Advanced GC/MS Techniques for Enhanced Analysis of Trace Compounds in Complex Matrices

- Deconvolution Reporting Software (DRS)
  - Retention Time Locking
  - AMDIS deconvolution and NIST search
- Improving Chromatography by Capillary Flow Technology
  - Backflush and other Benefits
- Twodimensional GC only if time is left

Moritz Hebestreit, PhD
Applications Chemist
Agilent Technologies
Europe, Middle East, Africa and India
Agilent GC/MS Portfolio

A family of solutions designed for your workflow

- **Ultimate combination of qualitative and quantitative capability**
  - Q-TOF GC/MS

- **High speed & sensitivity; New MassHunter SW**
  - Triple Quad GC/MS

- **Greater flexibility between EI, CI, and MS/MS modes**
  - Ion Trap GC/MS

- **Industry-standard workhorse**
  - Single Quad GC/MS

- **Foundation: Industry-leading performance, usability, reliability, and support**

- **Demanding analyses** (R&D and routine)
  - (metabolomics, food, toxicology)

- **High-throughput, high sensitivity**
  - Targeted quantitative analyses
  - (food, drugs, environmental)

- **Routine analyses**
  - (environmental, food, pharmaceutical, chemical, materials)
7890A/5975C GC/MSD: Industry’s Best Advanced GC & MS Features

- Improved signal-to-noise with Trace Ion Detection
- Improved CFT for backflush and 2-dimensional GC
- Application specific Retention Time Locked Databases
- High sensitivity and library searching with synchronous SIM/scan
- Safety and performance tested with hydrogen gas
- Deconvolution (DRS) for analysis of complex samples
- Gain normalized methods for consistent sensitivity from MSD to MSD
- EM Saver eliminating EM damage from response to high matrix
MSD Deconvolution Reporting Software

A tool to Aid in the Identification and Quantitation of Target Compounds

with a brief overview of Retention Time Locking (RTL)
RetentionPolicy Locking

What Is Retention Time Locking (RTL)?

The ability to match precisely chromatographic retention times in any system to those in another chromatographic system with the same nominal method and column.
Different Columns, Same GC

Retention times vary from column-to-column by as much as 0.5 min...
Results for 5 Retention Time Locking Runs on GC/MS

1. RTLOCK1.D: 17.345 -20 % pressure or flow
2. RTLOCK2.D: 16.940 -10 % pressure or flow
3. RTLOCK3.D: 16.567 nominal pressure or flow
4. RTLOCK4.D: 16.214 +10 % pressure or flow
5. RTLOCK5.D: 15.885 +20 % pressure or flow

Note: Relocking e.g. after column maintenance only needs one run.
Retention Time Locking

Retention times match from column-to-column AND instrument-to-instrument to 0.030 min or better...

GC1  Column 1  
GC2  Column 2  
GC3  Column 3  
GC4  Column 4
Benefits of RTL

- Never have to change SIM nor MRM group times
- Never have to change integration events
- Never have to change ion extraction windows
- Leak detection
- Correct column?
- Rapid data comparison
- Uniform methods and method transfer
Locking a GC/MS Method

• Analyst gets a “good” chromatogram
• Five runs are made of a locking mixture, automatically, each at a different pressure
• Analyst picks a peak to lock on
• GC/MS auto-finds peak in each run
• Calibration curve is calculated and method is locked
• This process is only done once; relocking after e.g. column-cut is only one injection
DRS - TICs of surface water extracts

How many pesticides are in these samples?
Seventeen (17) surface water samples

<table>
<thead>
<tr>
<th></th>
<th>CDFA*</th>
<th>Agilent DRS with 927 Compound Database</th>
</tr>
</thead>
<tbody>
<tr>
<td>Targets Found (not counting ISTD)</td>
<td>37</td>
<td>Same 37 +99 more</td>
</tr>
<tr>
<td>False Positives</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Processing Time</td>
<td>~8 hrs (ChemStation Only)</td>
<td>34 min</td>
</tr>
</tbody>
</table>

*CDFA is the California Department of Food and Agriculture. Data files courtesy of Dr. Mark Lee and Steve Siegel.
DRS = MSD + AMDIS + NIST Search

- MSD Chemstation
  - RTLock and ID targets based on r.t. and 4 ions and quantitate
- AMDIS
  - ID targets based on deconvoluted full spectra and qualify targets based on r.t.
- NIST Search
  - search targets using deconvoluted full spectra from AMDIS against NIST library
- Automate the above and produce an easy to read Report
What is **AMDIS**?

