Trace Contaminant Analysis of Water with On-line SPE LC/MS/MS

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Outline

Why On-line SPE?

Hardware

Column considerations

Linearity

Ion suppression

Regulations
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

**Pharmaceuticals**
- Veterinary and human antibiotics: Trimethoprim, erythromycin, lincomycin, sulfamethoxazole
- Analgesics, anti-inflammatory drugs: Codein, ibuprofene, acetaminophen, acetylsalicylic acid, diclofenac, fenoprofen
- Psychiatric drugs: Diazepam
- Lipid regulators: Bezafibrate, clofibric acid, fenofibrate acid
- β-blockers: Metoprolol, propanolol, timolol
- X-ray contrasts: Iopromide, iopamidol, diatrizoate

**Steroids and hormones**
- Personal care products: Estradiol, estrone, estriol, diethylstilbestrol
- Fragrances: Nitro, polycyclic and macrocyclic musks
- Sun-screen agents: Benzophenone, methylbenzylidene camphor
- Insect repellents: N,N-diethyltoluamide
- Antiseptics: Triclosan, Chlorophene

**Surfactants and surfactant metabolites**
- Alkylphenol ethoxylates, 4-nonylphenol, 4-octylphenol, alkylphenol carboxylates

**Flame retardants**
- Polybrominated diphenyl ethers (PBDEs), Tetrabromo bisphenol A, C_{10}-C_{13} chloroalkanes, Tris (2-chloroethyl) phosphate

**Industrial additives and agents**
- Chelating agents (EDTA), aromatic sulfonates

**Gasoline additives**
- Dialkyl ethers, Methyl- t-butyl ether (MTBE)
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*
Hardware
Three Unattended Steps, from Sample to Result
Automated Online Sample Preparation, HPLC/UHPLC Separation, MS QQQ or QTOF Detection
Agilent 1260 LC + 6520 QTOF

1260 Binary pump
1260 Isocratic pump for reference mass solution
1260 Quaternary pump for sample loading
6-column select valve for column switching
6/2 flow control valve

Dual electrospray source
± 2 ppm mass accuracy
Setup of the Agilent Online-SPE System

**SPE system:**
- Quaternary pump
- G1329A autosampler with 900 µl head
- 6 port 2 position valve
- 14 port 6 position valve
- 13 port 12 position stream selection valve (optional)

**RRLC system:**
- Binary pump SL
- Well plate sampler SL (not used)
- Column department SL
Method Implementation in Mass Hunter Acquisition – Graphic Valve Icons
Equilibration of SPE cartridges, SPE and separation of pesticides is overlapped and results in a total runtime for the method of 20 minutes.
Column Considerations
Online Sample Preparation for LC-MS/MS Analysis

Online SPE (trace enrichment-SPE)

- 100% of the prepared sample is loaded
- Volume can be <5 mL
- Combined with more sensitive detection (MS/MS)

- Logistics of getting sample to the lab is simplified
SPE column vs SPE + Analytical Column

PLRP
SPE column only
2 minute load

PLRP as SPE
100 mm Eclipse Plus
2 minute load
20 ng Estradiol on analytical column only
10 μL
20 ng Estradiol on analytical column only
200 μL
20 ng Estradiol on **PLRP** + analytical column
200 µL
20 ng Estradiol on **PS-AQ** + analytical column
200 μL
20 ng Estradiol on **new PLRP + analytical column**
200 μL
Estradiol: 10x Serial Dilutions
500-uL injection with 3-minute wash

Large-volume injections maintain excellent peak shape and linearity
Search results from 130-compound database
Ion Suppression (500 uL)
Regulations
Requirements for Identification and Confirmation

- New guidelines for identification of pesticide residue analysis in food and feed available since January 1st 2010 (SANCO/10684/2009)
- High resolution/high mass accuracy MS instruments other than magnetic sector instruments are specified for the first time
- Minimum resolution: \( \frac{D_m}{m} > 10,000 \) (@ 10% valley)  
  \(~ 20,000 @ \) FWHM
- Positive identification: \( \geq 2 \) diagnostic ions  
  (including one fragment)  
  Mass accuracy \(< 5 \) ppm
- TOF/QTOF instruments are typically factor 10 less sensitive than QQQ instruments
- Online SPE and TOF/QTOF is an ideal combination
Agilent’s Ultra High Definition Accurate Mass Q-TOFs

Exceptional accurate mass, sensitivity, dynamic range and resolution … perfect match for 1290 Infinity UHPLC

- 500 ppb mass accuracy
- Femtogram sensitivity
- 5 decades dynamic range
- 40,000 resolving power
- 20 Spectra/sec
- Excellent Linearity and Isotopic Fidelity
- Supports Agilent Jet Stream and HPLC-Chip
Ultra High Definition QTOF

Maintaining Resolving Power – Across the Mass Range

Scan Rate Independent
Requirements for Environmental Screening

- EU water framework directive and drinking water legislation requires analysis of pollutants in water samples at concentrations of 10 ng/L or below.

