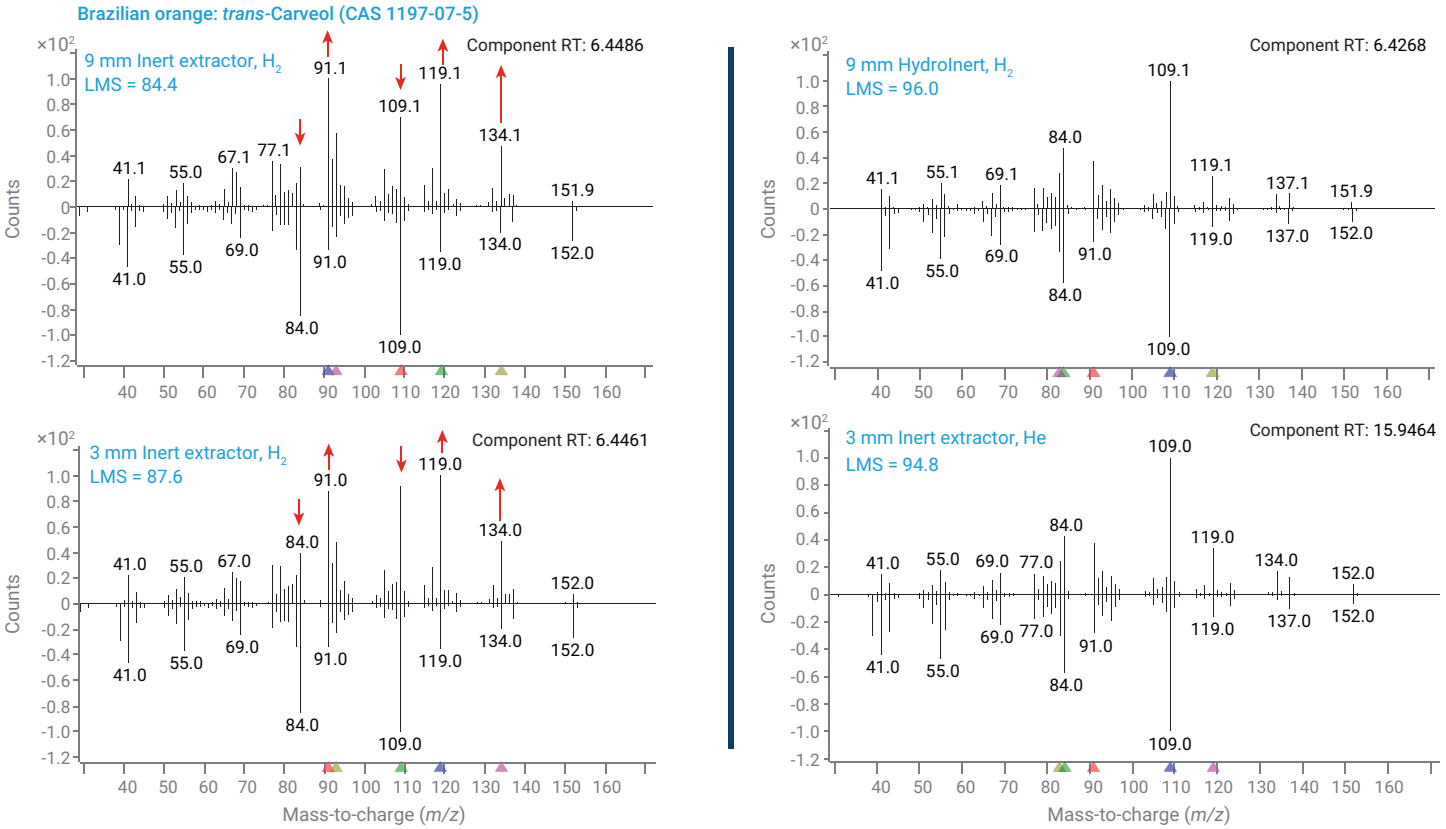
In this section, the spectra of several compounds from the Brazilian orange and neroli oils are examined. For each compound, the spectrum obtained using helium and the 3 mm inert extractor source is presented with the spectrum for each compound obtained using hydrogen with the 9 mm HydroInert source, and the inert extractor source with the 9 and 3 mm extractor lenses. As shown in Figures 7 to 11, changes in the hydrogen spectra without the HydroInert source are clear, resulting in reduced LMS values and reduced confidence in identification.



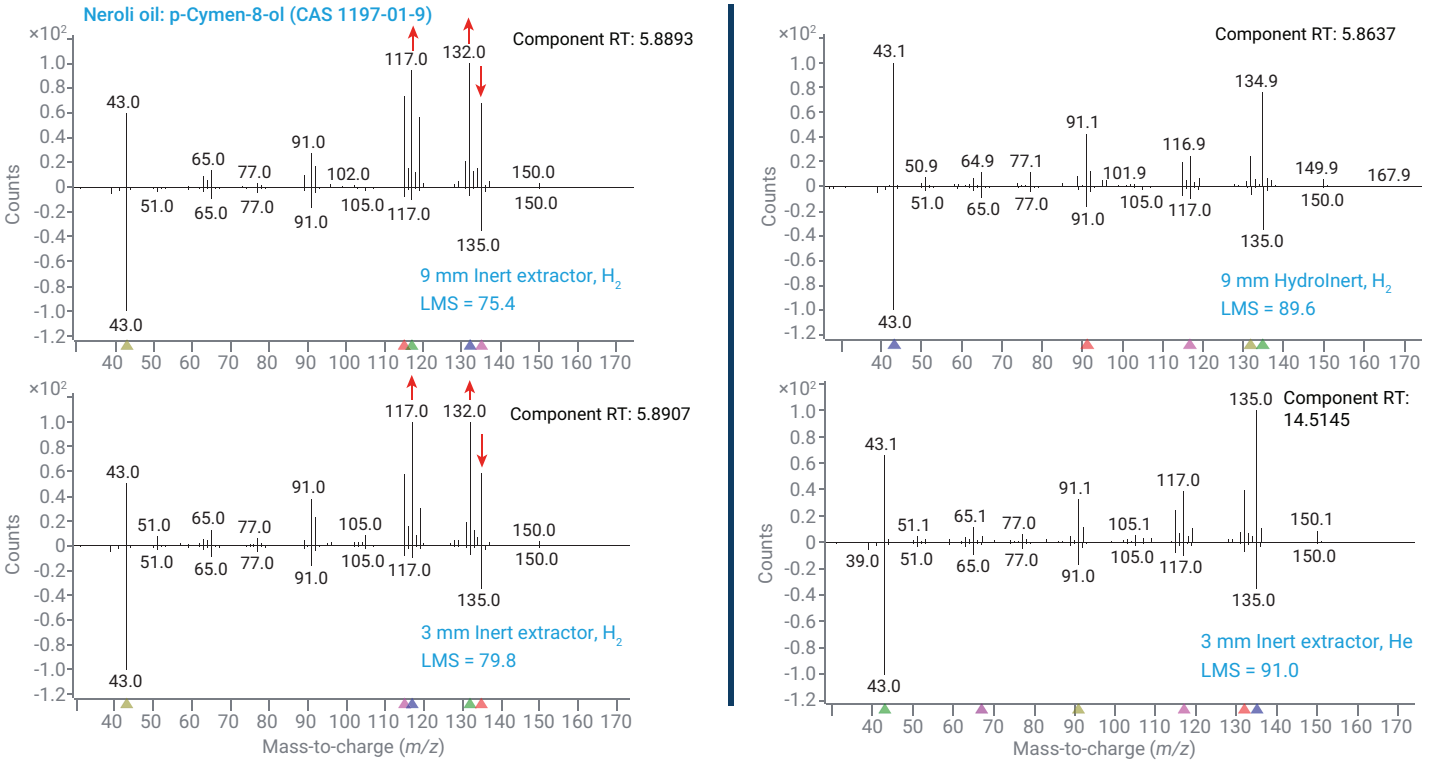
Appendix Figure 7. Linalool in Brazilian orange oil. Red arrows indicate spectral changes observed with hydrogen carrier and non-HydroInert sources.



