Analysis of Emerging Organic Contaminants in water by fully-automated online SPE LC-MS/MS

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Using online SPE to solve Environmental Analytical issues
Case Studies

• Trace Organic Compounds analysis in water and wastewater

• Glyphosate and AMPA analysis in drinking and surface water
What are Trace Organic Compounds

WERF (2008)
Why do we care about TOrCs

Pharmaceuticals lurking in U.S. drinking water
AP probe found traces of meds in water supplies of 41 million Americans

Study: India's Water Contains Highest Levels of Pharmaceuticals in World

Published January 26, 2009 / Associated Press

£30bn bill to purify water system after toxic impact of contraceptive pill
Drug firms oppose an EU call for controls on potent chemicals that have been blamed for the gender mutation of freshwater fish

The Washington Post
Six years later, gender-bending fish in our water supply remain a mystery

271M Lbs Of Pharmaceuticals In Our Water

the guardian
Conventional SPE methods
T0rCs analysis

Sample collection

Surrogate addition

Evaporation

Extraction (SPE)

Filtration (for wastewater analysis)

LC-MS Analysis
Sample & Solvent Volume Reduction
Agilent Infinity Flexcubce OSPE system

Schematic system set-up
1260 Infinity LC – 6460 MS(MS)

Solvents required for SPE
- rinsing, conditioning

Solvents for LC

Flexible Cube
Autosampler
Thermostat

Quaternary Pump

6460 MS

Triple quad MS for quantification
1200 Infinity Series Online SPE product and concept

Solvent selection valve for up to three solvents

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

up to two Quick-Change valves, according to application
Online SPE valve setup
LOAD position

[Diagram of SPE valve setup]
Online SPE valve setup
ELUTE position
Simultaneous analysis of 30 TOrCs in positive and negative ESI

Injection Volume: 1.5 mL

Cycle time (Extraction + Analysis): 18.5 min

Analytical Column: Poroshell 120 EC, 2.1x50 mm

Method Performance
# Method Performance

<table>
<thead>
<tr>
<th>Analyte</th>
<th>MRL (ng/L)</th>
<th>Analyte</th>
<th>MRL (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atenolol</td>
<td>10</td>
<td>Gemfibrozil</td>
<td>1.5</td>
</tr>
<tr>
<td>Atrazine</td>
<td>5</td>
<td>Ibuprofen</td>
<td>10</td>
</tr>
<tr>
<td>Benzophenone</td>
<td>15</td>
<td>Meprobamate</td>
<td>0.5</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>10</td>
<td>Naproxen</td>
<td>10</td>
</tr>
<tr>
<td>Caffeine</td>
<td>0.5</td>
<td>PFBS</td>
<td>10</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>2.5</td>
<td>PFOA</td>
<td>10</td>
</tr>
<tr>
<td>DEET</td>
<td>0.1</td>
<td>PFOS</td>
<td>5</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>10</td>
<td>Primidone</td>
<td>15</td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>1</td>
<td>Diltiazem</td>
<td>0.5</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>Trimethoprim</td>
<td>5</td>
<td>Triclocarban</td>
<td>1</td>
</tr>
<tr>
<td>TCPP</td>
<td>0.5</td>
<td>Triclosan</td>
<td>5</td>
</tr>
</tbody>
</table>

**Spike Recovery**

- DI Water 20 ppt spike
- Tucson GW 20 ppt spike

**Sample Size**

\( n=5 \)

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*Image credit: Agilent*
Wastewater Analysis
Effluent samples collected in South-West USA
Wastewater Analysis

Sample size:
- n=3 for online SPE
- n=1 for conventional SPE
Ultivo Triple Quad LC/MS

• Designed for the user!
  • Easy maintenance
  • Compact size

• Robust, reliable and accurate

• An ideal tool for the food and environmental laboratory.

Agilent Infinity Flexcube
Trace Organic Compounds in Drinking Water

- 31 representative TOrCs
- 1260 Infinity II HPLC
  - Column: Zorbax Eclipse plus C18 3.0x50mm, 1.8µm
  - Mobile Phase: H₂O and ACN, 0.2mM NH₄F
  - Flow Rate: 0.350 ml/min
- Flex Cube Online SPE
  - 750ul injection volume.
  - No bench-top sample pretreatment.
  - SPE Cartridge: Zorbax Extend C18, 4.6x12.5mm, 5µm
  - Load: H₂O, 0.1% Formic Acid
  - Elution: 1:1 ACN/IPA
- Fast → 16 min total!
Trace Organic Compounds in Drinking Water

- **Ultivo Triple-Quad MS**
  - Sensitivity enhanced further with Agilent Flex Cube Online SPE.
  - No complex sample preparation needed.

