Sample Preparation Techniques for Emerging Contaminants in Water

Golnar Javadi, Application Engineer

Dr. Sheher Bano Mohsin, Senior Scientist

Agilent Technologies
Today’s Agenda

• Analysis of emerging contaminants in water – an overview
• Offline sample preparation options for drinking water analysis
  – Solid-supported liquid/liquid extraction
  – Solid phase extraction
• New developments in online SPE
• Summary of each approach
• Question and answer period
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Why the Interest in Emerging Contaminants?

• > 3,000 drugs and PPCPs
• Interest and fate of drugs, hormones
• Limited knowledge of treatability for all contaminants
• High public visibility and concern
## Examples of EC Concerns

<table>
<thead>
<tr>
<th>Pharmaceuticals</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Veterinary and human antibiotics</td>
<td>Trimethoprim, erythromycin, lincomycin, sulfamethoxazole</td>
</tr>
<tr>
<td>Analgesics, anti-inflammatory drugs</td>
<td>Codein, ibuprofen, acetaminophen, acetylsalicylic acid,</td>
</tr>
<tr>
<td></td>
<td>diclofenac, fenoprofen</td>
</tr>
<tr>
<td>Psychiatric drugs</td>
<td>Diazepam</td>
</tr>
<tr>
<td>Lipid regulators</td>
<td>Bezafibrate, clofibric acid, fenofibrac acid</td>
</tr>
<tr>
<td>β-blockers</td>
<td>Metoprolol, propanolol, timolol</td>
</tr>
<tr>
<td>X-ray contrasts</td>
<td>Iopromide, iopamidol, diatrizoate</td>
</tr>
<tr>
<td>Steroids and hormones</td>
<td>Estradiol, estrone, estriol, diethylstilbestrol</td>
</tr>
<tr>
<td>Personal care products</td>
<td>Nitro, polycyclic and macrocyclic musks,</td>
</tr>
<tr>
<td>Fragrances</td>
<td>Benzophenone, methylbenzylidene camphor</td>
</tr>
<tr>
<td>Sun-screen agents</td>
<td>N,N-diethylytoluamide</td>
</tr>
<tr>
<td>Insect repellents</td>
<td>Antiseptics</td>
</tr>
<tr>
<td></td>
<td>Triclosan, Chlorophene</td>
</tr>
<tr>
<td>Surfactants and surfactant metabolites</td>
<td>Alkylphenol ethoxylates, 4-nonylphenol,</td>
</tr>
<tr>
<td></td>
<td>4-octylphenol, alkylphenol carboxylates</td>
</tr>
<tr>
<td>Flame retardants</td>
<td>Polybrominated diphenyl ethers (PBDEs),</td>
</tr>
<tr>
<td></td>
<td>Tetrabromo bisphenol A, C₁₀-C₁₃ chloroalkanes</td>
</tr>
<tr>
<td></td>
<td>Tris (2-chloroethyl)phosphate</td>
</tr>
<tr>
<td>Industrial additives and agents</td>
<td>Chelating agents (EDTA), aromatic sulfonates,</td>
</tr>
<tr>
<td>Gasoline additives</td>
<td>Dialkyl ethers, Methyl-t-butyl ether (MTBE)</td>
</tr>
</tbody>
</table>

**Pesticides/Herbicides**

**Sugar Substitutes**
Requirements for Emerging Contaminant Analysis Methods

• Selectivity/Specificity
• Sensitivity: MRL determination
• Target List
• QA/QC
• Ruggedness/matrix effects
• Cost
Innovative LC-MS/MS Approaches to EC Analysis

- Cost-effective screen for as many analytes as possible
- Reporting levels: 5 to 10 ng/L (ppt)
- Known target analytes and identification from experts
- Accuracy and precision comparable to existing drinking water methods: 70-130% recovery
- Use multiple ion transitions (MRM) to prevent misidentification
Today’s Agenda

- Analysis of emerging contaminants in water – an overview
- Offline sample preparation options for drinking water analysis
  - Solid-supported liquid/liquid extraction
  - Solid phase extraction
- New developments in online SPE
- Advantages of each approach
- Question and answer period
Historical Sample Preparation Scheme for Low Level Analyte Detection

- Large sample vessels transported to/from sampling sites
- Collection of water samples, typically 1000 mL
- Transport promptly to laboratory for analysis
- Preservation of backlog in walk-in coolers
- Bench-top serial sample preparation
- Instrumental analysis and reporting

Turnaround time?  Typically long
Sample prep cost?  Typically high
Sample Preparation