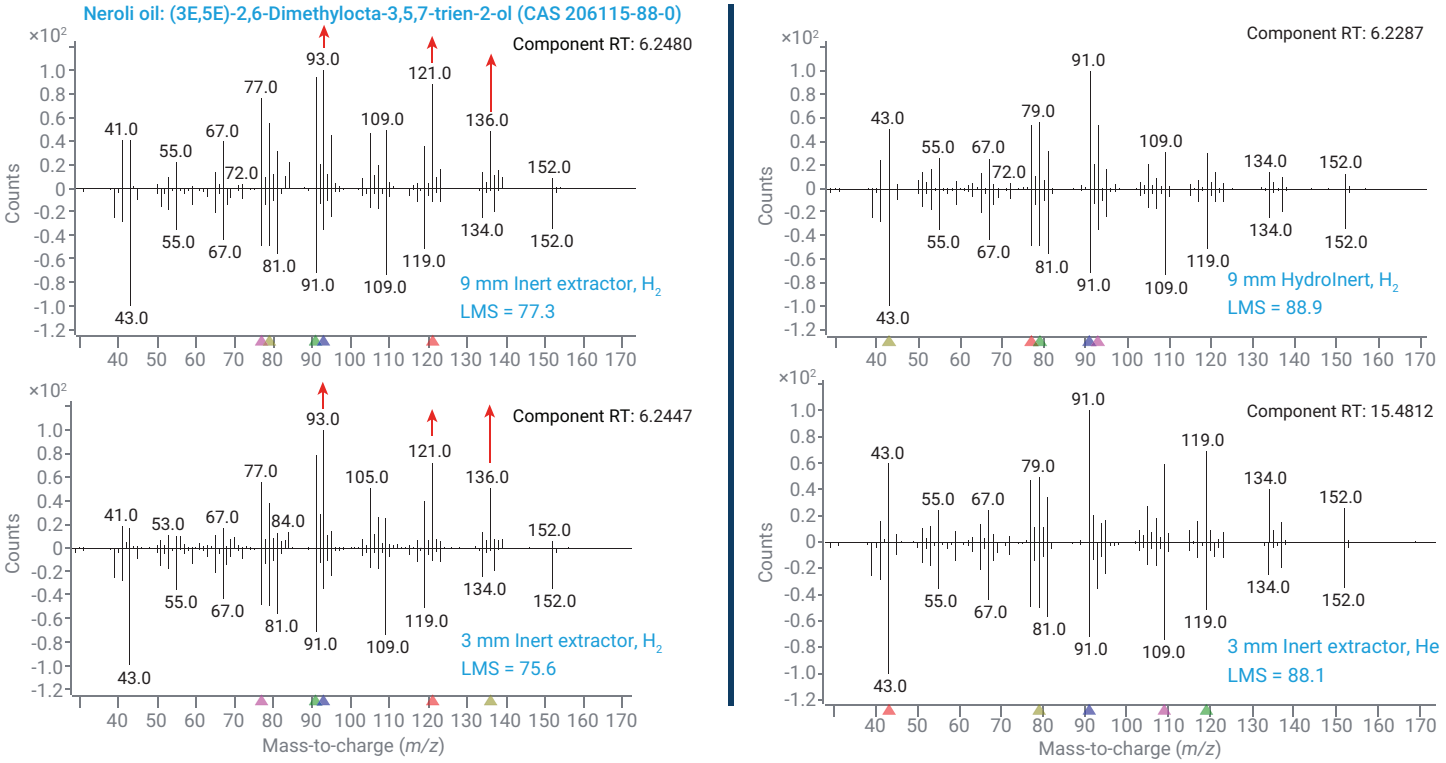
Appendix Figure 8. *cis*-Carveol in Brazilian orange oil. Red arrows indicate spectral changes observed with hydrogen carrier and non-HydroInert sources.



Appendix Figure 9. *trans*-Carveol in Brazilian orange oil. Red arrows indicate spectral changes observed with hydrogen carrier and non-HydroInert sources.



Appendix Figure 10. p-Cymen-8-ol in neroli oil. Red arrows indicate spectral changes observed with hydrogen carrier and non-HydroInert sources.



Appendix Figure 11. (3E,5E)-2,6-Dimethylocta-3,5,7-trien-2-ol in neroli oil. Red arrows indicate spectral changes observed with hydrogen carrier and non-HydroInert sources.

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Printed in the USA, January 26, 2024
5994-7058EN

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Application Note

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Estimation of Ethylene Oxide and 2-Chloroethanol in Spices and Oilseeds Using QuEChERS Extraction and GC/MS/MS

Agilent 8890/7010 triple quadrupole GC/MS system

Authors

Praveen Arya, Soma Dasgupta, and Vivek Dhyani
Agilent Technologies, Inc.

Abstract

This application note demonstrates the use of an Agilent 8890 GC system coupled with an Agilent 7010 GC/MS triple quadrupole mass spectrometry system to detect and quantify ethylene oxide (EtO) and 2-chloroethanol (2-CE) simultaneously in foodstuffs such as flaxseed, cumin powder, and red chili powder. Sample preparation was done using QuEChERS extraction and a dispersive cleanup process followed by injection into GC/MS/MS through liquid injection. A limit of quantification (LOQ) of 10 ppb for both compounds was achieved in matrix. Average recoveries ranged from 75 to 86% for both compounds.

Introduction

EtO is used to sterilize foods to eliminate insects and bacteria, such as salmonella. Ethylene chlorohydrin, or 2-CE, is a derivative produced by the reaction of EtO with chlorine ions present in foodstuff. Use of EtO is banned in the European Union (EU) due to its carcinogenic and toxic properties.

Previously, methods for analysis of EtO (EtO and 2-CE) have been developed that include acidic conversion of EtO to 2-CE. These methods are time-consuming, labor-intensive, and require large quantities of harmful solvents. Due to the volatile nature of EtO, sample preparation is crucial. In December 2020, the EU Reference Laboratories (EURL) for Residues of Pesticides recommended a single-residue method for the analysis of EtO and 2-CE in sesame seeds using QuEChERS extraction followed by GC/MS/MS analysis.

The method adopted in this work demonstrates the use of an automated liquid sampler for sample introduction to the 8890 GC system coupled with a 7010 GC/MS triple quadrupole mass spectrometry system.

Table 1. GC/TQ parameters.

Parameter	Value
GC/MS/MS Method Parameters	
GC	Agilent 8890 GC with G4513 autosampler
Mass Spectrometer	Agilent 7010 triple quadrupole mass spectrometry system
Analytical Column	Agilent J&W DB-VRX (60 m x 0.25 mm, 1.4 µm)
Column Flow	Helium: 1.0 mL/min, constant flow
Injection Mode	Pulsed split (4:1)
Injection Volume	2 µL
Injection Program	Starts at 90 °C (hold for 0.8 min), ramped at 450 °C/min to 250 °C, hold for 10 min
Oven Program	Starts at 40 °C (hold for 1.0 min), ramped at 10 °C/min to 160 °C, and then at 30 °C/min to 245 °C, hold for 5 min
MS Parameters	Ionization mode: EI; Ion source temperature: 230 °C; Quadrupole temperature (Q1 and Q2): 150 °C
MRM Transitions	
EtO	44 & 14 (CE:20) 44 & 28 (CE:5) 44 & 29 (CE:5)
2-CE	80 & 31 (CE:5) 80 & 43 (CE:5) 82 & 31 (CE:5)

Experimental

Standard preparation

Due to the high volatility of EtO, its standard solutions were prepared at a low temperature (< 10 °C). As a diluent, acetonitrile was placed in a freezer (at –20 °C) for at least 15 minutes before use. The cold analytical standard solutions were diluted with cold acetonitrile to prepare the stock standard solutions of EtO and 2-CE, which had a concentration of 1 mg/mL each.

The stock standard solution was further diluted with acetonitrile to obtain a working standard solution which had a concentration of 10 µg/ml for EtO and 2-CE. All the calibration standards (2.0, 5.0, 10.0, 20.0, 50.0, 100, and 200 ng/mL) were freshly prepared by diluting the stock solution with acetonitrile. Matrix-matched standards of flaxseeds, cumin powder, and red chili powder were prepared by post-spiking the requisite amount of solution to extracts of each matrix. Before analysis, all stock solutions were preserved at a temperature of −20 °C to avoid degradation.

Sample preparation

Homogenized samples of flaxseed, cumin powder, and red chili powder were processed using the QuEChERS extraction procedure according to the EN 15662 procedure (Figure 1). Approximately 2.00 ± 0 .01 g of each sample was weighed in 50 mL centrifuge tubes. Ten mL of cold water was added to the centrifuge tubes followed by capping and vortex mixing for 1 minute to ensure sample hydration, followed by the addition of 10.0 mL of cold acetonitrile along with two ceramic homogenizers to improve the extraction efficiency. The centrifuge tubes were capped and shaken for 10 minutes. The QuEChERS extraction salt (4 g of $MgSO_4$, 1 g of NaCl, 1 g of sodium citrate, and 0.5 g of disodium citrate sesquihydrate) was added, and the tubes were shaken for another 3 minutes. The samples were then centrifuged for 5 minutes at 6,000 rpm. The upper acetonitrile layer (6.0 mL) was transferred to QuEChERS Dispersive Kit 15 mL tubes (150 mg of PSA, 150 mg of C18EC , and 900 mg $MgSO_4$). The tubes were vortexed for 30 seconds followed by centrifugation at 5,000 rpm for 5 minutes. After centrifugation, the supernatant was transferred into GC vials for analysis.