- **Automatic Mass spectral Deconvolution and Identification Software**
  - Developed to automatically detect chemicals in violation of Chemical Weapons Convention
  - Identify *target* compounds at low concentration levels in complex matrices
  - Developed by National Institute of Standards and Technology
  - Retention Time can be used as a qualifier, so RTL is a big advantage
A Single Chromatographic Peak May Contain Multiple Components

TIC & Spectrum

Components and Mixed Spectra

- Component 1
- Component 2
- Component 3
AMDIS Deconvolution Pulls Out Individual Components and their Spectra

Components and Mixed Spectra

Deconvoluted components and spectra

TIC

Component 1
Component 2
Component 3

Deconvolution

matrix

interference

target
DRS Report from 3 Integrated Processes

Total ion chromatogram

- AMDIS deconvolutes component spectra and searches target MS database using locked RT as a qualifier
- Confirmed AMDIS hits
- AMDIS Quant Result
- Confirmed NIST08 hits

Targets are identified by comparison to locked R.T.s and 3 qualifier ion ratios, then quantified using target ion area vs ISTD cal table

Combined quantitative and qualitative HTML summary report

Deconvoluted Target spectra confirmed by AMDIS, searched against NIST08 MS database

10/11/2011
CERTECH
Emissions and Odours from Materials - 2011
Spinach Report, results from 3 DRS processes

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>18.4431</td>
<td>84742</td>
<td>Di-n-butylphthalate</td>
<td>7.04</td>
<td>95</td>
<td>1.7</td>
<td>91</td>
<td>2</td>
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<td>23.9754</td>
<td>80057</td>
<td>Bisphenol A</td>
<td></td>
<td>97</td>
<td>8.7</td>
<td>91</td>
<td>1</td>
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<tr>
<td>24.0705</td>
<td>72559</td>
<td>p,p'-DDE</td>
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<td>74</td>
<td>3.0</td>
<td>82</td>
<td>1</td>
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<tr>
<td>25.7154</td>
<td>72548</td>
<td>p,p'-DDD</td>
<td>52</td>
<td>52</td>
<td>1.8</td>
<td>65</td>
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<tr>
<td>26.9932</td>
<td>50293</td>
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<td>53</td>
<td>0.7</td>
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<td>27.0103</td>
<td>85687</td>
<td>Butyl benzyl phthalate</td>
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<td>54</td>
<td>0.2</td>
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<td>27.9265</td>
<td>51036</td>
<td>Piperonyl butoxide</td>
<td>37.79</td>
<td>96</td>
<td>1.6</td>
<td>94</td>
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<tr>
<td>29.6685</td>
<td>117817</td>
<td>Bis(2-ethylhexyl)phthalate</td>
<td></td>
<td>93</td>
<td>1.2</td>
<td>85</td>
<td>3</td>
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<tr>
<td>31.6131</td>
<td>52645531</td>
<td>Permethrin II</td>
<td></td>
<td>90</td>
<td>3.8</td>
<td>91</td>
<td>3</td>
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<tr>
<td>13.718</td>
<td></td>
<td>Phenanthrene-d10</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ISTD, used for targets amount

Match against AMDIS library

RT diff in sec. vs expected locked RT

NIST 08 Match and Hit number of the top 100 hits from 191,000+ compounds
DRS User Benefits

Ease of use  no need to learn deconvolution software

Automation  part of a method or part of a sequence, analyst can be doing other things

Quality  program results are not subject to changes in “mood/attention” of analyst

Sensitivity  AMDIS will find answers that an analyst might miss

Confidence  DRS will report the fewest false positive / negatives in the shortest time
DRS App Notes Available

- “Screening for Hazardous Chemicals in Homeland Security and Environmental Samples Using GC/MS/ECD/FPD with a 731 Compound DRS Database” pub # 5989-4834
- “Improved Forensic Toxicology Screening Using A GC/MS/NPD System with a 725-Compound DRS Database” pub # 5989-8582
- “Semivolatiles Retention Time Locked (RTL) Deconvolution Databases for Agilent GC/MSD Systems “ pub # 5989-7875
- Can "Deconvolution" Improve GC/MS Detectability?” 5990-5052
DRS App Notes Available