Offline SPE

- 1000 mL transported to the lab
- Trace enrich onto a cartridge or disk
- Elute with few mL solvent, dry, reconstitute in solvent amenable to LC or GC for separation
- 1 mL sample loaded into autosampler vial
- 2-20 µL injected for instrumental analysis
Sample Preparation – Supported LLE (SLE)

**Hydromatrix** - diatomaceous earth sorbent

- Composed of fossilized diatoms
- Purified at high temperatures
- High surface area for water adsorption
- Very polar surface

**Chem Elut** - pre-assembled cartridges with Hydromatrix
The SLE Process

Before Extraction

Dry sorbent

Apply Sample

Aqueous layer

Extract with Organic Solvent

Organic layer
The Chem Elut Method

Aqueous sample being applied

Solid support adsorbs water onto high surface area particles

Organic extraction solvent
Solid Supported LLE - Traditional Benefits

- No emulsions
- Less glassware
- Less time
- Reduced technique dependence
- Increased reproducibility
- Automatable; batch processing
Solid Phase Extraction (SPE)

- **Types of SPE**
  - Reversed phase SPE
  - Cation exchange SPE
  - Anion exchange SPE
  - Mixed mode SPE
  - Specialty SPE

- **Capabilities**
  - Very selective
  - Clean samples
  - Wide range of applicability
  - Automation friendly
  - Concentration of analytes with removal of background
### Solid Phase Extraction Protocol

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Acids</th>
<th>Neutrals</th>
<th>Bases</th>
</tr>
</thead>
<tbody>
<tr>
<td>LogP &gt; 1.0</td>
<td>LogP &gt; 1.5</td>
<td>LogP &gt; 0.8</td>
<td></td>
</tr>
<tr>
<td>pK_a &lt; 5</td>
<td>pK_a 3.5</td>
<td>pK_a 6-10</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Pre-Treat Sample
- Sample Pre-treatment:
  - Plexa PAX: 2% NH₂OH
  - Plexa (Acid load method): 1% HCO₃H
  - Plexa (Base load method): 2% NH₂OH
  - Plexa PCX: 2% H₃PO₄

#### Condition
- Sorbent Condition:
  - 100% MeOH

#### Equilibrate
- Equilibration:
  - 100% H₂O

#### Load
- Load:
  - Apply pre-treated sample

#### Wash
- Wash:
  - 100% H₂O

#### Elute
- Elution 1/Wash 2:
  - 100% MeOH
- Elution 2:
  - 5% HCO₃H in MeOH

#### Prep for Analysis
- Analysis:
  - Prepare extracts for instrumental analysis
Solid Phase Extraction Application Example – Haloacetic Acids in Drinking Water

1. Methyl chloroacetate
2. Methyl bromoacetate
3. Methyl dichloroacetate
4. Dalapon methyl ester
5. Methyl trichloroacetate
6. 1,2,3-Trichloropropane (IS)
7. Methyl bromochloroacetate
8. Methyl 2-bromobutanoate (SS)
9. Methyl bromodichloroacetate
10. Methyl dibromoacetate
11. Methyl dibromochloroacetate
12. Methyl tribromoacetate
**Plexa Method – Easy Extraction Method Covers a Wide Range of Analytes**

SPE Method using 200 mg/6 mL Bond Elut Plexa polymeric SPE

<table>
<thead>
<tr>
<th>Step</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Condition with 10 mL MeOH</td>
</tr>
<tr>
<td>2.</td>
<td>Condition with 10 mL H₂O</td>
</tr>
<tr>
<td>3.</td>
<td>Load 800 mL water sample</td>
</tr>
<tr>
<td>4.</td>
<td>Dry sorbent with air for 10 minutes</td>
</tr>
<tr>
<td>5.</td>
<td>Soak and collect 2.5 mL fraction using EtOAc</td>
</tr>
<tr>
<td>6.</td>
<td>Collect 1 mL fraction using EtOAc</td>
</tr>
<tr>
<td>7.</td>
<td>Soak and collect 2.5 mL fraction using DCM</td>
</tr>
<tr>
<td>8.</td>
<td>Collect 1 mL fraction using DCM</td>
</tr>
</tbody>
</table>

Collect into same sample vial

HPLC-FL/UV for determination of PAHs
GC/MS for Chloro-Pesticides
LC-MS/MS for Triazines
## Pesticides and PAHs from Drinking Water on Plexa