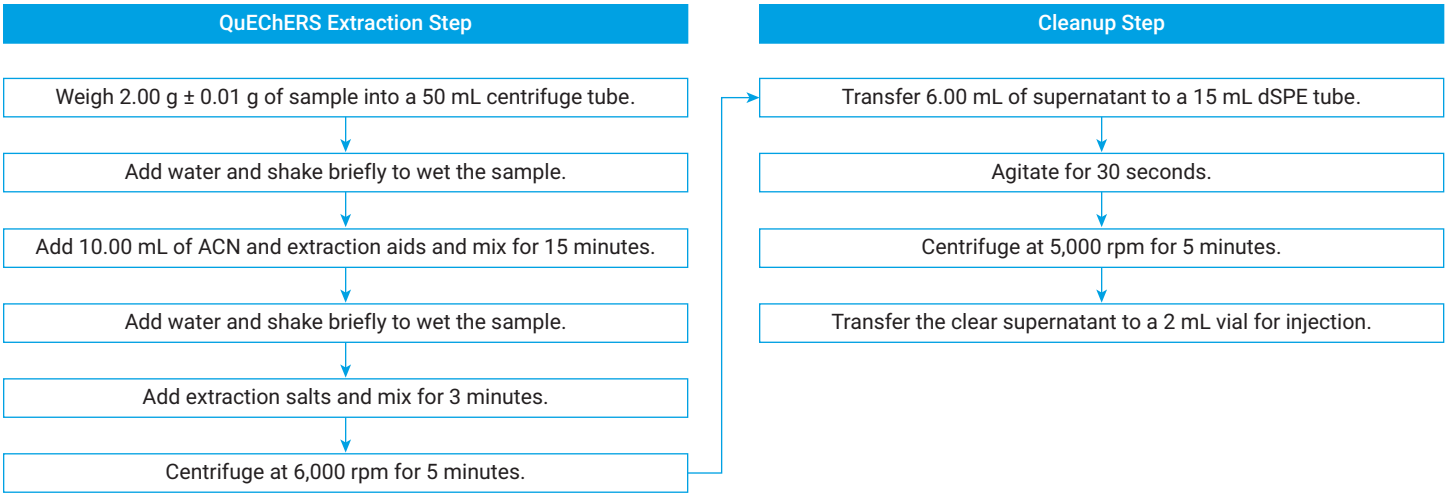

Figure 1. QuEChERS workflow for the extraction and cleanup of samples.

Results and discussion

Calibration

Matrix calibrations were performed for EtO and 2-CE at concentrations of 2, 5, 10, 20, 50, and 100 ng/mL in solvent. Similar calibrations were performed in post-spike matrix extracts for flaxseed, cumin powder, and red chili powder. Excellent values for $R^2 > 0.99$ were obtained.

Figures 8 and 9 show linearity of EtO and 2-CE respectively in acetonitrile. Figures 10 and 11 show linearity of EtO and 2-CE respectively in a flaxseed matrix.

Standard area repeatability

A repeatable elution was obtained by injecting a 10 ppb concentration of EtO and 2-CE in a matrix extract. As shown in Table 2, a % RSD data of EtO and 2-CE is calculated from peak areas of 6 replicate injections of 10 ppb matrix standards in flaxseed, cumin powder, and red chili powder.

Recovery

EtO and 2-CE were spiked in samples of flaxseed, cumin powder, and red chili powder at concentration levels of 20 and 50 ng/g. Acceptable recoveries were obtained by quantification through post-spike matrix-based calibration. Results are highlighted in Table 3.

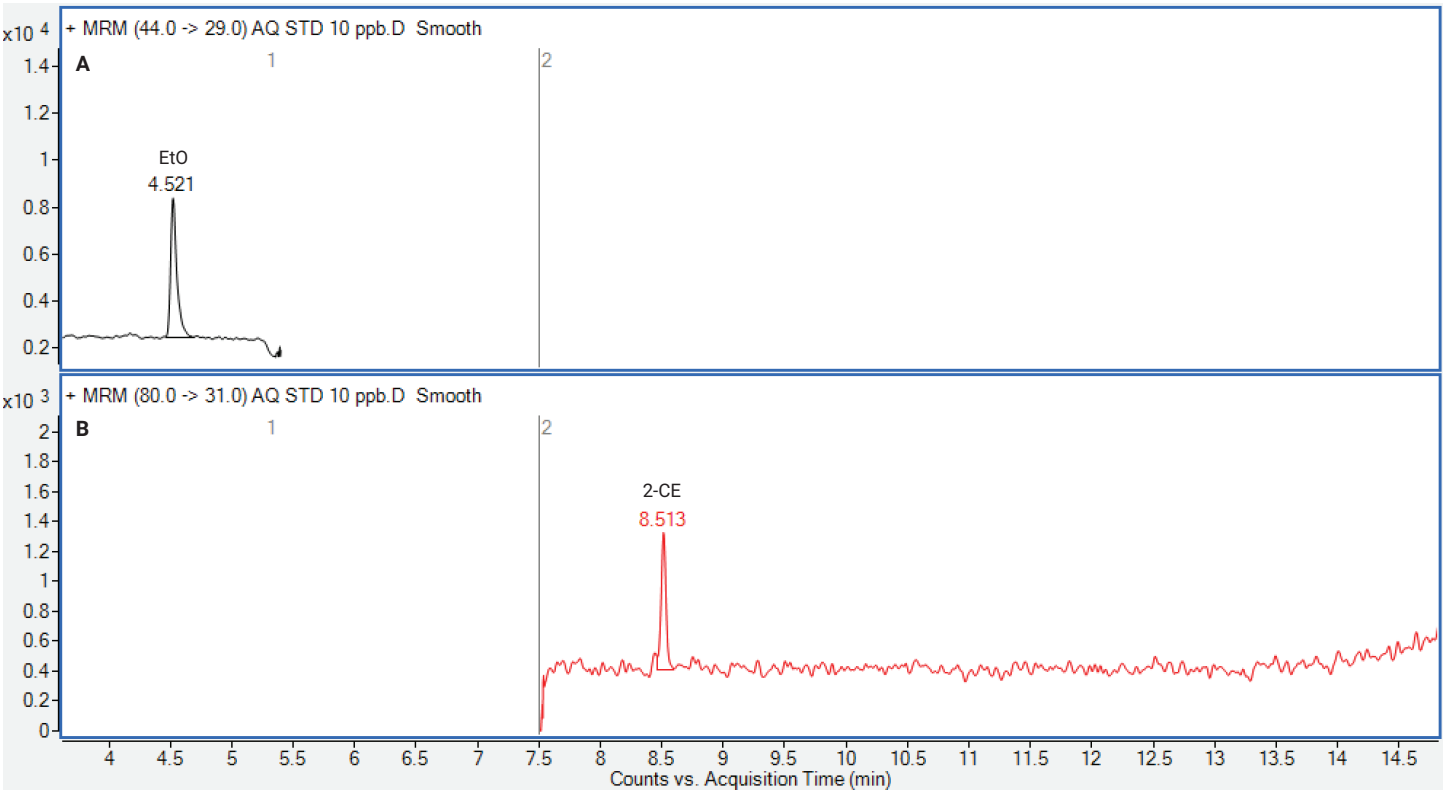

Figure 2. Chromatograms for (A) EtO at the 10 ng/mL level and (B) 2-CE at the 10 ng/mL level.

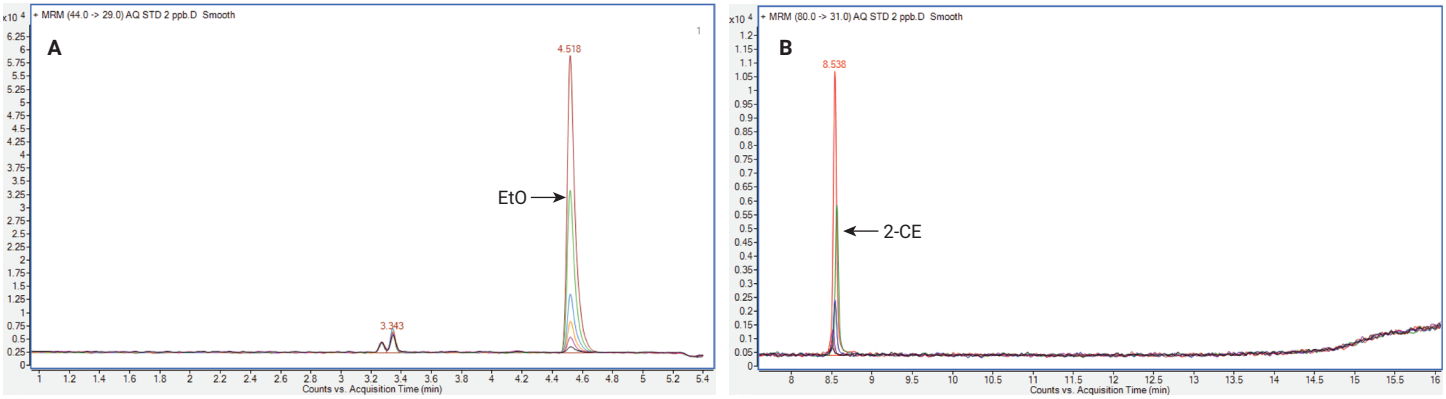

Figure 3. Overlaid chromatograms from a set of calibration standards from 2 ng/mL to 100 ng/mL; (A) EtO and (B) 2-CE.

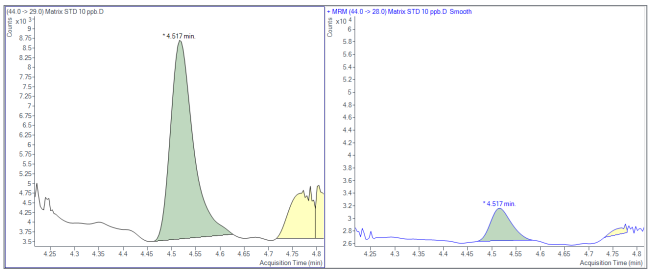

Figure 4. Quantifier and qualifier peaks of EtO at 10 ng/g matrix standard in flaxseed.

