LC-MS workflows for emerging contaminants

Dwain Cardona
Environmental Marketing Manager, Thermo Fisher Scientific
• Organic Contaminant Analysis Using LC-MS Workflow (5 min)

• Cyanotoxin Analysis Using Triple Quadrupole (10 min)
  • Thermo Scientific™ TSQ Altis™ MS and TSQ Quantis™ MS

• Known and Unknown Screening and Quantitation in Waste Water (10 min)
  • Orbitrap technology

• Questions & Discussion (5 min)
Organic Environmental Contaminant Analysis

Comprehensive Organic Contaminant Analysis Solutions

Sample Extraction
- SPE, Quechers
- Thermo Scientific™ Dionex™ ASE™ 350 Accelerated Solvent Extractor
- Thermo Scientific™ Dionex™ AutoTrace™ 280 Solid-Phase Extraction

Consumables
- GC Columns & Consumables
- LC Columns & Consumables
- IC Columns & Consumables

Routine monitoring
- GC, GCMS
- GC Columns & Consumables
- LC, LCMS
- IC, ICMS

Targeted & Non-Targeted monitoring
- Thermo Scientific™ Orbitrap™ GC-MS/MS
- Orbitrap LC-MS/MS

Data analysis & Interpretation
- SW & LIBRAIRIES

Laboratory Information Management Systems (LIMS)
LC-MS Analysis for Organic Contaminants

Sample prep / consumables
- Automation for sample preparation
- Consumables that simplify and improve results

Liquid chromatography (LC)
- Accuracy, Precision, Speed for Productivity
- Innovating LC for routine / demanding applications

LC Mass spectrometry
- Lower limits of detection for regulatory compliance
- Robust performance for simple and complex matrices

Data analysis Software
- Compliance-ready data management, with unified instrument control
- Environmental specific reporting

Workflow Solutions for Environmental Sample Analysis
LC-MS Analysis for Organic Contaminants

Targeted Screening and Quantitation

Thermo Scientific TSQ Altis/Quantis Triple Quadrupole MS

Unknown / Known Screening, Identification & Quantitation

Orbitrap LC-MS
MS Platforms to Choose From

✅ When QQQ?

Robustness, Reproducibility in a routine environment
Most sought after in routine, targeted quantitation of a variety of samples

Ultimate Sensitivity
For a host of molecule types

Reducing cost/sample
Robust, reproducible workflow for multiple samples

From regulated environment to established Methods
Easy method development for all molecule types

✅ When HRAM?

Confirmation of analyte structure
Confident start for both identification and confirmation

Analysis of unknowns
Extend the scope of analysis to include unexpected pesticides

Retrospective search for new compounds
High resolution full scan data to shape your studies

Addressing sensitivity requirements
Add flexibility to your workflow by using one technology for both qual/quan & routine quantitative analysis

HRAM OR QQQ?
# Introduction to TSQ Altis MS and TSQ Quantis MS

<table>
<thead>
<tr>
<th></th>
<th>TSQ Altis MS</th>
<th>TSQ Quantis MS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mass Range</strong></td>
<td>5-2000</td>
<td>5-3000</td>
</tr>
<tr>
<td><strong>SRM/sec</strong></td>
<td>600</td>
<td>600</td>
</tr>
<tr>
<td><strong>Selectivity (H-SRM)</strong></td>
<td>0.2 Da FWHM</td>
<td>0.4 Da FWHM</td>
</tr>
<tr>
<td><strong>Sensitivity (HESI Reserpine 1 pg)</strong></td>
<td>500,000:1</td>
<td>150,000:1</td>
</tr>
<tr>
<td><strong>Targeted Market</strong></td>
<td>Omics, Research, Pharma/Biopharma, Clinical Research and Forensic Toxicology</td>
<td>Environmental and Food Safety, Clinical Research, and Forensic Toxicology</td>
</tr>
</tbody>
</table>

**Robustness, Reproducibility, Speed, Ease-of-Use, Flexibility**
Active Ion Management Plus (AIM+) - The next step in precision design delivers the ultimate in ion management, inception to detection, from the OptaMax™ ion source housing to the enhanced electron multiplier. Incorporates segmented quadrupoles with hyperbolic surface and enhanced RF Electronics to further optimize ion management precision, reliability, speed, and reproducibility.