• “Comprehensive Pesticide Screening by GC/MSD using Deconvolution Reporting Software” pub # 5989-1157
• “New Tools for Rapid Pesticide Analysis in High Matrix Samples” pub # 5989-1716
• “A Blind Study of Pesticide Residues in Spiked and Unspiked Fruit Extracts Using Deconvolution Reporting Software” pub # 5989-1654
• “Building and Editing RTL Screener/Quant Databases and Libraries” pub # 5989-0916
• “Building Agilent GC/MSD Deconvolution Reporting Libraries for Any Application” pub # 5989-2249 (32 pages, so, use the videos !!!)
• “Screening for 171 Volatile Organic Air Pollutants Using GC/MS with Deconvolution Reporting Software and a New Indoor Air Toxics Library” 5989-5435
### Commercial and RTL Databases

<table>
<thead>
<tr>
<th>MS Library</th>
<th>Application Area</th>
<th>Number of spectra (approx.)</th>
<th>Product Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIST 2008 MS Library</td>
<td>General purpose</td>
<td>220,000+</td>
<td>G1033A</td>
</tr>
<tr>
<td>Wiley 8th/NIST 2008 MS Library</td>
<td>General purpose</td>
<td>562,000+</td>
<td>G1035B</td>
</tr>
<tr>
<td>Stan Pesticides MS Library</td>
<td>Pesticides</td>
<td>340</td>
<td>G1038A</td>
</tr>
<tr>
<td>Maurer/Pfleger/Weber MS Library 2007</td>
<td>Drugs and metabolites</td>
<td>7,840 data sets</td>
<td>G1039D</td>
</tr>
<tr>
<td>Hazardous Chemicals RTL Library</td>
<td>Environmental</td>
<td>731</td>
<td>G1671AA</td>
</tr>
<tr>
<td>Pesticides RTL Library</td>
<td>Pesticides and endocrine disrupters*</td>
<td>926</td>
<td>G1672AA</td>
</tr>
<tr>
<td>Indoor Air Toxics RTL Library</td>
<td>Environmental</td>
<td>171</td>
<td>G1673AA</td>
</tr>
<tr>
<td>Forensic Toxicology RTL Library</td>
<td>Forensic</td>
<td>723</td>
<td>G1674AA</td>
</tr>
<tr>
<td>Japanese Positive List Pesticide RTL Library</td>
<td>Pesticides</td>
<td>431</td>
<td>G1675AA</td>
</tr>
<tr>
<td>Fiehn GC/MS Metabolomics RTL Library</td>
<td>Metabolomics</td>
<td>1,000+</td>
<td>G1676AA</td>
</tr>
<tr>
<td>Environmental Semi-Voas RTL Library</td>
<td>Environmental</td>
<td>273</td>
<td>G1677AA</td>
</tr>
</tbody>
</table>

*Mass spectra were collected on an Agilent 6890/5973 GC-MSD system under retention-time locked conditions.

**In Preparation: RTL Library of VOCs Relevant to Materials Emissions**
## Free RTL Databases


<table>
<thead>
<tr>
<th>Database Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RTL GC/MS Databases for Organotin Derivatives</strong></td>
<td>Includes methyl, ethyl, and pentyl derivatives</td>
</tr>
<tr>
<td><strong>VOC MS Library and RTL Databases for GC and MS</strong></td>
<td>Includes the GC RT-locked database, the MS screener database, and full-spectra library of 65 volatile organic compounds</td>
</tr>
<tr>
<td><strong>PCB Congener GC/MSD RTL Databases</strong></td>
<td>Includes two RT-locked screener databases and MS library for 209 PCB congeners.</td>
</tr>
<tr>
<td><strong>Forensic Toxicology GC/MSD RTL Databases</strong></td>
<td>Includes an RT-locked screener database and MS library for 277 drugs and other substances</td>
</tr>
<tr>
<td><strong>Fatty Acid Methyl Ester GC and GC/MSD RTL Databases</strong></td>
<td>Includes RT-locked GC retention time databases and GC/MSD spectral libraries and screener databases for 37 common FAMEs</td>
</tr>
<tr>
<td><strong>Flavors RTL Databases for GC/FID and GC/MS</strong></td>
<td>Includes RT-locked GC/FID and GC/MS retention time databases and mass spectral library containing 409 compounds.</td>
</tr>
<tr>
<td><strong>Allergens DRS Database for GC/MS</strong></td>
<td>Includes RT-locked GC/MS method, retention time databases and mass spectral library containing 24 regulated allergens and their isomers (total 32 target compounds and 1 internal standard), installation guide and related application note.</td>
</tr>
</tbody>
</table>
Capillary Flow Technology