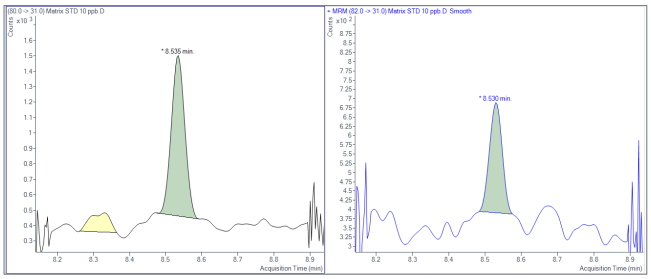

Figure 5. Quantifier and qualifier peaks of 2-CE at 10 ng/g matrix standard in flaxseed.

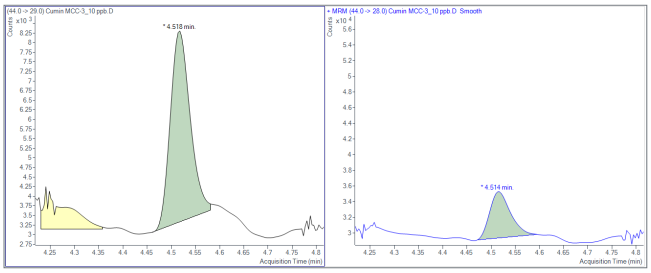

Figure 6. Quantifier and qualifier peaks of EtO at 10 ng/g matrix standard in cumin powder.

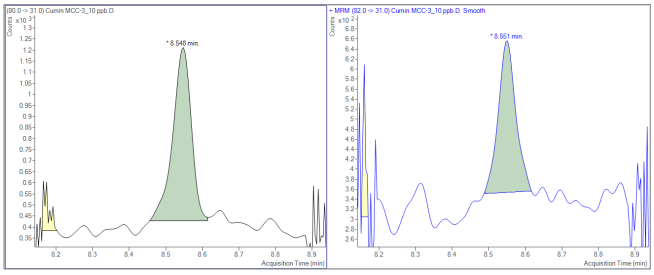

Figure 7. Quantifier and qualifier peaks of 2-CE at 10 ng/g matrix standard in cumin powder.

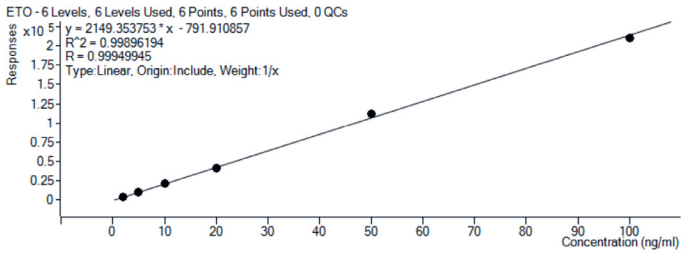

Figure 8. Calibration curve for EtO in acetonitrile (R^2 > 0.998).

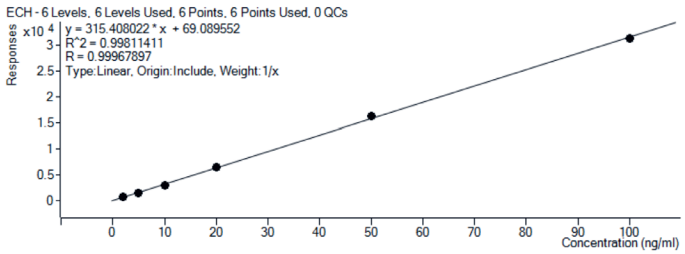

Figure 9. Calibration curve for 2-CE in acetonitrile (R^2 > 0.998).

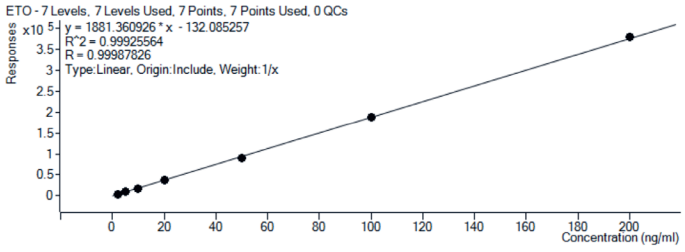

Figure 10. Calibration curve for EtO in flaxseed (R^2 > 0.999).

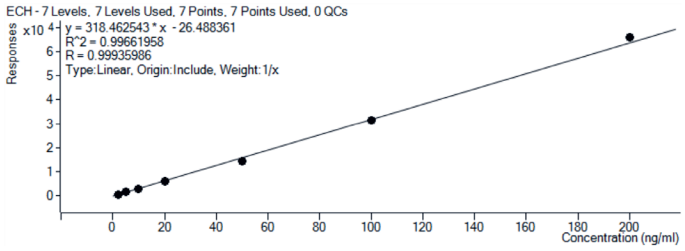

Figure 11. Calibration curve for 2-CE in flaxseed (R^2 > 0.996).

Table 2. Standard area repeatability for 6 replicates of 10 ppb matrix standard in flaxseed, cumin powder, and red chili powder.

Number of Injections (Replicates)	EtO (Peak Area)			2-CE (Peak Area)		
	Flaxseed	Cumin Powder	Chili Powder	Flaxseed	Cumin Powder	Chili Powder
	10 ng/mL					
Injection 1	2,266	2,193	3,135	1,065	893	735
Injection 2	2,264	2,205	3,445	1,049	1,035	910
Injection 3	2,545	2,179	3,425	1,129	1,125	880
Injection 4	2,562	2,162	3,440	993	1,048	755
Injection 5	2,392	2,228	3,385	1,236	1,168	845
Injection 6	2,526	2,135	3,335	1,100	979	785
Mean	2,426	2,184	3,361	1,095	1,041	818
SD	138.32	32.76	118	83.04	99.01	70.68
RSD (%)	5.70	1.50	3.51	7.58	9.51	8.64

Table 3. Percent Recovery of EtO and 2-CE in flaxseed and cumin powder spiked at 20 and 50 ng/g levels, respectively.

Food Matrix	EtO			2-CE		
	Spiked Concentration (ng/g)	Average Obtained Concentration (ng/g)	Recovery (%)	Spiked Concentration (ng/g)	Average Obtained Concentration (ng/g)	Recovery (%)
Flaxseed	20	15.12	75.6	20	15.84	79.2
Cumin Powder	50	39.02	78.0	50	43.29	86.6
Chili Powder	50	38.52	77.04	50	42.47	84.9

Conclusion

An accurate and rugged method was developed which meets the requirements of the EURL for a single-residue method that uses QuEChERS extraction followed by GC/MS/MS analysis for the analysis of EtO and 2-CE in flaxseed, cumin powder, and red chili powder. The LOQ of the method was demonstrated at 10 ng/g for all the tested matrices. Repeatable results are found for 6 successive replicates of matrix-based standards at 10 ng/g concentration levels for EtO and 2-CE. Excellent recoveries were obtained for both EtO and 2-CE in all tested matrices at 20 and 50 ng/g spiked concentration levels. Thus, this study demonstrates the usefulness of the developed method for the routine analysis of food samples for EtO and 2-CE at trace levels.

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DE-000364

Printed in the USA, September 3, 2024
5994-7720EN

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Analysis of Aldehydes in Beer by Agilent PAL3 Autosampler and 5977C GC/MSD

Authors

Yufeng Zhang and
Lay Peng Tan
Agilent Technologies Inc.

Abstract

This application note describes a method for quantitation of four aldehydes (hexanal, furfural, phenylacetaldehyde, and *trans*-2-nonenal) responsible for off-flavors in beer using an Agilent 8890/5977C GC/MSD with an Agilent PAL3 (SPME) autosampler. The method used fully automated, solvent-free extraction and on-fiber derivatization. The aldehyde compounds were derivatized with the derivatization agent O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) using the on-fiber derivatization procedure. The derivatization agent was first adsorbed onto an Agilent 65 µm PDMS/DVB fiber. Next, the fiber was inserted into a 20 mL headspace vial containing a 2 mL beer sample with agitation at 60 °C for 30 minutes. Both extraction and derivatization procedures were automatically performed using the PAL3 autosampler. The method demonstrated excellent sensitivity, with detection limits of 0.0009 µg/L for hexanal, 0.52 µg/L for furfural, 0.015 µg/L for phenylacetaldehyde, and 0.003 µg/L for *trans*-2-nonenal. The limits of the quantification for the four compounds were 0.003, 1.72, 0.05, and 0.01 µg/L, respectively. The four aldehydes were successfully quantified in four beer samples purchased from a supermarket. Good repeatability was demonstrated with RSD < 4.9% based on three replicate injections of the four beer samples for all four aldehydes.

Introduction

Aldehydes are a critical group of compounds in beer that significantly influence its flavor and aroma profile. These volatile organic compounds, even at low concentrations, can impact undesirable sensory characteristics such as cardboard, grassy, or stale flavors, which negatively affect the overall quality and consumer acceptance of the product. The presence of aldehydes is often linked to oxidation processes during brewing, packaging, and storage, making them key indicators of beer freshness and stability.[1] Hexanal, furfural, phenylacetaldehyde, and *trans*-2-nonenal are among the most recognized aldehydes responsible for off-flavors, with flavor thresholds of 350 µg/L, 15.157 mg/L, 105 µg/L, and 0.1 µg/L, respectively.[2]

Monitoring and controlling aldehyde levels in beer are essential for maintaining product consistency and ensuring a high-quality drinking experience. By accurately quantifying aldehydes, brewers can identify potential issues in their production processes, such as oxygen exposure or ingredient degradation, and implement corrective measures to minimize off-flavors. Additionally, understanding the aldehyde profile of beer can aid in the development of new brewing techniques and formulations that enhance flavor stability and shelf life.