**Active collision cell with axial DC field** facilitates more SRMs/sec

**Best in class** with hyperbolic surface for enhanced performance with both SRM and H-SRM (0.2 FWHM)

**OptaMax™ NG** APCI ready

**NEW!**

**Enhanced dual-mode electron multiplier detector** Ensures excellent linearity and dynamic range

**NEW!**

**Active collision cell with axial DC field** facilitates more SRMs/sec

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**NEW!**

**Enhanced dual-mode electron multiplier detector** Ensures excellent linearity and dynamic range

**NEW!**
Reduced injection volume applied to the quantitation of cylindrospermopsin and anatoxin-a in drinking water according to EPA Method 545
Reduced Injection Volume Applied to the Quantitation of Cylindrospermopsis and Anatoxin-a in Drinking Water According to EPA Method 545

- **Cyanobacteria** naturally occur in surface waters. Under certain conditions, such as in warm water containing an abundance of nutrients, they can rapidly form harmful algal blooms (HABs).

- HABs can produce toxins known as cyanotoxins, which can be harmful to humans and animals.

- **Anatoxin-a** (also known as Very Fast Death Factor) is a neurotoxin with acute toxic effects and subject to monitoring and regulation efforts in several countries, including the US.

- **Cylindrospermopsis** is toxic to liver and kidney tissues and as a result, the USEPA has developed EPA Method 545 for the UCMR 4 program.
Reduced Injection Volume Applied to the Quantitation of Cylindrospermopsin and Anatoxin-a in Drinking Water According to EPA Method 545

- 2 mL of sample was filtered through a 0.2 μm pore size PVDF disposable filter to address the potential presence of intact algal cells in finished water samples.

- 1 mL of sample was mixed with the phenylalanine-d5 and uracil-d4 internal standards and measured by direct injection-LC/ESI-MS/MS.

<table>
<thead>
<tr>
<th>Ion Source Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spray Voltage</td>
<td>3500 V</td>
</tr>
<tr>
<td>Sheath Gas</td>
<td>45 Arb</td>
</tr>
<tr>
<td>Aux Gas</td>
<td>10 Arb</td>
</tr>
<tr>
<td>Sweep Gas</td>
<td>0 Arb</td>
</tr>
<tr>
<td>Ion Transfer Tube Temperature</td>
<td>325 °C</td>
</tr>
<tr>
<td>Vaporizer Temperature</td>
<td>275 °C</td>
</tr>
</tbody>
</table>

**Table 2. Optimized SRM conditions.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Polarity</th>
<th>Precursor (m/z)</th>
<th>Product (m/z)</th>
<th>Collision Energy (V)</th>
<th>RF Lens (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anatoxin-a</td>
<td>Positive</td>
<td>166</td>
<td>131</td>
<td>18</td>
<td>100</td>
</tr>
<tr>
<td>Cylindrospermopsin</td>
<td>Positive</td>
<td>416</td>
<td>176</td>
<td>35</td>
<td>120</td>
</tr>
<tr>
<td>Phenylalanine-d5 IS for Anatoxin-a</td>
<td>Positive</td>
<td>171</td>
<td>125</td>
<td>12</td>
<td>80</td>
</tr>
<tr>
<td>Uracil-d4 IS for Cylindrospermopsin</td>
<td>Positive</td>
<td>115</td>
<td>98</td>
<td>10</td>
<td>55</td>
</tr>
</tbody>
</table>
Reduced Injection Volume Applied to the Quantitation of Cylindrospermopsin and Anatoxin-a in Drinking Water According to EPA Method 545

Table 3. Low background noise for all EPA Method 545 analytes.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>MRL (µg/L)</th>
<th>1/3 MRL (µg/L)</th>
<th>5 µL</th>
<th>10 µL</th>
<th>25 µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anatoxin-a</td>
<td>0.03</td>
<td>0.01</td>
<td>NF</td>
<td>NF</td>
<td>NF</td>
</tr>
<tr>
<td>Cylindrospermopsin</td>
<td>0.09</td>
<td>0.03</td>
<td>NF</td>
<td>NF</td>
<td>NF</td>
</tr>
</tbody>
</table>

NF = Not found
Reduced injection volume applied to the quantitation of cylindrospermopsin and anatoxin-a in drinking water according to EPA Method 545