- Screening and quantitation in this concentration range usually requires off-line SPE to preconcentrate the target compounds in the sample solution.

- For LC/MS water is the “ideal” sample and sensitivity of QQQ instruments is almost sufficient to meet the requirements (with large volume injection ~ 100 µL).
6550 as Ultimate Detector

DL on 6520 ~ 5 pg on-column

On 6550, use this level to optimize!

Example:

Using Large Volume Injections with on-line SPE:

10 fg / 900 uL ~ **10 fg/mL (PPQ)**
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
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 ~ 25 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 MRMs scheduled by peak retention times instead of time segments
- Triggered MRM for confirmation

Agilent Jet Stream
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

The Measure of Confidence
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>Anticonvulsant</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>
### Surrogate Standards

<table>
<thead>
<tr>
<th>Compound</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeine-$^{13}$C$_3$</td>
<td>PFOA-$^{13}$C$_4$</td>
</tr>
<tr>
<td>Trimethoprim-d$_3$</td>
<td>Gemfibrozil-d$_6$</td>
</tr>
<tr>
<td>Atrazine-d$_5$</td>
<td>PFOS-$^{13}$C$_4$</td>
</tr>
<tr>
<td>Primidone-d$_5$</td>
<td>Triclocarban-$^{13}$C$_6$</td>
</tr>
<tr>
<td>Sulfamethoxazole-d$_6$</td>
<td>Ibuprofen-d$_3$</td>
</tr>
<tr>
<td>Meprobamate-d$_7$</td>
<td>Bisphenol A-$^{13}$C$_{12}$</td>
</tr>
<tr>
<td>Fluoxetine-d$_5$</td>
<td>Naproxen-$^{13}$C$_1$d$_3$</td>
</tr>
<tr>
<td>Carbamazepine-d$_{10}$</td>
<td></td>
</tr>
<tr>
<td>DEET-d$_6$</td>
<td></td>
</tr>
<tr>
<td>Triclocarban-$^{13}$C$_6$</td>
<td></td>
</tr>
</tbody>
</table>

17 isotopically labeled surrogate standards used for increased accuracy and precision in quantitation.
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>Atrazine</td>
<td>5</td>
<td>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>DEET</td>
<td>0.1</td>
<td>Primidone</td>
<td>15</td>
</tr>
<tr>
<td>Estrone</td>
<td>20</td>
<td>Simazine</td>
<td>1.5</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>10</td>
<td>Sulfamethoxazole</td>
<td>2.5</td>
</tr>
<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
Quantitative LC/MS/MS Analysis of glyphosate and its metabolite AMPA in potable and wastewaters using on-line SPE enrichment.

Application note : 5990-8136EN
Glyphosate is a global herbicide which is widely used in agriculture and urban landscape management.

In the environment glyphosate is metabolized to its metabolite aminomethyl phosphonic acid (AMPA).

Both compounds are extremely polar due to their bipolar structure.

For the analysis of both compounds a derivatization is widely accepted.
Robust Method for Glyphosate and AMPA

- Derivatization works at basic pH-values with high excess of FMOC and both reduces the lifetime of the chromatographic column and the robustness of the method
- Online-SPE allows for clean-up and enrichment to comply with screening

**Experimental:**
- 1 mL of sample is fortified with 100 ng ISTD (Glyphosate $1,2^{13}$C-$^{15}$N, AMPA $^{13}$C)
- Borate buffer (pH 10) and FMOC (5 mg/mL) is added and reaction takes place over night (> 4 h)
- Acetic acid (58%) is added to neutralize sample
Glyphosate/AMPA

- Offline sample preparation using FMOC reagent
- On-line enrichment of a 900ul injection on polymeric trapping cartridges.
- Routine method used for potable and wastewater.
- Final effluents and crude sewage diluted x10
- Target LoD’s: clean water 10ng/l, wastewater 100ug/l
Glyphosate and AMPA 1.0ug/l
## Summary of Performance

<table>
<thead>
<tr>
<th>Matrix</th>
<th>GLY %Rec</th>
<th>GLY LOD (ug/L)</th>
<th>AMPA %Rec</th>
<th>AMPA LOD (ug/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well water</td>
<td>98.6</td>
<td>0.002</td>
<td>102.7</td>
<td>0.003</td>
</tr>
<tr>
<td>Tap water</td>
<td>102.7</td>
<td>0.002</td>
<td>103.6</td>
<td>0.002</td>
</tr>
<tr>
<td>River water</td>
<td>100.9</td>
<td>0.003</td>
<td>103.8</td>
<td>0.006</td>
</tr>
<tr>
<td>Final Effluent</td>
<td>101.5</td>
<td>0.044</td>
<td>100.7</td>
<td>0.277</td>
</tr>
<tr>
<td>Crude Sewage</td>
<td>102.0</td>
<td>0.034</td>
<td>100.0</td>
<td>0.185</td>
</tr>
</tbody>
</table>
Summary and Conclusions