Solid-phase microextraction (SPME) is a widely used, solvent-free sample preparation technique that operates on the principles of adsorption and desorption. It uses a fiber coated with an extractive phase to concentrate analytes from a sample. Various types of fibers are available, including PDMS, acrylate, carbon WR, DVB, and combinations of these sorbents, to address the differing polarities of analytes. SPME is extensively used in a range of analyses, including environmental characterization, food and flavor analysis, pharmaceutical studies, and forensics investigations. It is well suited for automated sample preparation, resulting in reduced preparation time per sample, minimized risk of human error, and the freeing up of lab personnel from repetitive tasks.

This application note demonstrates the use of a PAL3 RTC sampler with SPME tool and 8890/5977C GC/MSD for the analysis of the four aldehyde compounds in beer. The SPME sampling tool is equipped with its own read-and-write chip with preset parameters including the stationary phase and usage tracking. Both sample extraction and derivatization were automated with the PAL3 RTC sampler.

Experimental

Reagents and samples

Aldehyde standards (hexanal, furfural, phenylacetaldehyde, and *trans*-2-nonenal) and derivatization reagent PFBHA were purchased from Sigma-Aldrich. HPLC-grade methanol was from Merck. The water was Milli-Q Ultrapure. Four different brands of beer were purchased from a local supermarket.

Standards preparation

A mixed stock solution consisting of 10 µg/mL hexanal, 10 µg/mL phenylacetaldehyde, 1,000 µg/mL furfural, and 1 µg/mL *trans*-2-nonenal was prepared in methanol.

A secondary mixed stock solution consisting of 100 µg/L hexanal, 100 µg/L phenylacetaldehyde, 10 µg/mL furfural, and 10 µg/L *trans*-2-nonenal was prepared in ultrapure water.

Using the secondary mixed stock solution, a series of calibration standards were prepared with a concentration range of 0.05 to 10 µg/L for hexanal, 5 to 1,000 µg/L for furfural, 0.1 to 50 µg/L for phenylacetaldehyde, and 0.025 to 5 µg/L for *trans*-2-nonenal.

Two milliliters of each calibration standard was added to a 20 mL headspace vial and immediately capped for analysis.

PFBHA derivatization reagent with a concentration of 60 mg/L was prepared by weighing 30.1 mg of powder PFBHA into a 500 mL volumetric flask and dissolving in ultrapure water up to the 500 mL mark. Ten milliliters of the 60 mg/L PFBHA solution was added to a headspace vial to be used for sample derivatization.

Sample preparation

Beer samples were stored in a refrigerator at 4 to 6 °C prior to analysis. A 250 mL portion of the beer was poured into a clean plastic bottle and capped. The sample was degassed by hand shaking the capped bottle five times followed by opening the cap to release the carbon dioxide (CO_2). The degassing of the samples was performed a total of three times. A 2 mL degassed beer sample was transferred to a 20 mL headspace vial, which was immediately capped for analysis.

PAL3 RTC and GC/MSD parameters

The analysis parameters of the PAL3 RTC sampler are shown in Table 1.

Table 1. Agilent PAL3 autosampler and GC/MSD parameters for beer analysis.

Agilent PAL3 (SPME)	
Fiber Type	Agilent 65 µm PDMS/DVB (p/n 5610-5873)
Fiber Conditioning Temperature	250 °C
Preconditioning Time	5 min
Incubation Time	20 min
Incubation Temperature	60 °C
Derivatization Time	10 min
Agitator Speed	250 rpm
Sample Extraction Time	30 min
Sample Desorption Time	1 min
Post Conditioning Time	5 min
Gas Chromatograph	
Model	Agilent 8890 GC
GC Column	Agilent J&W DB-5ms UI, 30 m × 0.25 mm, 0.25 µm (p/n 122-5532UI)
Column Pneumatics	Constant flow
Carrier Gas	Helium
Injector Mode	Splitless
Purge Flow to Split Vent	50 mL/min at 2 min
Inlet Temperature	250 °C
Injector Liner	Agilent Ultra Inert splitless liner (p/n 5190-4047)
Flow Rate	1.2 mL/min
Oven Temperature Program	60 °C for 2 min
	10 °C/min to 140 °C
	7 °C/min to 250 °C, hold 3.0 min
Equilibration Time	3 min
Mass Spectrometer	
Model	Agilent 5977C GC/MSD
Ionization Mode	EI, 70eV
Acquisition Mode	Scan
Scan Speed	N = 2
Scan Range	50 to 520 amu
GC Transfer Line Temperature	250 °C
Ion Source Temperature	230 °C
Quad Temperature	150 °C

Results and discussion

Compound identification and retention time confirmation

The 10 µg/L calibration standard was analyzed in full scan data acquisition mode, and the total ion chromatogram (TIC) is shown in Figure 1.

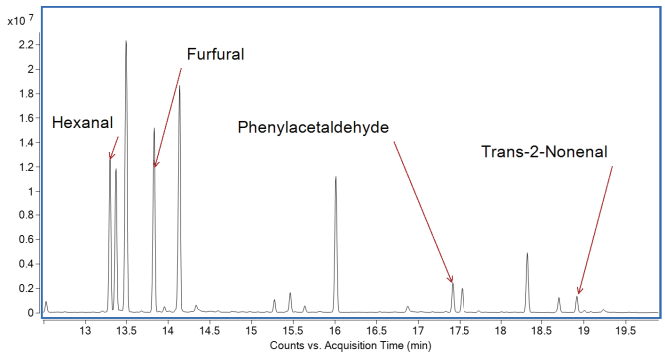

Figure 1. TIC of 10 µg/L aldehydes with Agilent SPME on-fiber PFBHA derivatization.

The data file for the 10 µg/L sample was processed using MassHunter Unknowns Analysis software. Automatic deconvolution of the data was performed using Unknowns Analysis to identify the components present exclusively in the sample. From the resulting list of components, the four target aldehydes were identified through library matching against the NIST 23 spectral library, achieving match scores above 80. The retention times (RTs) of the four aldehyde derivatives were identified as 13.299, 13.830, 17.419, and 18.914 minutes, respectively (Figures 2 to 5).

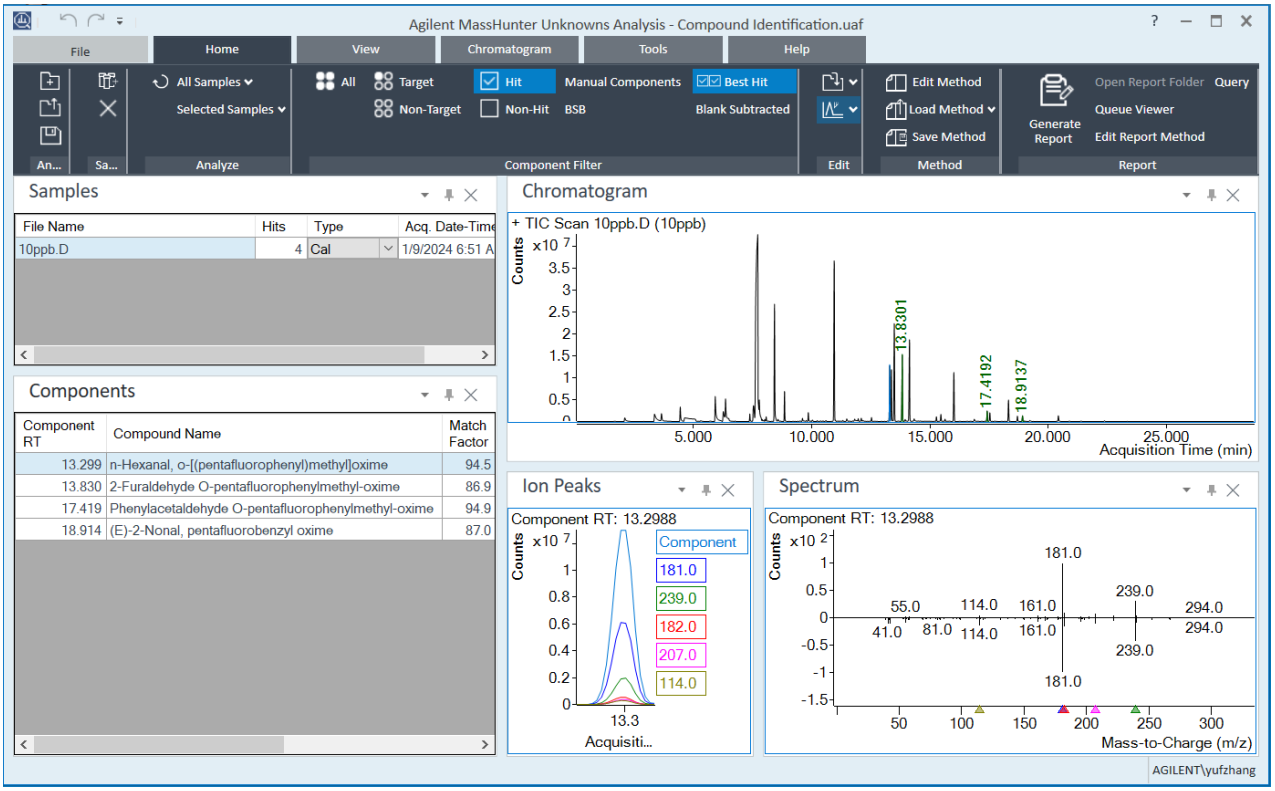

Figure 2. Hexanal derivative identified with an RT of 13.299 minutes.