### Chloro-Pesticides

<table>
<thead>
<tr>
<th>Alachlor</th>
<th>Aldrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDD o-p’</td>
<td>DDD p-p’</td>
</tr>
<tr>
<td>DDE o-p’</td>
<td>DDE p-p’</td>
</tr>
<tr>
<td>DDT p-p’</td>
<td>DDT o-p’</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>Endosulfan I (alfa)</td>
</tr>
<tr>
<td>Endosulfan II (beta)</td>
<td>Endosulfan sulfato</td>
</tr>
<tr>
<td>Endrin</td>
<td>HCH-alfa</td>
</tr>
<tr>
<td>HCH-beta</td>
<td>HCH-delta</td>
</tr>
<tr>
<td>HCH-gamma</td>
<td>Heptachloro</td>
</tr>
<tr>
<td>Heptachloro Epoxide trans.</td>
<td>Hexachlorobenceno</td>
</tr>
</tbody>
</table>

### Triazines

| DESISPROPYLATRAZINE           | DESETHYLATRAZINE               |
| CIANAZINE                    | SIMAZINE                      |
| ATRAZINE                     | TERBUTRINE                    |
| PROPAZINE                    | TERTBUTYLZINE                 |

### PAHs

- Benzo(a)anthracene
- Benzo(b)fluoranthene
- Benzo(k)fluoranthene
- Benzo(a)pyrene
- Dibenz(a,h)anthracene
- Benzo(ghi)perylene
- Indeno(1,2,3-cd)pyrene
- Chrysene

**Using Plexa all three compound classes can be extracted on a single cartridge**
**Triazines Extracted from Drinking Water with Bond Elut Plexa**

**HPLC column:** C18, 2.1 x100mm 1.7 µm

- **Concentration of spiked triazines:** 0.4 µg/L
- **Max. conc. in validation:** 4 µg/L
- **LQ:** 0.05 µg/L
- **Recoveries > 98%** (rel. recoveries calculated with IS Simazine D10)

MRM of 17 Channels ES+ TIC

1.15e7

2.58

1.71

4.56

3.67

5.52

5.36

EM

0.00 0.50 1.00 1.50 2.00 2.50 3.00 3.50 4.00 4.50 5.00 5.50 6.00 6.50 7.00 7.50 8.00 8.50 9.00

**Time**

**%**

0

100

07110961

MRM of 17 Channels ES+

1.15e7
### Relative Recoveries of Chloro-Pesticides

![Graph showing relative recoveries of various chloro-pesticides.]

<table>
<thead>
<tr>
<th>Chlorpesticide</th>
<th>% Recovery 0.1µg/L</th>
<th>% Recovery 0.04µg/L</th>
<th>% Recovery 0.02µg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCH-alfa</td>
<td>84</td>
<td>81.83</td>
<td>82.17</td>
</tr>
<tr>
<td>HCH-beta</td>
<td>89.5</td>
<td>104.5</td>
<td>104.67</td>
</tr>
<tr>
<td>Hexachlorobenzole</td>
<td>53.13</td>
<td>56.83</td>
<td>56.67</td>
</tr>
<tr>
<td>HCH-gamma</td>
<td>83.13</td>
<td>95</td>
<td>93</td>
</tr>
<tr>
<td>HCH-delta</td>
<td>89.25</td>
<td>101</td>
<td>103.67</td>
</tr>
<tr>
<td>Heptachloro</td>
<td>70.38</td>
<td>73.17</td>
<td>70.67</td>
</tr>
<tr>
<td>Alachlor</td>
<td>101.25</td>
<td>120</td>
<td>118.33</td>
</tr>
<tr>
<td>Aldrin</td>
<td>62.25</td>
<td>71</td>
<td>69.67</td>
</tr>
<tr>
<td>Heptachloro Epoxido</td>
<td>92.13</td>
<td>109</td>
<td>104.17</td>
</tr>
<tr>
<td>DDE o-p’</td>
<td>68.75</td>
<td>78.5</td>
<td>81.67</td>
</tr>
<tr>
<td>Endosulfan I</td>
<td>96</td>
<td>116.5</td>
<td>105.33</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>97.88</td>
<td>117.5</td>
<td>125</td>
</tr>
<tr>
<td>DDE p-p’</td>
<td>67.75</td>
<td>79.33</td>
<td>82.33</td>
</tr>
<tr>
<td>DDD o-p’</td>
<td>92.88</td>
<td>114.67</td>
<td>111.67</td>
</tr>
<tr>
<td>Endrin</td>
<td>99.63</td>
<td>124.5</td>
<td>122.5</td>
</tr>
<tr>
<td>Endosulfan II</td>
<td>87.75</td>
<td>86.33</td>
<td>98.5</td>
</tr>
<tr>
<td>DDD p-p’ -1</td>
<td>105.13</td>
<td>128.17</td>
<td>115.67</td>
</tr>
<tr>
<td>DDT o-p’-2</td>
<td>78.88</td>
<td>95</td>
<td>96.67</td>
</tr>
<tr>
<td>Endosulfan sulfato</td>
<td>111.5</td>
<td>139.67</td>
<td>142.83</td>
</tr>
<tr>
<td>DDT p-p’</td>
<td>88.75</td>
<td>109.17</td>
<td>109.83</td>
</tr>
</tbody>
</table>