- Online SPE combined with a LC-QQQ system allows a relatively simple, fast and reliable determination of trace organics in the low ng/L range.
- The entire system is fully controlled and automated with the MassHunter acquisition software.
- Good recovery values and reproducibilities can be achieved even in complex samples and for very polar compounds.
- Online SPE provides significant time and labor savings compared to off line SPE!
WHAT’S NEW WITH ONLINE SPE?

Flexcube with Built in Valves and a Pump - all Controlled through Mass Hunter
Online SPE with the Flex Cube

Autosampler with 900uL loop and extension seat capillary
Agilent Online SPE System

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

Replace with Flexcube
1290 Infinity Flexible Cube

The Flexible Cube offers the following features:

• Single piston pump to deliver three different solvents exhibiting different solvent strengths

• Any valve application including two valves, e.g. SPE sample enrichment
Flex Cube and Autosampler

- Single Piston Pump
- 6 port valve
- 10 port valve
- Autosampler
High sensitivity analysis of Drinking water

System configuration

Online-SPE system consisting of

- Flexcube
- Autosampler with 900 µl head and needle seat extention
- 6 port 2 pos valve
- 10 port 2 pos valve

Housed Inside the Flexcube

HPLC system consisting of

- 1290 Infinity binary pump
- 1290 Infinity autosampler (not used)
- 1290 Infinity thermostatted column compartment

6460AA Triple Quadrupole Mass Spectrometer
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
Mass Hunter B 06.00 with Flexcube Control
Flexcube Valve Controls During a Run

- **Left valve (2 / 6, 5067-4117)**
  - Position
    - Use current valve position
    - Use valve position
    - Position 2 (Port 1 -> 2)
  - Position after run
    - Do not switch
    - Switch to position at beginning of run
    - Increase valve position
    - Use valve position
    - Position 1 (Port 1 -> 6)

- **Right valve (2 / 10, 5067-4144)**
  - Position
    - Use current valve position
    - Use valve position
    - Position 1 (Port 1 -> 10)
  - Position after run
    - Do not switch
    - Switch to position at beginning of run
    - Increase valve position
    - Use valve position
    - Position 1 (Port 1 -> 10)
# Flexcube Events

## Timetable (5/100 events)

<table>
<thead>
<tr>
<th>Time</th>
<th>Function</th>
<th>Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Pump volume</td>
<td>Pump 5mL, Flow: 1mL/min, Channel A: A1, Channel B: Method setting</td>
</tr>
<tr>
<td>0</td>
<td>Left valve change position</td>
<td>Position 2 (Port 1 -&gt; 2)</td>
</tr>
<tr>
<td>5</td>
<td>Left valve change position</td>
<td>Position 1 (Port 1 -&gt; 6)</td>
</tr>
<tr>
<td>6</td>
<td>Pump volume</td>
<td>Pump 10mL, Flow: 1mL/min, Channel A: A2, Channel B: Method setting</td>
</tr>
<tr>
<td>17</td>
<td>Pump volume</td>
<td>Pump 5mL, Flow: 1mL/min, Channel A: A1, Channel B: Method setting</td>
</tr>
</tbody>
</table>
Results
TIC 100 ppt and 10 ppt (Bottom)

- Methomyl
- Quinoline
- Tebuconazole
- Tebufenozide
- Thiodicarb
- 4,4’-methyleneedianiline
- 3-hydroxycarbofuran
- Bensulide/
- Clethodim
- Fenamiphos
- Fenamiphos sulfone
- Fenamiphos sulfoxide
Fenamiphos Sulfone – 10 ppt and 1 ppt
Thiodicarb – 10ppt and 1 ppt
Tebufenozide – 10 ppt and 1 ppt

+ESI MRM Frag=85.0V CF=0.000 DF=0.000 CID@2.0 (353.2000 -> 297.0000) 1ppt-001.d
Noise (RMS) = 91.72; SNR (13.333min) = 31.3

Counts vs. Acquisition Time (min)
Thank you!

Please join us for our next event

Analysis of Environmental Samples with Ultra High Definition LC/QTOF-MS and Accurate Mass: How Much Resolving Power is Enough?

Thursday January 17, 2013
Two Live Events:
Europe: 3:00pm Berlin Time
Americas: 1:00pm Eastern Time