- High linearity with $R^2 > 0.99$ for quantitation range 0.5-200ng/L

- **Quantitation Limit <0.5-5 ng/L** for most analytes.
**Glyphosate Application and Properties**

- Thought to be the most widely used pesticide in the world
  - A non-selective herbicide, absorbed rapidly by plants
  - Sold as ‘Roundup’ by Monsanto in US
- Expected to have low toxicity and high efficiency
- AMPA is chief degradation product
- Chronic toxicity of both is unknown
Glyphosate & AMPA Presence in water

- Presence detected in several US streams and rivers\(^1\)

- Detected in European groundwater sources\(^2\)

- Maximum allowable concentration in drinking water set by the European Community for several polar pesticides of 0.1 µg/L

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Derivatization reaction with FMOC

Workflow

Collect 25 mL sample

Derivatization

Flexcube 900 uL

6460 LC/MS/MS

Glyphosate

25 ml sample

250 uL of 10 ug/L surrogate

1 ml EDTA
1 ml Borate buffer
2.5 ml ACN

0.5 ml of 20 mM FMOC

Heat at 100 C for 30 min

50 uL Phosphoric Acid

AMPA
Automated Sample Prep (Derivatization) – Agilent 7696 Workbench
LC Column: Poroshell EC 120 C-18, 3x50 mm, 2.7 µm
Flowrate: 0.35 mL/min
Injection Volume: 900 µL
Column Compartment Temperature: 30°C

LC Mobile Phase:
A – Water + 5mM Ammonium Acetate
B – Acetonitrile

Flexcube Solvents:
A - [95/5(v/v): HPLC Water/Acetonitrile] + 0.1% Acetic Acid
B - 1/1/1 (v/v/v): Acetonitrile/Methanol/Isopropanol
### Gradient Profiles

#### Flexcube

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Function</th>
<th>Volume</th>
<th>Flowrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Pump Solvent A</td>
<td>4 mL</td>
<td>1 mL/min</td>
</tr>
<tr>
<td>4</td>
<td>Valve Change Position to Elution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Pump Solvent B</td>
<td>4 mL</td>
<td>1 mL/min</td>
</tr>
<tr>
<td>10</td>
<td>Pump solvent A</td>
<td>2 mL</td>
<td>1 mL/min</td>
</tr>
</tbody>
</table>

#### Analytical

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile Phase</th>
<th>Flow rate (mL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5% B</td>
<td>0.4</td>
</tr>
<tr>
<td>4</td>
<td>5% B</td>
<td>0.4</td>
</tr>
<tr>
<td>8</td>
<td>55% B</td>
<td>0.4</td>
</tr>
<tr>
<td>9</td>
<td>95% B</td>
<td>0.4</td>
</tr>
<tr>
<td>10</td>
<td>95% B</td>
<td>0.4</td>
</tr>
<tr>
<td>10.1</td>
<td>5% B</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Stop time: 12 min
Post time: 1 min
Sample Chromatogram

- Glyphosate 392 -> 179
- Glyphosate 392 -> 88
- AMPA 334 -> 179
- AMPA 334 -> 112

10 ng/L in Mili-Q water
Samples analyzed from a ground water source in AZ and a surface water (Colorado river).

- Surface Water (n=4): Glyphosate – 1.5 ng/L ; AMPA – 0.5 ng/L
- Ground Water (n=4): Glyphosate – ND ; AMPA - ND

Samples were spiked at two levels (20 ng/L and 100 ng/L) to determine method recoveries.
### Method Performance

#### Ground Water

<table>
<thead>
<tr>
<th>Analyte</th>
<th>20 ng/L spike (n=5)</th>
<th>100 ng/L spike (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recovery (%)</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>Glyphosate</td>
<td>88.6</td>
<td>2.7</td>
</tr>
<tr>
<td>AMPA</td>
<td>98.9</td>
<td>7.7</td>
</tr>
</tbody>
</table>

#### Surface Water

<table>
<thead>
<tr>
<th>Analyte</th>
<th>20 ng/L spike (n=5)</th>
<th>100 ng/L spike (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recovery (%)</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>Glyphosate</td>
<td>95.2</td>
<td>1.1</td>
</tr>
<tr>
<td>AMPA</td>
<td>78.4</td>
<td>2.4</td>
</tr>
</tbody>
</table>

- Recoveries for AMPA and glyphosate in both waters was 75-100%
- RSD (%) was <10% in all spikes
## Comparison of Conventional & Online SPE

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Conventional SPE</th>
<th>Online SPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labor</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>Solvent Use</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>Materials Cost</td>
<td>*</td>
<td>**</td>
</tr>
<tr>
<td>Detection Limits</td>
<td>***</td>
<td>**1/2</td>
</tr>
<tr>
<td>Ion Suppression</td>
<td>*</td>
<td>**1/2</td>
</tr>
<tr>
<td>Volume of sample</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>Total Cycle Time</td>
<td>*</td>
<td>***</td>
</tr>
</tbody>
</table>

* Least favorable  
** Moderately favorable  
*** Optimal
Conclusions

- Sensitive and robust methods for analysis of TOrCs; Glyphosate & AMPA have been developed using the Agilent Flexcube & Ultivo Triple Quad LC/MS system

- The Ultivo Triple Quad LC/MS/M offers extremely sensitive performance while having a much lower footprint.

- The Flexcube allows automated online sample enrichment thus reducing labor time while drastically reducing required sample volume and cost.

- Methods have been proven to be robust in several different water matrixes.

- Online SPE is significantly less affected by ion suppression compared with conventional offline extraction techniques.
References

Automated Online SPE for LC/MS/MS Analysis of Trace Organic Contaminants in Water Using the Agilent 1290 Infinity Flexible Cube Module
Application Note

5991-6115EN

Analysis of Glyphosate and AMPA in Drinking Water with the Agilent 1200 Infinity Series Online SPE Solution

5991-3208EN

Comparison of Online SPE Analysis and Direct Injection of Trace Level Estrogens in Drinking Water with the Agilent 6460 and Agilent 6490 Triple Quadrupole LC/MS Systems
The Agilent 1200 Infinity Series Online SPE Solution for the Highest Sensitivity

5991-3440EN

5991-8155EN