**IS Hexachlorobenzene C\(^{13}\)**

![Agilent Technologies Logo]
Advantages of Offline SPE

• Multiple published methods available utilizing the technique
• Wide variety of sorbent types suitable for different target analyte properties and to support different sample matrices
• Concentration of analytes to achieve lower detection limits in conjunction with the removal of background interferences
• Suitable for a wide variety of analytical instrumentation
• Offline preparation does not tie the instrument to the preparation, maintaining flexibility

*Flexibility and versatility for a wide range of sample types and target compounds*
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On-Line SPE with the Flexcube/6460 QQQ

Dr. Sheher Bano Mohsin
Mass Spectrometry Applications
September 5, 2013
• Introduction – Why Online SPE?
• Introducing the Flexcube for Online SPE
• Sensitivity of the Mass Spectrometers
• Application Examples
  - Water Samples
  - Samples with dirty Matrices
Solid Phase Extraction (SPE)

Wouldn’t it be great if you could automate SPE while reducing sample and solvent volumes?
Automated on-line SPE

1. Load 500ul-1800 µl water sample on the enrichment cartridge.
3. Separate on the analytical column and collect MRM tranistions on the QQQ.
4. Recondition enrichment cartridge during analysis.
5. Wash autosampler with Acetonitrile
6. Ready for next sample after ~ 20 minutes.
High sensitivity 6460 Triple Quadrupole

- Sub-femtogram, on-column sensitivity (1pg reserpine; s/n 2000:1)
- Five Orders of Linearity
- Fast Pos/Neg switching, 30 millisec
- “Dynamic Multiple Reaction Monitoring” for MRM schedules by peak retention times instead of time segments
- Triggered MRM for confirmation

Agilent Jet Stream

Enhanced efficiency nebulizer
Super-heated sheath gas
Nozzle voltage
Heated drying gas
Resistive sampling capillary

The super-heated sheath gas collimates the nebulizer spray and creates a dramatically “brighter source”
Agilent MassHunter Software

Instrument Control
Real-time monitoring
Method set-up
Autotune

Qualitative Analysis
Chromatographic results
Spectral results
Find compounds

Quantitative Analysis
User filters
Compound results
Calibration curve

Reporting
Easily Customizable
Based on Excel and XML technology
Agilent Online SPE System

- Switching valve with TEC
- Quaternary pump
- Autosampler
- Analytical column for separation
- Binary pump for gradient elution
- MS/MS
WHAT’S NEW WITH ONLINE SPE?

Flexcube with Built in Valves and a Pump - all Controlled through Mass Hunter
Agilent Online SPE System

- Switching valve with TEC
- Analytical column for separation
- MS/MS
- Quaternary pump
- Autosampler
- Binary pump for gradient elution

Replace with Flexcube
Agilent 1200 Infinity Series Online SPE solution

Solvent selection valve for up to three solvents

Reciprocating single-piston pump for flows up to 4 ml/min (60 bar)

Valves mounted in 1290 Infinity Flexible Cube

up to two Quick-Change valves, according to application
Online SPE with the Flex Cube

Autosampler with 900uL loop and extension seat capillary
Flex Cube and Autosampler

- 6 port valve
- 10 port valve
- Single Piston Pump
- Autosampler
Mass Hunter B 06.00 with Flexcube Control
System with alternating SPE loading:
Load sample to SPE1
System with alternating SPE loading:
Elute sample from SPE1 to column / Load sample 2 on SPE2
Application:

Quantitative LC/MS/MS analysis of 24 trace pharmaceuticals and consumer products in water using on-line SPE enrichment.