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Actual (µg/L)</th>
<th>LFB1 (µg/L)</th>
<th>LFB2 (µg/L)</th>
<th>LFB3 (µg/L)</th>
<th>LFB4 (µg/L)</th>
<th>%Rec</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>5 µL Injection</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anatoxin-a</td>
<td>0.3</td>
<td>0.315</td>
<td>0.326</td>
<td>0.286</td>
<td>0.29</td>
<td>101%</td>
<td>6%</td>
</tr>
<tr>
<td>Cylindrospermopsin</td>
<td>0.9</td>
<td>1.011</td>
<td>0.952</td>
<td>1.182</td>
<td>1.12</td>
<td>118%</td>
<td>10%</td>
</tr>
<tr>
<td>IS-Phenylalanine-d5</td>
<td>90%</td>
<td>96%</td>
<td>123%</td>
<td>117%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IS-Uracil-d4</td>
<td>122%</td>
<td>119%</td>
<td>127%</td>
<td>119%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>10 µL Injection</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anatoxin-a</td>
<td>0.3</td>
<td>0.297</td>
<td>0.306</td>
<td>0.309</td>
<td>0.309</td>
<td>102%</td>
<td>2%</td>
</tr>
<tr>
<td>Cylindrospermopsin</td>
<td>0.9</td>
<td>1.09</td>
<td>0.994</td>
<td>1.02</td>
<td>1.02</td>
<td>114%</td>
<td>4%</td>
</tr>
<tr>
<td>IS-Phenylalanine-d5</td>
<td>90%</td>
<td>99%</td>
<td>100%</td>
<td>98%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IS-Uracil-d4</td>
<td>89%</td>
<td>101%</td>
<td>93%</td>
<td>83%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>25 µL Injection</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anatoxin-a</td>
<td>0.3</td>
<td>0.347</td>
<td>0.319</td>
<td>0.324</td>
<td>0.315</td>
<td>109%</td>
<td>4%</td>
</tr>
<tr>
<td>Cylindrospermopsin</td>
<td>0.9</td>
<td>0.956</td>
<td>0.899</td>
<td>1.072</td>
<td>1.055</td>
<td>111%</td>
<td>8%</td>
</tr>
<tr>
<td>IS-Phenylalanine-d5</td>
<td>88%</td>
<td>94%</td>
<td>96%</td>
<td>94%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IS-Uracil-d4</td>
<td>93%</td>
<td>101%</td>
<td>80%</td>
<td>78%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>IS criteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%Recovery</td>
<td>50–150%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%RSD</td>
<td>&lt;20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Reduced injection volume applied to the quantitation of cylindrospermopsin and anatoxin-a in drinking water according to EPA Method 545

**Conclusions**

- The TSQ Quantis Triple Quadrupole MS provided sensitive, accurate, reproducible, and reliable quantitation of cylindrospermopsin, and anatoxin-a in drinking water

- Adequate sensitivity was obtained with 5 μL, 10 μL, and 25 μL injection volumes for drinking water