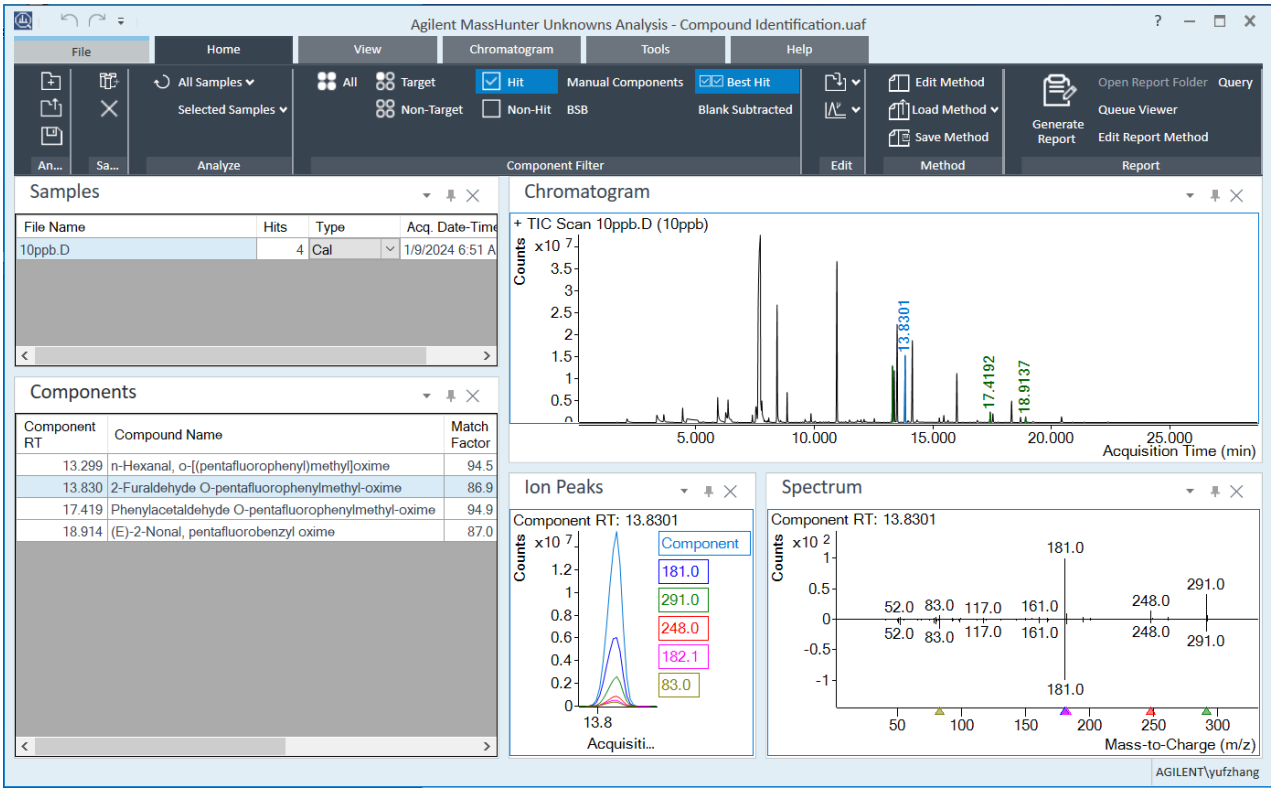

Figure 3. Furfural derivative identified with an RT of 13.830 minutes.

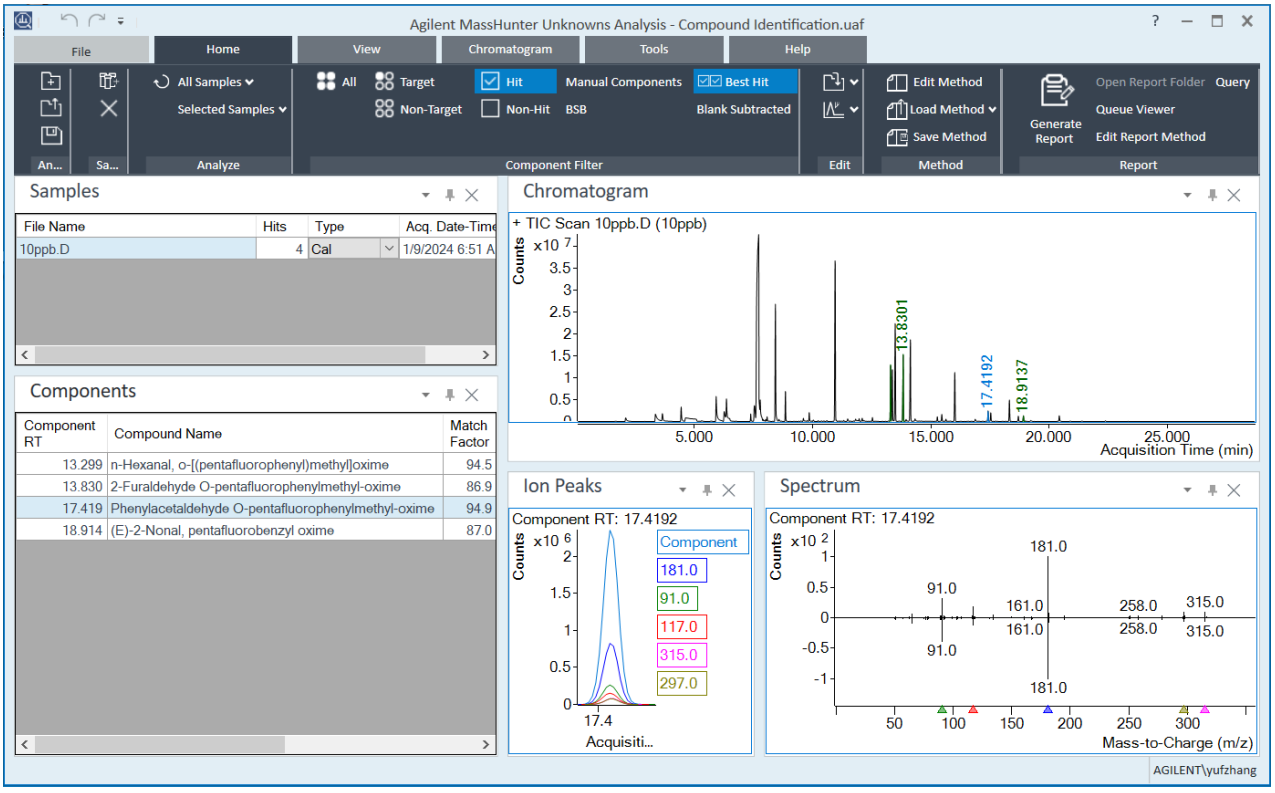

Figure 4. Phenylacetaldehyde derivative identified with an RT of 17.419 minutes.

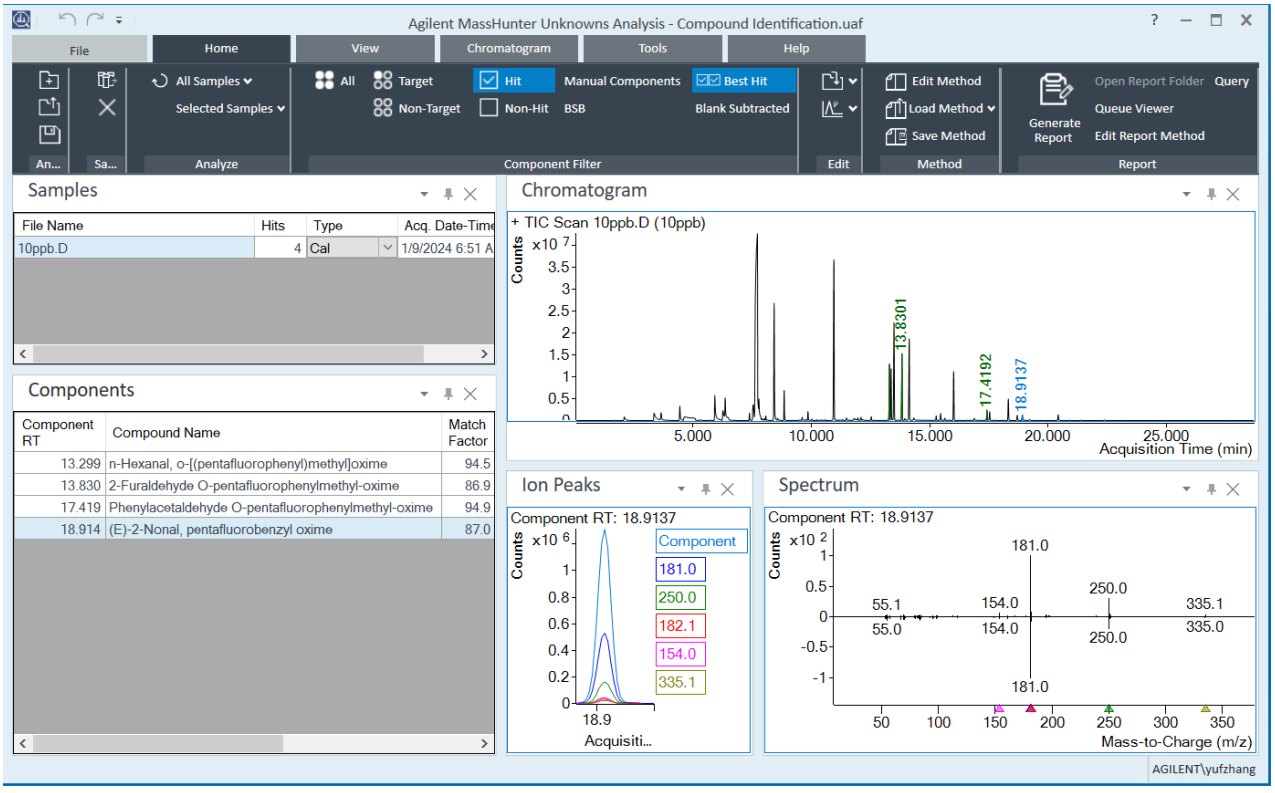

Figure 5. *trans*-2-Nonenal derivative identified with an RT of 18.914 minutes.