Tarun Anumol and Shane Snyder University of Arizona

Sheher Mohsin – Agilent Applications Scientist
## Compounds Analyzed

<table>
<thead>
<tr>
<th>Compound</th>
<th>Class</th>
<th>Compound</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrazine</td>
<td>Herbicide</td>
<td>PFBS</td>
<td>Fluoro-surfactant</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>Plasticizer</td>
<td>PFOA</td>
<td>Fluoro-surfactant</td>
</tr>
<tr>
<td>Caffeine</td>
<td>Stimulant</td>
<td>PFOS</td>
<td>Fluoro-surfactant</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Anti-seizure</td>
<td>Primidone</td>
<td>Anticovulsant</td>
</tr>
<tr>
<td>DEET</td>
<td>Insect-repellant</td>
<td>Simazine</td>
<td>Herbicide</td>
</tr>
<tr>
<td>Estrone</td>
<td>Hormone</td>
<td>Sulfamethoxazole</td>
<td>Antibiotic</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>Anti-depressant</td>
<td>Atenolol</td>
<td>B-blocker</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>Anti-cholesterol</td>
<td>TCPP</td>
<td>Flame-retardant</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>Analgesic</td>
<td>Testosterone</td>
<td>Hormone</td>
</tr>
<tr>
<td>Meprobamate</td>
<td>Anti-anxiety</td>
<td>Triclocarban</td>
<td>Anti-microbial</td>
</tr>
<tr>
<td>Naproxen</td>
<td>Pain-reliever</td>
<td>Triclosan</td>
<td>Anti-microbial</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>Anti-histamine</td>
<td>Trimethoprim</td>
<td>Antibiotic</td>
</tr>
</tbody>
</table>
Summary Experimental Methods

1. Collect 5 mL sample and store at 4°C for preservation.

2. Filter sample through 0.2 µm filters.


4. Analyze by UHPLC/MS/MS with the Agilent 1290 LC coupled to an Agilent 6460 Mass Spectrometer.
Simultaneous analysis of 24 PPCPs in both ESI+ & ESI- mode.

Injection Volume: 1.5 mL

Cycle time (Extraction + Analysis): 18.5 min

Trace Enrichment Cartridge: PLRP-S, 15 µm

Analytical Column: Poroshell 120 EC, 2.1x50 mm
# Method Reporting Limits (ng/l)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>MRL</th>
<th>Analyte</th>
<th>MRL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atenolol</td>
<td>15</td>
<td>Meprobamate</td>
<td>0.5</td>
</tr>
<tr>
<td>\textit{Atrazine}</td>
<td>5</td>
<td>\textit{Naproxen}</td>
<td>10</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>10</td>
<td>PFBS</td>
<td>10</td>
</tr>
<tr>
<td>Caffeine</td>
<td>0.5</td>
<td>PFOA</td>
<td>10</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>2.5</td>
<td>PFOS</td>
<td>10</td>
</tr>
<tr>
<td>\textit{DEET}</td>
<td>0.1</td>
<td>\textit{Primidone}</td>
<td>15</td>
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<tr>
<td>Estrone</td>
<td>20</td>
<td>Simazine</td>
<td>1.5</td>
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<tr>
<td>Fluoxetine</td>
<td>10</td>
<td>\textit{Sulfamethoxazol}</td>
<td>2.5</td>
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<tr>
<td>Gemfibrozil</td>
<td>1.5</td>
<td>TCPP</td>
<td>0.5</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>10</td>
<td>Triclocarban</td>
<td>1</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>5</td>
<td>Triclosan</td>
<td>5</td>
</tr>
</tbody>
</table>

S/N > 10 (by height) for 3 successive injections
Evaluation of On-line Solid Phase Extraction and Direct Injection-LC/MS/MS for the Analysis of Chemicals on U.S. EPA’s Contaminant Candidate List 3

Sheher Bano Mohsin
Chemicals for LC/MS/MS Evaluation*

- 4,4’-methyleneedianiline
- 3-hydroxycarbofuran
- bensulide
- clethodim
- fenamiphos
- fenamiphos sulfone
- fenamiphos sulfoxide
- methomyl
- quinoline
- tebuconazole
- tebufenozide
- thiodicarb

9 CCL3 chemicals
3 CCL3 degradates

*Slide Taken From: Evaluation of On-line Solid Phase Extraction-LC/MS/MS for the Analysis of Chemicals on U.S. EPA’s Contaminant Candidate List 3 - Jody A. Shoemaker and Daniel R. Tettenhorst
Agilent 1200 Infinity Series Online-SPE solution system comprising:
G1312B Binary Pump with modular degasser
G1329B Standard Autosampler
G4227A Flexible Cube with 2-position/10-port valves, G1316C Thermostatted Column Compartment.
6460 Triple Quadrupole LC/MS with Jet Stream Technology
LC/MS/MS Parameters

Binary Pump:
• Solvent A: Water + 0.1% formic acid.
• Solvent B: ACN + 0.1% formic acid.
Analysis Flow rate: 0.4 mL/min.
Column: ZORBAX EclipsePlus C18 3.0x50mm 1.8 μm, 50 °C.