- 10-fold reduction in the injection volume

- Less matrix contribution

  = Enhanced sensitivity  
  Reduced maintenance intervals

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**Table 6. Monrovia water sample analyzed using the TSQ Quantis triple quadrupole MS.**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Actual (μg/L)</th>
<th>FS</th>
<th>LFSM</th>
<th>LFSMD</th>
<th>%Rec</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>5 μL Injection</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anatoxin-a</td>
<td>0.3</td>
<td>0</td>
<td>0.337</td>
<td>0.367</td>
<td>117%</td>
<td>6%</td>
</tr>
<tr>
<td>Cylindrospermopsin</td>
<td>0.9</td>
<td>0</td>
<td>0.954</td>
<td>0.966</td>
<td>107%</td>
<td>1%</td>
</tr>
<tr>
<td>IS-Phenylalanine-d5</td>
<td>120%</td>
<td></td>
<td>111%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IS-Uracil-d4</td>
<td>142%</td>
<td></td>
<td>130%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>10 μL Injection</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anatoxin-a</td>
<td>0.3</td>
<td>0</td>
<td>0.347</td>
<td>0.352</td>
<td>117%</td>
<td>1%</td>
</tr>
<tr>
<td>Cylindrospermopsin</td>
<td>0.9</td>
<td>0</td>
<td>1.162</td>
<td>1.085</td>
<td>125%</td>
<td>5%</td>
</tr>
<tr>
<td>IS-Phenylalanine-d5</td>
<td>119%</td>
<td></td>
<td>109%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IS-Uracil-d4</td>
<td>89%</td>
<td></td>
<td>113%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>25 μL Injection</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date Analyzed</td>
<td>4/7/2017</td>
<td></td>
<td>4/7/2017</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anatoxin-a</td>
<td>0.3</td>
<td>0</td>
<td>0.35</td>
<td>0.341</td>
<td>115%</td>
<td>2%</td>
</tr>
<tr>
<td>Cylindrospermopsin</td>
<td>0.9</td>
<td>0</td>
<td>0.788</td>
<td>0.843</td>
<td>91%</td>
<td>5%</td>
</tr>
<tr>
<td>IS-Phenylalanine-d5</td>
<td>96%</td>
<td></td>
<td>87%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IS-Uracil-d4</td>
<td>122%</td>
<td></td>
<td>107%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IS criteria</td>
<td>50–150%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%Recovery</td>
<td>70–130%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%RSD</td>
<td>&lt;30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FS stands for Field Sample. LFSM stands for Laboratory Fortified Sample Matrix. LFSMD stands for Laboratory Fortified Sample Matrix Duplicate.
In 2014, half-a-million people were without drinking water in the state of Ohio due to high toxin level!

EPA 544 released in February 2015

~ 4 x average sensitivity improvement over TSQ Endura
Soil Analysis for Organic Contaminants: LC-MS

Targeted Screening and Quantitation

Unknown / Known Screening and Targeted Quantitation

Thermo Scientific TSQ Altis/Quantis Triple Quadrupole MS

Orbitrap LC-MS
Thermo Scientific™ Exactive™ Series Portfolio

**Q Exactive & Q Exactive Plus**
- Orbitrap analyzer
- Mass Range m/z 50 - 6000
- Mass Accuracy <1ppm
- Max. Mass Resolution >140,000
- Scan speed up to 12Hz
- Spectral Multiplexing
- AQT & AABG (QE Plus only)
- Optional Intact Protein Mode and Enhanced Resolution (280k) for QE Plus only

**Q Exactive HF**
- Ultra High Field Orbitrap analyzer
- Mass Range m/z 50 - 6000
- Mass Accuracy <1ppm
- Max. Mass Resolution >240,000
- Scan speed up to 18Hz
- Spectral Multiplexing
- AQT & AABG
- Optional Protein Mode

**Q Exactive Focus**
- Orbitrap analyzer
- Mass Range m/z 50 - 2000
- Mass Accuracy <1ppm
- Max. Mass Resolution >70000
- Scan speed up to 12Hz
- vDIA scan mode*

**Exactive Plus EMR**
- Orbitrap analyzer
- Mass Range m/z 50 - 6000
- Mass Range EMR: 300 - 20,000
- Mass Accuracy: <1ppm
- Mass Resolution >140,000
- Scan Speed up to 12 Hz

* vDIA scan mode is an optional feature available on Q Exactive Focus.
Thermo Scientific™ Q Exactive™ Focus Hybrid Quadrupole-Orbitrap™ Mass Spectrometer

- Mass range: 50-2,000
- Resolving power: Up to 70,000 (at m/z 200)
- Mass accuracy: < 1 ppm (internal)
  < 3 ppm (external)
- Scan rate: Up to 12 Hz at R = 17,500
- Full MS and MS/MS analysis with high resolution and high mass accuracy
- Positive/negative ion switching on a chromatographic timescale (one full cycle in <1 sec; one full positive mode scan and one full negative mode scan at a resolution setting of 35,000)
- Multiple approaches for quantitation:
  - Selected ion monitoring (SIM)
  - Parallel reaction monitoring (PRM)
  - Data Independent Acquisition (DIA)
- HCD, all-ion fragmentation (AIF), data-independent acquisition (DIA) in HCD cell with high resolution and high mass accuracy detection

Ideal for labs performing routine analysis in food safety, environmental analysis, forensic toxicology, sports doping, clinical research, metabolomics, and pharmaceutical analyses
Screening and Quantitation of Micro-pollutants from Sewage Water in the Process of Bank Filtration Using UHPLC-HRAM
• When surface water from a river or lake enters a ground water system, organic compounds are often degraded in the process—this is known as ‘bank filtration’