Calibration curves

Based on the response of the calibration standard solutions, the calibration curves were plotted for the four aldehyde derivatives. The results are shown in Table 2 and Figures 6 to 9.

Table 2. The calibration range and R^2 for the four aldehydes.

No.	Compound Name	Calibration Range (µg/L)	R^2
1	Hexanal	0.05 to 10	0.999
2	Furfural	5 to 1,000	0.998
3	Phenylacetaldehyde	0.1 to 50	0.996
4	*trans*-2-Nonenal	0.025 to 5	0.998

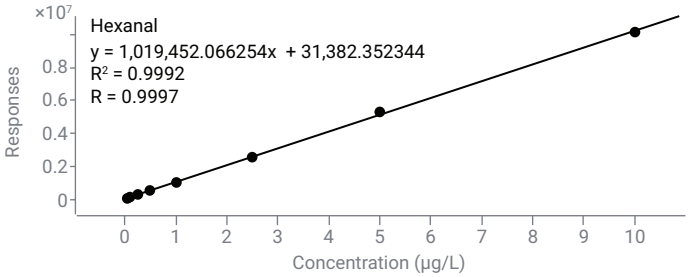

Figure 6. Calibration curve for hexanal 0.05 to 10 µg/L.

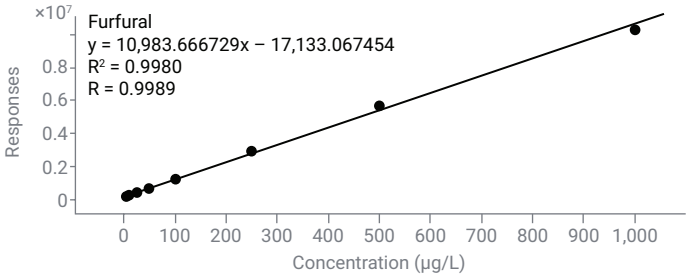

Figure 7. Calibration curve for furfural 5 to 1,000 µg/L.

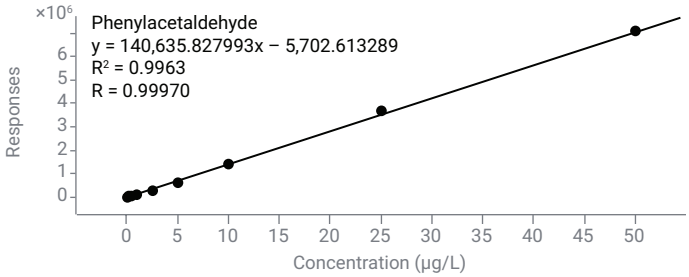

Figure 8. Calibration curve for phenylacetaldehyde 0.1 to 50 µg/L.

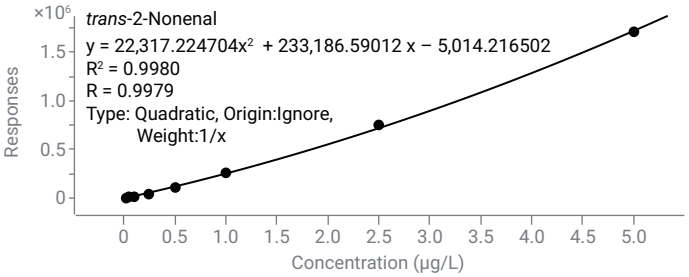

Figure 9. Calibration curve for *trans*-2-nonenal 0.025 to 5 µg/L.

Quantification results of beer samples

Based on the calibration curve setup, quantification was performed for the four aldehydes in the four brands of beer samples that were tested. The quantification results are summarized in Tables 3 to 6. All the aldehydes in the four beer samples were below their respective flavor thresholds. Among the four brands of beer samples that were tested, Brand 4 contained the lowest levels of aldehydes, with 0.45 µg/L hexanal, 6.26 µg/L furfural, 6.64 µg/L phenylacetaldehyde, and 0.031 µg/L *trans*-2-nonenal.

Table 3. Quantification results of hexanal in four brands of beer samples.

No.	Beer	Hexanal Concentration (µg/L)			Average Concentration (µg/L)	Concentration %RSD (n = 3)
1	Brand 1	0.67	0.67	0.69	0.68	1.7
2	Brand 2	1.53	1.54	1.61	1.56	2.8
3	Brand 3	0.99	1.03	1.04	1.02	2.6
4	Brand 4	0.45	0.46	0.45	0.45	1.3

Table 4. Quantification results of furfural in four brands of beer samples.

No.	Beer	Furfural Concentration (µg/L)			Average Concentration (µg/L)	Concentration %RSD (n = 3)
1	Brand 1	18.45	18.75	18.45	18.55	0.9
2	Brand 2	49.46	49.39	52.27	50.37	3.3
3	Brand 3	24.81	26.53	25.04	25.46	3.7
4	Brand 4	6.10	6.37	6.30	6.26	2.2

Table 5. Quantification results of phenylacetaldehyde in four brands of beer samples.

No.	Beer	Phenylacetaldehyde Concentration (µg/L)			Average Concentration (µg/L)	Concentration %RSD (n = 3)
1	Brand 1	11.07	10.41	10.05	10.51	4.9
2	Brand 2	8.36	8.26	8.07	8.23	1.8
3	Brand 3	8.72	8.63	8.92	8.76	1.7
4	Brand 4	6.84	6.59	6.48	6.64	2.8

Table 6. Quantification results of *trans*-2-nonenal in four brands of beer samples.

No.	Beer	Trans-2-Nonenal Concentration (µg/L)			Average Concentration (µg/L)	Concentration %RSD (n = 3)
1	Brand 1	0.034	0.035	0.034	0.034	1.7
2	Brand 2	0.061	0.063	0.063	0.062	2.8
3	Brand 3	0.034	0.034	0.035	0.034	1.7
4	Brand 4	0.030	0.031	0.031	0.031	1.3

With three replicate injections for each beer sample, the concentration %RSDs were calculated to be below 4.9%. The TIC overlays of the three replicate injections are shown in Figures 10 to 13.

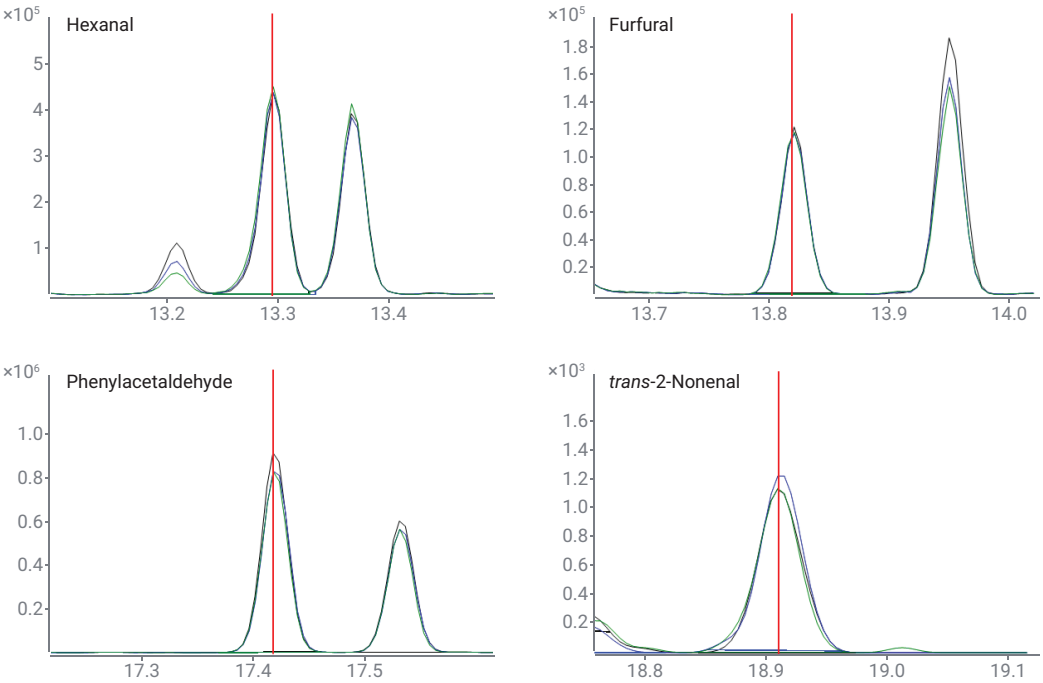

Figure 10. TIC overlay of the four aldehydes in the Brand 1 beer sample.