Flexible Cube:
Valves: 2 of 2-position/10-port Quick-Change valves.
Flow rate: 1.0 mL/min.
Solvent:
A1: 3% MeOH/Water,
B1: ACN/IPA/MeOH/Water 1/1/1/1 v/v/v/v.
Standard Autosampler fitted with 900 μL metering head
Auto-SPE Injection volume: 900 μL using a draw and eject speed of 1,000 μL/min.
Direct Injection volume: 90 μL

Triple Quadrupole MS method with Agilent Jet Stream thermal gradient focusing technology:
• Gas temperature: 300 °C. Gas flow: 10 L/min.
• Nebulizer: 45 psi
• Sheath gas temperature: 350 °C. Sheath gas flow: 11 L
• Capillary: 4,000 Volt. Nozzle: 0 Volt
<table>
<thead>
<tr>
<th>Cmpd Name</th>
<th>Prec Ion</th>
<th>Prod Ion</th>
<th>Frag (V)</th>
<th>CE (V)</th>
<th>Cell Acc (V)</th>
<th>Ret Time (min)</th>
<th>Ret Window</th>
<th>Polarity</th>
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<tbody>
<tr>
<td>4,4'-diaminodiphenylmethane</td>
<td>199.1</td>
<td>106</td>
<td>120</td>
<td>28</td>
<td>3</td>
<td>6.74</td>
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<tr>
<td>Bensulide</td>
<td>398</td>
<td>356</td>
<td>100</td>
<td>1</td>
<td>2</td>
<td>11.06</td>
<td>1.11</td>
<td>Positive</td>
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<tr>
<td>Bensulide</td>
<td>398</td>
<td>314</td>
<td>100</td>
<td>6</td>
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<td>11.06</td>
<td>1.11</td>
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<tr>
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<td>158</td>
<td>100</td>
<td>26</td>
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<td>Carbofuran-3-hydroxy</td>
<td>238.1</td>
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<td>120</td>
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<tr>
<td>Clethodim</td>
<td>360.1</td>
<td>166</td>
<td>130</td>
<td>27</td>
<td>4</td>
<td>11.67</td>
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<td>Positive</td>
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<tr>
<td>Fenamiphos</td>
<td>304.1</td>
<td>234</td>
<td>150</td>
<td>15</td>
<td>3</td>
<td>10.28</td>
<td>1.03</td>
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<tr>
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<tr>
<td>Fenamiphos-sulfone</td>
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<td>308</td>
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<td>15</td>
<td>3</td>
<td>9.13</td>
<td>0.91</td>
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</tr>
<tr>
<td>Fenamiphos-sulfone</td>
<td>336.1</td>
<td>266</td>
<td>130</td>
<td>18</td>
<td>3</td>
<td>9.13</td>
<td>0.91</td>
<td>Positive</td>
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<tr>
<td>Fenamiphos-sulfone</td>
<td>336.1</td>
<td>188</td>
<td>130</td>
<td>24</td>
<td>3</td>
<td>9.13</td>
<td>0.91</td>
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<tr>
<td>Fenamiphos-sulfoxide</td>
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<tr>
<td>Fenamiphos-sulfoxide</td>
<td>320.1</td>
<td>233</td>
<td>150</td>
<td>23</td>
<td>3</td>
<td>8.64</td>
<td>0.99</td>
<td>Positive</td>
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<tr>
<td>Fenamiphos-sulfoxide</td>
<td>320.1</td>
<td>171</td>
<td>150</td>
<td>18</td>
<td>3</td>
<td>8.64</td>
<td>0.99</td>
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<tr>
<td>Methomyl</td>
<td>163</td>
<td>107</td>
<td>170</td>
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<tr>
<td>Quinoline</td>
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<td>103</td>
<td>120</td>
<td>30</td>
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<td>6.97</td>
<td>1.81</td>
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<tr>
<td>Quinoline</td>
<td>130</td>
<td>77</td>
<td>120</td>
<td>30</td>
<td>3</td>
<td>6.97</td>
<td>1.81</td>
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<tr>
<td>Tebuconazole</td>
<td>308.1</td>
<td>125</td>
<td>130</td>
<td>42</td>
<td>3</td>
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<tr>
<td>Tebuconazole</td>
<td>308.1</td>
<td>70</td>
<td>130</td>
<td>23</td>
<td>3</td>
<td>10.49</td>
<td>1.05</td>
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<tr>
<td>Tebufenozide</td>
<td>353.2</td>
<td>297</td>
<td>85</td>
<td>1</td>
<td>3</td>
<td>10.85</td>
<td>1.09</td>
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<tr>
<td>Tebufenozide</td>
<td>353.2</td>
<td>133</td>
<td>85</td>
<td>15</td>
<td>3</td>
<td>10.85</td>
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<tr>
<td>Thiodicarb</td>
<td>355</td>
<td>163</td>
<td>80</td>
<td>2</td>
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<td>9.17</td>
<td>1.28</td>
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<tr>
<td>Thiodicarb</td>
<td>355</td>
<td>108</td>
<td>80</td>
<td>14</td>
<td>3</td>
<td>9.17</td>
<td>1.28</td>
<td>Positive</td>
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<tr>
<td>Thiodicarb</td>
<td>355</td>
<td>88</td>
<td>80</td>
<td>17</td>
<td>3</td>
<td>9.17</td>
<td>1.28</td>
<td>Positive</td>
</tr>
</tbody>
</table>
Superimposed chromatograms Showing Reproducibility of Retention time as well as Area Counts for two runs Alternating Between two Different SPE Cartridges.