-- solves difficult application problems easily & opens up many new (and old) possibilities for GC & GC/MS
Capillary Flow Technology

• IF we only had a technology that provided easy, reliable flow structures in the GC oven, it would open up many new (and old) capabilities
  – Column connections (connect pre-column)
  – Change MSD columns (without venting)
  – Backflush (Reverse flow through column)
  – Detector splitter (effluent split to two or more detectors)
  – Merge flows (2 columns to 1 MSD)
  – Deans switch (heart cut selected peaks to 2nd column)
  – Comprehensive 2-D GC (cut all peaks to 2nd column)
  – etc.
### Types of Connectors Used In The GC Oven

<table>
<thead>
<tr>
<th>Connector Type</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metal Fittings</td>
<td>Packed columns, reliable</td>
<td>Not inert, no ferrule for capillary columns</td>
</tr>
<tr>
<td>Press Fit Glass</td>
<td>Low dead volume, inert, low cost</td>
<td>Difficult to assemble, comes apart</td>
</tr>
<tr>
<td>Graphite</td>
<td>High temperature</td>
<td>Sheds active graphite particles into sample path</td>
</tr>
<tr>
<td>Polyimide</td>
<td>Low initial leakage</td>
<td>Loosens and leaks with oven cycling, solvent tailing</td>
</tr>
</tbody>
</table>
Challenges For Inside the Oven Devices

- **Inertness** (it is in the sample path)
- **Low dead volume** (it is in the separation path)
- **Leak free** (especially with repeated temp cycling)
- **Fast thermal response** (follow rapid oven ramping)
- **High temp tolerance** (GC oven can go over 350 °C)
- **Reliable and easy to use**
5 Key Developments in Capillary Flow Technology

- **Metal Ferrules**: Easy to use, do not loosen or leak with oven cycling to 400°C
- **Manifold Plates**: Complex flow structures with low thermal mass
- **Deactivation of Metal**: Makes metal surfaces as inert as column
- **EPC**: Backflushing now possible, change MSD columns without venting, known column outlet pressure
- **Calculators**: Accurately predict flows and pressures BEFORE installing devices
The Metal Ferrule

Does not loosen (leak) even with *thousands* of runs to 350C

Does not shed particles
Capillary Flow Technology - Design

... a proprietary Agilent Technology

- Photolithographic chemical milling for low dead volume
- Diffusion bond two halves to form a single flow plate
- Small, thin profile provides fast thermal response
- Projection welded connections for leak tight fittings
- Deactivation of all internal surfaces for inertness
3-Way Splitter With Makeup

Effluent Splitter
(3 Way)

- Det3 out
- Det2 out
- Det1 out
- Column in
- Aux EPC in
Capillary Flow Technology-Capabilities

- Solvent Bypass
- Heart Cutting (Deans Switch)
- Backflush
- Detector Splitting
- Column Change without Vent
- Modulation (GCXGC)
Agilent Capillary Flow Technology - Backflush

- **Split/Splitless, MMI or PTV Injection Port**
- **Vent**
- **7890A GC**
- **Pressure/Flow Controller**
- **Z mL/min very low flow; set against column backpressure**
- **Y ml/min**
- **Y+Z ml/min**
- **Z ml/min**
- **Capillary Flow Technology Device**
- **2.7m pre-column (0.25mm id x 0.0µm)**
- **30m HP-5MS UI (0.25mm id x 0.25µm)**
- **Purged Union**
- **Column 1 and 2 could be different phases and different dimensions**

Emissions and Odours from Materials - 2011
Forward Flow
Backflush Flow

Inlet EPC

P1

Split Vent

Column 1
(or Retention Gap)

PUU

P2

Aux EPC

Column 2
(or Restrictor)

MSD
Milk Extract

It took additional 33 mins and column to 320°C to remove these high boilers.

Run stopped at 42 min and backflushed at 280°C for 7 mins.

Blank run after backflushing showing the column was clean.
Summary CFT

Capillary Flow Technology solves difficult application problems easily.

It opens up many new (and old) possibilities for GC and GC/MS systems.
Thank you for your attention!