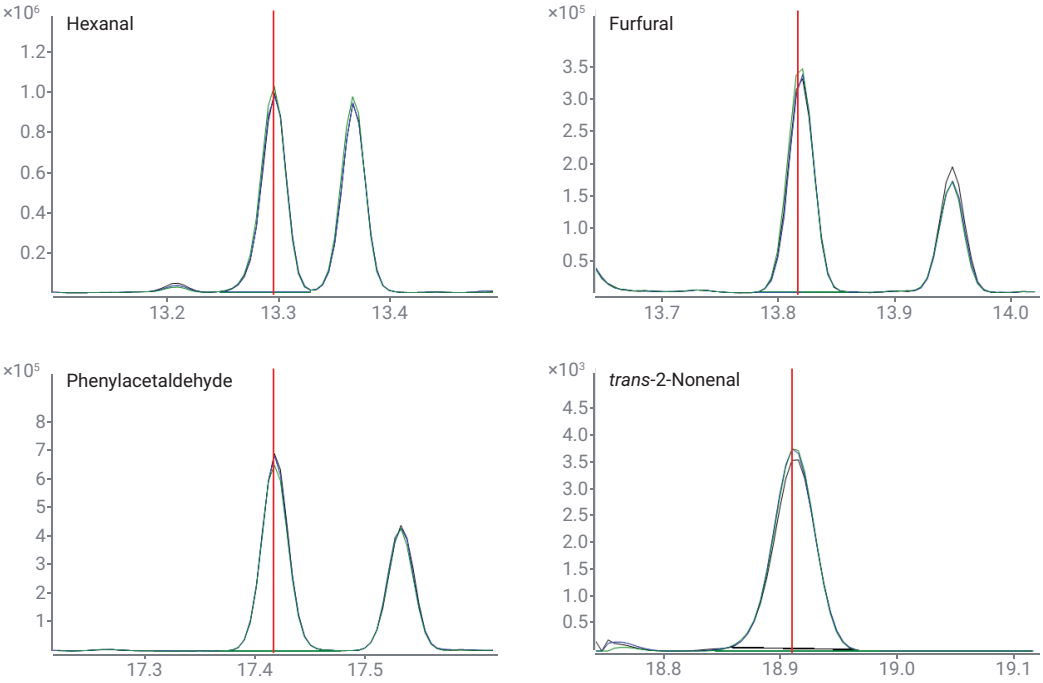

Figure 11. TIC overlay of the four aldehydes in the Brand 2 beer sample.

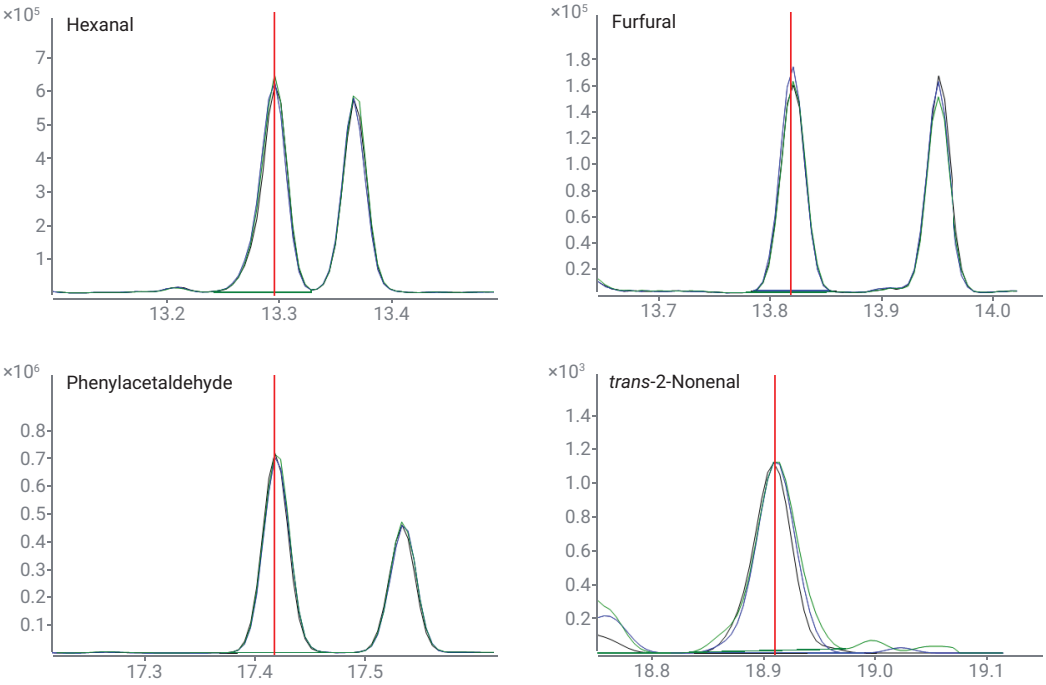

Figure 12. TIC overlay of the four aldehydes in the Brand 3 beer sample.

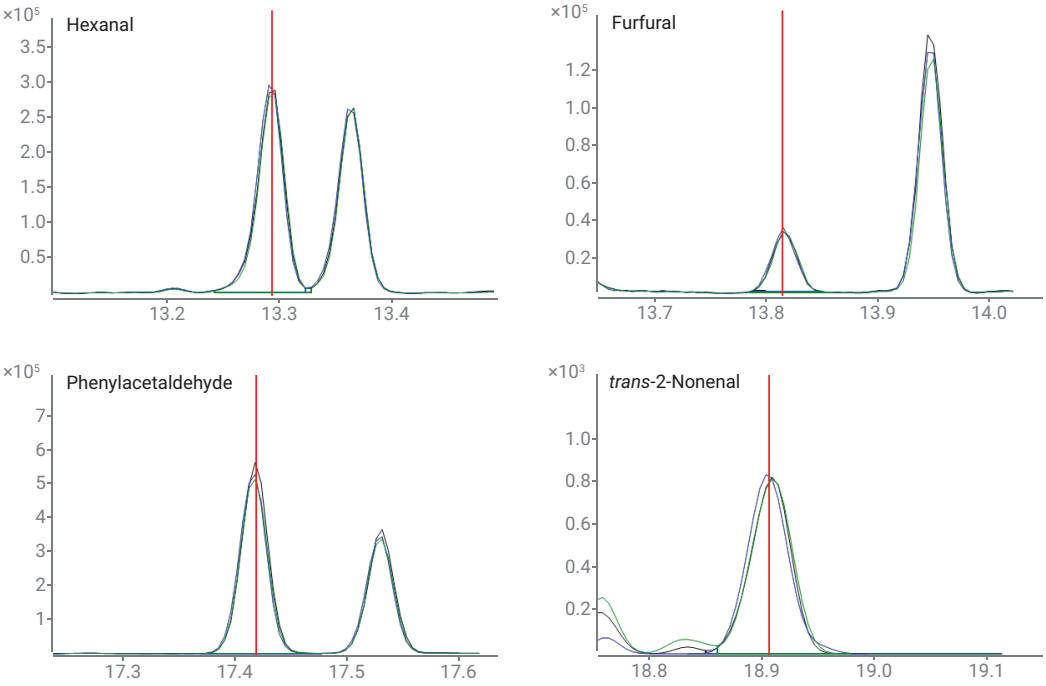

Figure 13. TIC overlay of the four aldehydes in the Brand 4 beer sample.

Determination of detection limits

Using the lowest calibration concentration of each compound, the signal-to-noise ratio (S/N) in full scan mode was calculated. The limit of quantification (LOQ) was determined at a S/N of 10, and the limit of detection (LOD) was determined at a S/N of 3. A summary of the LOQ and LOD results is presented in Table 7.

Table 7. LOQ and LOD of the aldehydes.

Aldehyde	S/N	LOQ (µg/L)	LOD (µg/L)
Hexanal (0.05 µg/L)	161	0.003	0.0009
Furfural (5 µg/L)	29	1.72	0.52
Phenylacetaldehyde (0.1 µg/L)	20	0.05	0.015
trans-2-Nonenal (0.025 µg/L)	24	0.01	0.003

Conclusion

This application note describes the quantitative analysis of four aldehydes (hexanal, furfural, phenylacetaldehyde, and *trans*-2-nonenal) responsible for off-flavors in beer using an Agilent 8890/5977C GC/MSD with a PAL3 (SPME) autosampler. This method offers the advantages of full automation, rapid analysis, solvent-free extraction, and on-fiber derivatization. With this automated solution, excellent sensitivity was demonstrated for the detection of hexanal (0.0009 µg/L), furfural (0.52 µg/L), phenylacetaldehyde (0.015 µg/L), and *trans*-2-nonenal (0.003 µg/L). Four different brands of beer were analyzed, with hexanal detected in the range of 0.45 to 1.56 µg/L, furfural in the range of 6.62 to 50.37 µg/L, phenylacetaldehyde in the range of 6.64 to 10.51 µg/L, and *trans*-2-nonenal in the range of 0.031 to 0.062 µg/L. Good repeatability was demonstrated with RSD < 4.9% based on three replicate injections of the four beer samples for all four aldehydes.

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Published in the USA, November 19, 2024
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