The calibration standard used has a concentration of 10 ppt (ng/L) each for all 12 compounds with quantifier and qualifier ions. Each run alternates between two different SPE cartridges.
Mix at 1 ppt
Mix at 0.1 ppt
1 ppt and 0.1 ppt
Direct Injection and online-SPE
Same amount on column

Direct Injection

Online SPE
Thiodicarb

Thiodicarb - 7 Levels, 7 Levels Used, 14 Points, 14 Points Used, 0 QCs

\[ y = 9712.750738 \times x - 371.407180 \]

R^2 = 0.99900590

Type: Linear, Origin: Ignore, Weight: None

Online SPE
Tebuconazole

Counts vs. Acquisition Time (min)

+ESI MRM Frag=130.0V CF=0.000 DF=0.000 CID@23.0 (308.1000 -> 70.0000) 0-1ppt-r001.d

Noise (RMS) = 10.64; SNR (10.484min) = 427.1

0.1 ppt

0.5 ppt

1 ppt
Tebuconazole
0.1 ppt to 100 ppt

Tebuconazole - 7 Levels, 7 Levels Used, 14 Points, 14 Points Used, 0 QCs
y = 29206.177838 * x + 9683.405016
R^2 = 0.99961760
Type: Linear, Origin: Ignore, Weight: None

Online SPE
Water Samples Analysis

Water samples from a lake-fed municipal water supply (tap), residential well (ground), a suburban collection basin (WestLake) and a flood control reservoir (Spring Creek) were analyzed. Notably, both WestLake and Spring Creek are in the drainage basin of nearby public and private golf courses.

Samples were filtered with 0.45 um disk filters prior to analysis.
Tebuconazole in Water

- Spring Creek: 50 ppt
- WestLake: 37 ppt
- Ground: 0.9 ppt
- Tap: 0.004 ppt
Lake Water and Ground water

Results are based on the average of duplicate analyses, for the one quantifier transition (308.1 to 70.0) and one qualifier transition (308.1 to 125.0). Tebuconazole is used as a fungicide for turf control. Both WestLake and Spring Creek are in the drainage basin of nearby public and private golf courses.
Performance data for all compounds present in the study based on the calculated signal to noise at 0.1 ppt and assuming an LOQ at approximately 10:1 signal-to-noise. The signal to noise calculation is based on peak area and noise is RMS x 3.

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Level (ppt)</th>
<th>Signal to Noise (S/N)</th>
<th>Nominal LOQ @ 10:1 S/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>4,4-diaminodiphenylmethane</td>
<td>0.1</td>
<td>25</td>
<td>0.04</td>
</tr>
<tr>
<td>Bensulide</td>
<td>0.1</td>
<td>10.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Carbofuran-3-hydroxy</td>
<td>0.1</td>
<td>34.4</td>
<td>0.03</td>
</tr>
<tr>
<td>Clethodim</td>
<td>0.1</td>
<td>7</td>
<td>0.15</td>
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<tr>
<td>Fenamiphos</td>
<td>0.1</td>
<td>301.2</td>
<td>0.005</td>
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<tr>
<td>Fenamiphos-sulfone</td>
<td>0.1</td>
<td>7.9</td>
<td>0.15</td>
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<tr>
<td>Fenamiphos-sulfoxide</td>
<td>0.1</td>
<td>8.2</td>
<td>0.15</td>
</tr>
<tr>
<td>Methomyl</td>
<td>0.1</td>
<td>8.3</td>
<td>0.15</td>
</tr>
<tr>
<td>Quinoline*</td>
<td>0.1</td>
<td>500</td>
<td>0.002</td>
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<tr>
<td>Tebuconazole</td>
<td>0.1</td>
<td>427</td>
<td>0.0025</td>
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<tr>
<td>Tebufenozide</td>
<td>0.1</td>
<td>9.4</td>
<td>0.15</td>
</tr>
<tr>
<td>Thiodicarb</td>
<td>0.1</td>
<td>9</td>
<td>0.15</td>
</tr>
</tbody>
</table>