Questions?

moritz_hebestreit@agilent.com
2-D GC, Comparing a Deans Switch with Traditional Hardware

More Reliable

- 3 times fewer fittings
- New Capillary Column Connector
  - No graphite/vespel capillary connectors
- No leaks at high temperatures

Better Performance

- 4 times lower thermal mass
  - Shorter flow paths, optimized for capillary GC
- Fully inert flow path
  - Better peak shape, less tailing
Deans Switch

Heartcutting 2-D GC provides extremely high chromatographic resolution

Auto-sampler

FID1

Cut

FID2

Deans Switch

Column 1

7890A GC

Column 2
Heart Cutting 2-D GC – How It Works

Valve off, no heart cutting—inject sample, initial separation on column 1

Column 1: HP-1MS

Sample

FID

3.0 mL/min

Restrictor

<< 1 mL/min

PCR

Off

S/S Inlet

38 psi

2.0 mL/min

Column 2: DB-17MS

MSD

3.0 mL/min

Column 1: HP-1MS

Sample

30 psi

2.0 mL/min

Purge restrictor

4.0 mL/min

26 psi
Heart Cutting 2-D GC – How It Works

Valve on – start heart cut from column 1 to column 2

FID

3.0 mL/min

S/S Inlet

38 psi

2.0 mL/min

Column 1: HP-1MS

Restrictor

1mL/min

3.0 mL/min

Column 2: DB-17MS

4.0 mL/min

Purge restrictor

<<1mL/min

MSD

38 psi

26 psi

PCM

0n

Valve on – start heart cut from column 1 to column 2.
Selected Cuts on Column 1 (30m DB-1MS)
Interfacing LTM to an Agilent 6890/7890 GC

Modules outside isothermal GC oven for fast heating/cooling

Use same GC injectors, detectors, autosamplers, software, …

Independent and simultaneous temperature programming of:
1-2 col modules (standard, fast cool)
1-4 col modules (small)

2 LTM Column Modules
(standard width shown)

LTM Control System
Keypad User Interface
Agilent Control SW now available

Note: Front thermal shield removed to show LTM column modules
Entire thermal shield should always be in place during operation
Low-Thermal-Mass GC Columns Allow for Two Independent Oven Programs

No practical limitations to capillary choices for GC/MS

Small size

Efficient, fast temperature programming

Rapid cooling (large surface area with small mass)
Agilent’s flow modulator design: Differential Flow

Flow modulator eliminates the need for cryo. Sample compression controlled by flow ratios occurs in the collection loop and is quickly injected into the second column, resulting in very narrow and tall peaks.

Differential flow concept is designed by John V. Seeley, Oakland University
Load or collect step

Load time must not be longer than time to fill collection channel

*Seeley JV, Micyus NJ, McCurry JD, Seeley SK, AMERICAN LABORATORY 38 (9): 24-26 Suppl. N APR 2006
Inject step

Collection channel is quickly “injected” into second column in about 0.1 seconds

*Seeley JV, Micyus NJ, McCurry JD, Seeley SK, AMERICAN LABORATORY 38 (9): 24-26 Suppl. N APR 2006
Peak capacity

GC x GC peak capacity is:
1000 x 25 = 25000 in this example

Conventional GC

ALS

n= 1000

Column 1

Modulator

Fast GC

n = 25

Fast GC

Column 2

Detector
N-butylbenzene

unmodulated

modulated
Flow modulation: (GC x GC) of diesel fuel: 7890A

GC x GC Chromatogram:
- Showing the normal B.P. distribution (1st dimension)
- Also shows hydrocarbon classes in clusters
- Consistent RT for alkanes in 1st dimension showing precise modulation
- Comparable peak in 2nd dimension band shows minimum peak broadening with flow modulation
Agilent Flow Modulation GC x GC

- **Reliable Setup:** Based on CFT, easy to setup, high performance chromatography, and reliable.
- **No Cryogen Required:** Flow modulation means no tanks of Liquid N\textsubscript{2} or CO\textsubscript{2}
- **7890A Enabled GC x GC:** Capillary-flow-technology ready, synchronized periodic events ensure precise modulation, control from a modified TCD board
- **Comparable resolution without Cryo:** CFT allows low dead volume and precise flow control, resulting in minimum peak broadening without cryo-focusing. Peak widths on the second column are typically 70 to 100 ms at half maximum.