• Lake Tegeler in Berlin contains up to 30% of effluent water from a municipal waste water treatment plant—identifying and quantitating the contaminants entering the ground water was needed to assess the effectiveness of this barrier

• A series of ground water probing sites (GWPS) were established between lake Tegeler and a ground water well used to draw raw ground water for the generation of drinking water for the city of Berlin

• For this investigation, specific workflows using HRAM were required for both target and non-target (suspect screening) of water samples drawn from both the GWPS and the ground water well
Pre-concentration and LC separation Conditions

- 1 mL sample injected on-line
- Pre-concentration column, C18 2.1 x 20 mm, 12 um particle size
- Separation: C18 2.11 x 50 mm, 1.8um
- Mobile Phase: A: 0.1 % FA in water
- Mobile Phase B: 0.1 % FA in MeOH
- Gradient: 2%B to 95%B in 6.7 min
MS HRAM Instrumentation- QE Focus

MS-Parameters for Screening
- Full Scan ESI(+) and ESI(-) separately
- R = 70,000
- m/z 100-1000
- ms2: DIA w/ CE=30 over specified ranges

MS-Parameters Quantitative Analysis
- Full Scan ESI (+/- switching)
- R = 35,000
- m/z 103-900
- Use of internal standards

Software for Data Analysis
- Thermo Scientific™ TraceFinder™
- Thermo Scientific™ Compound Discoverer Software
Target and Non-target Analysis Workflows

**Target Analysis**
- Water sample
- Analysis of the samples with UHPLC-HRAM
- Reference standard
- Quantitative processing

**Non-Target Analysis**
- **Suspect Screening**
  - Home built suspect database with >2000 entries
  - Identification
- **Non-Target Screening**
  - Online databases, spectra libraries, meta data
  - Reference standard
Overview - Bank Filtration of Surface Water

Improvement of Water Quality

Abstraction of Drinking Water

Groundwater

Bank Filtrate

Mixing

Impermeable Layer

Tegeler See

GWPS with bank filtrate

water well

GWPS inland side

NO₃

aquifer

aquitard

Mn

Mn/Fe

Fe

GWPS = ground water probing site
58 compounds were quantified

- **drug-metabolites (9)**
  - z.B. valsartan acid, 4-acetyl-aminoantipyrin (AAA), ...

- **sweeteners (2)**
  - sucralose, acesulfame

- **perfluorinated surfactants (2)**
  - PFOS, PFOA

- **miscellaneous (11)**
  - benzotriazole (anti corrosion), DEET (insect repellant), flame retardants (TBP, TCEP, TCPP), ...

- **drugs (34)**
  - *anti epileptic* (gabapentin, lamotrigine, carbamazepine...)
  - *blood pressure treatment* (metoprolol, bisoprolol, olmesartan, candesartan,...)
  - *anti depressive* (venlafaxine,...)
  - *neuroleptic* (amisulpride,...)
  - *analgetic* (phenazine, tramadol,...)
• Depending upon the GWPS, differing REDOX potentials could be identified, and this property has an impact on degradation of organic compounds

• Three groups of compounds were predominately degraded in one of the 3 regions

• The 3 regions identified were:
  • Nitrate Reducing (primarily aerobic degradation)
  • Manganese Reducing (primarily anaerobic degradation)
  • Iron Reducing
Results-Comparison of Samples

Aerobic degradation (NO\textsubscript{3}\textsuperscript{-}-reducing), as for:
- amisulpride
- clindamycin
- metoprolol
- venlafaxine

Anaerobic degradation (Mn-reducing), as for:
- candesartan
- carbamazepine
- sucralose

(Fe reducing) persistent for:
- gabapentin
- primidone
- valsartan acid
Conclusions

- Bank filtration is an important way to reuse surface water that is influenced by municipal and industrial waste water treatment plants.
- Contaminants found in the surface water were found to be persistent or degraded aerobically / anaerobically.
- Further water purification is therefore required for drinking water generation.
- LC-HRAM analysis with powerful software tools is a versatile method to quickly assess the quality of surface waters going into reuse as well as raw water used for drinking water.
- The on-line SPE approach saves time and minimizes the analytical effort required to pre-concentrate large volumes of sample off-line.
Thank You for Your Attention!

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Questions?