* uncertainty due to unresolved carryover
Trace level analysis of herbicides in drinking and surface water by online-SPE LC/triple quadrupole MS to the lower ppt range

Edgar Naegele, Agilent Technologies R&D and Marketing GmbH & Co. KG, Waldbronn, Germany
Chromatograms of a calibration standard with a concentration of 100 ppt (ng/L) each for all 28 pesticides measured by the final SPE-LC dynamic MRM method with quantifier and qualifier ion.
Agilent 1200 Infinity Series Online SPE solution

For online sample preparation, upgrade your system with Agilent 1290 Infinity Flexible Cube featuring:

- built-in pump to condition and flush re-usable cartridges
- solvent selection valve

Expand Your Versatility

- **Boost Performance**
  enrichment of analytes and matrix removal for utmost sensitivity

- **Save Time**
  individual configurations for broad automation flexibility

- **Reduce Costs**
  1290 Infinity Flexible Cube contains a built-in pump
Conclusions

We have demonstrated an integrated, cost effective scheme to do on-line SPE coupled to LC/MS/MS. The technical barriers and potential technology transfer problems associated with traditional online SPE system have been avoided.

The direct coupling of the SPE cartridges (PLRP-S, 2.1x12.5mm, 15-20um) to the LC/MS/MS system was done through the integrated valves. The use of Dynamic MRM enables complex mixtures to be efficiently analyzed.

We have demonstrated excellent performance with challenging contaminants on the contaminant list. LOQ for all components was nominally 1 ppt or lower.

Results for real samples, including ground water, lake and flood control reservoir, the latter two obviously containing visible swimming organisms, produced measurable tebuconazole results.
Advantages of Online SPE

• Utilizes entire prepared sample to achieve low detection limits
• Small sample volumes (mL) needed – lower logistics and storage costs
• Reduced chance of error caused by sample handling
• Save on solvent costs – both purchase and disposal of waste
• No evaporation step
• No reconstitution required
• Integrated system increases sample throughput

Exploit the native sensitivity of the LC-MS/MS system with online SPE
Today’s Agenda

• Analysis of emerging contaminants in water – an overview
• Sample preparation options for drinking water analysis
  – Solid-supported liquid/liquid extraction
  – Solid phase extraction
• New developments in online SPE
• Summary of each approach
• Question and answer period
## Review: Online versus Offline SPE

<table>
<thead>
<tr>
<th><strong>Online SPE</strong></th>
<th><strong>Offline SPE</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Utilizes entire prepared sample to achieve low detection limits</td>
<td>Only a fraction of concentrated sample is used</td>
</tr>
<tr>
<td>Small sample volumes (mL) needed – lower logistics and storage costs</td>
<td>Large initial sample volume required (100-1000 mL)</td>
</tr>
<tr>
<td>Reduced chance of error</td>
<td>Increased chance of error</td>
</tr>
<tr>
<td>No evaporation step</td>
<td>Concentration step may be necessary</td>
</tr>
<tr>
<td>No reconstitution required</td>
<td>Reconstitution may be required</td>
</tr>
<tr>
<td>Integrated system increases sample throughput</td>
<td>Sample preparation independent of analysis system - ↑ flexibility</td>
</tr>
<tr>
<td>Requires high-end instrumentation</td>
<td>Suitable for low-end instruments</td>
</tr>
</tbody>
</table>
Summary and Conclusions

- A wide range of offline SPE products support emerging contaminant analysis using established methods and protocols.
- Online SPE combined with a LC-QQQ system allows a relatively simple, fast and reliable determination of herbicides in the low ng/L range in drinking water samples.
- The whole system is fully controlled with the MassHunter acquisition software.
- Adding a more sensitive QQQ system allows for even better sensitivity for compounds which are weakly ionizable.
- Good recovery values and reproducibilities can be achieved even in complex samples and for very polar compounds.
- Online SPE not only increases the sensitivity but adds robustness to method.
- The use of online SPE with the QTOF allows for screening and quantification of unknown and unexpected contaminants in complex environmental samples.

Agilent offers a wide range of solutions for potable water analysis of Emerging Contaminants.
Technical Support - Sample Preparation Products

Technical Support*: 

Spp-support@agilent.com

800-227-9770, options 3, 3, 3

* (North America)
Today’s Agenda

• Analysis of emerging contaminants in water – an overview
• Sample preparation options for drinking water analysis
  – Solid-supported liquid/liquid extraction
  – Solid phase extraction
• New developments in online SPE
• Summary of each approach
• Question and answer period
Thank you for your time and attention