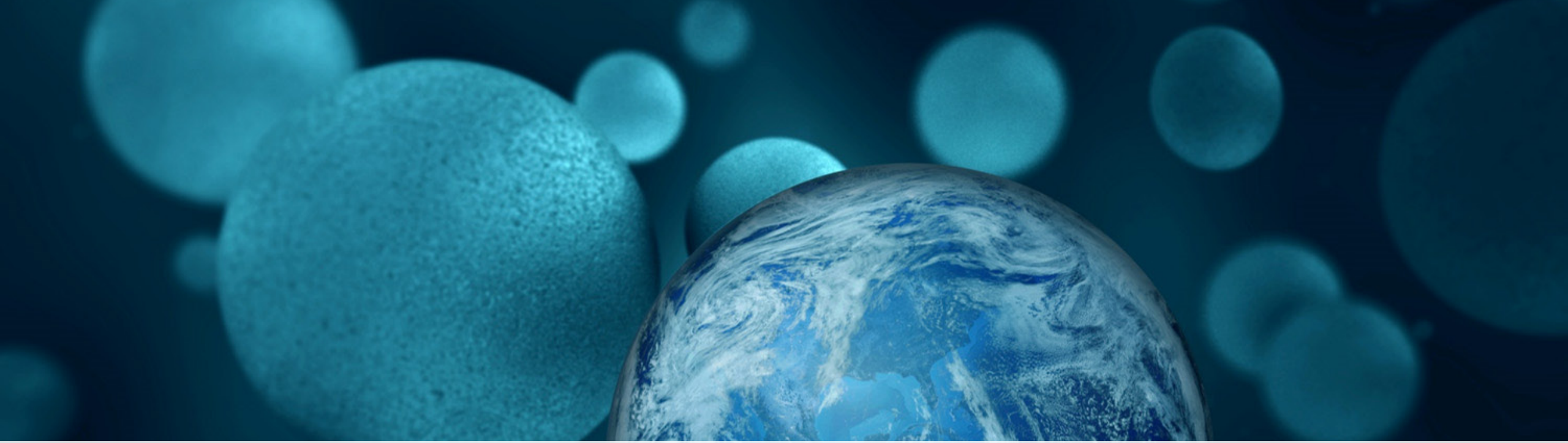


ThermoFisher SCIENTIFIC

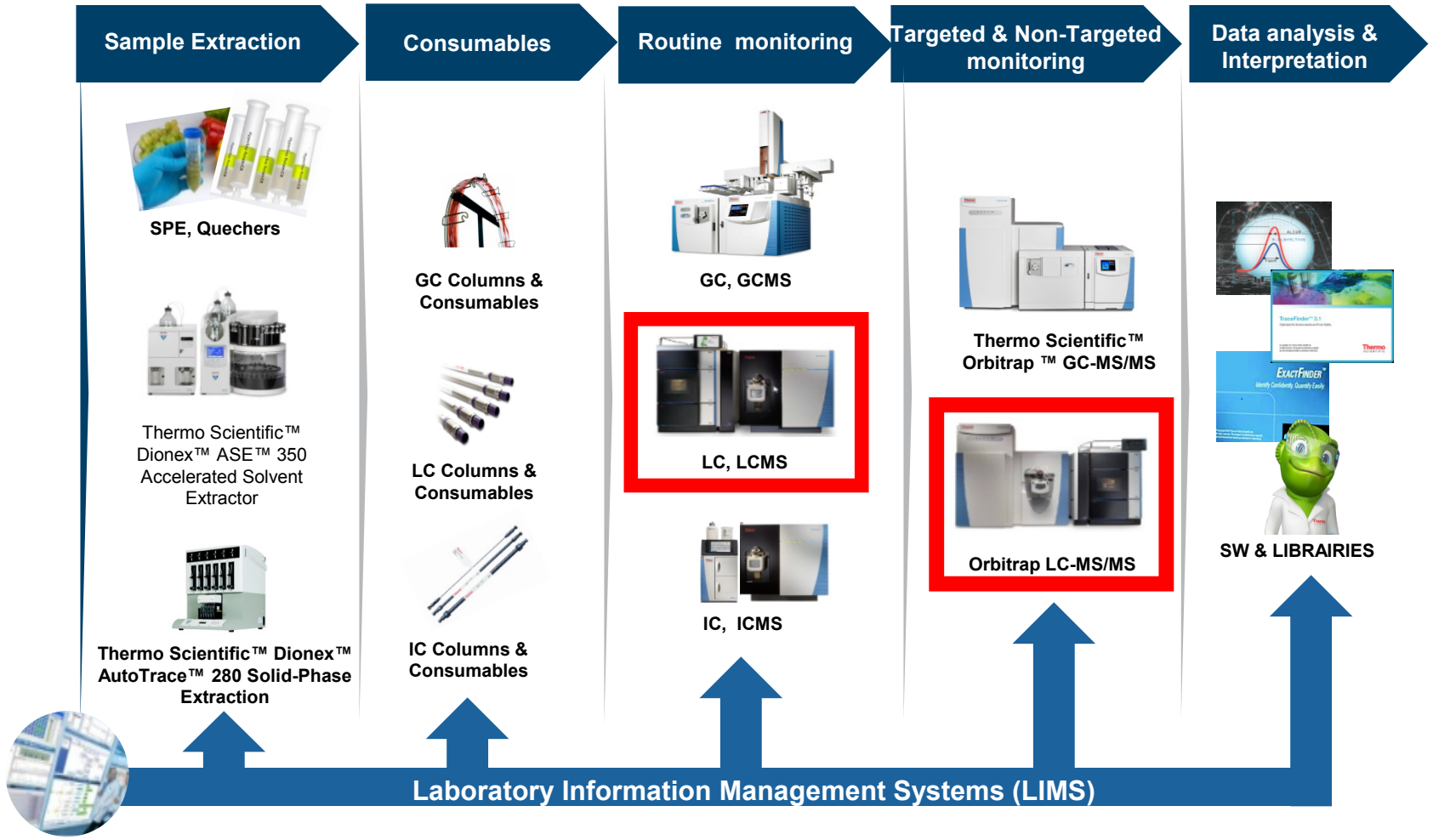
LC-MS workflows for emerging contaminants

Dwain Cardona
Environmental Marketing Manager, Thermo Fisher Scientific

Agenda

- **Organic Contaminant Analysis Using LC-MS Workflow (5 min)**
- **Cyanotoxin Analysis Using Triple Quadrupole (10 min)**
  - Thermo Scientifc™ TSQ Altis™ MS and TSQ Quantis™ MS
- **Known and Unknown Screening and Quantitation in Waste Water (10 min)**
  - Orbitrap technology
- **Questions & Discussion (5 min)**

Comprehensive Organic Contaminant Analysis Solutions



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LC-MS Analysis for Organic Contaminants

Sample prep / consumables

- Automation for sample preparation
- Consumables that simplify and improve results

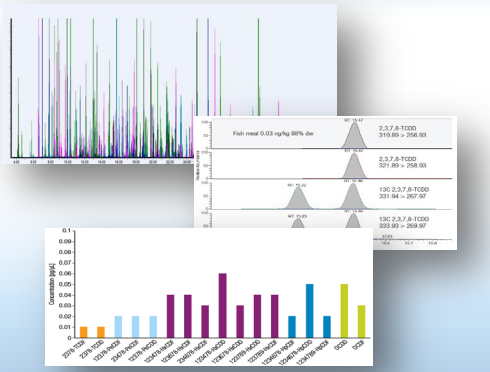
Liquid chromatography(LC)

- 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

ThermoFisher SCIENTIFIC

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

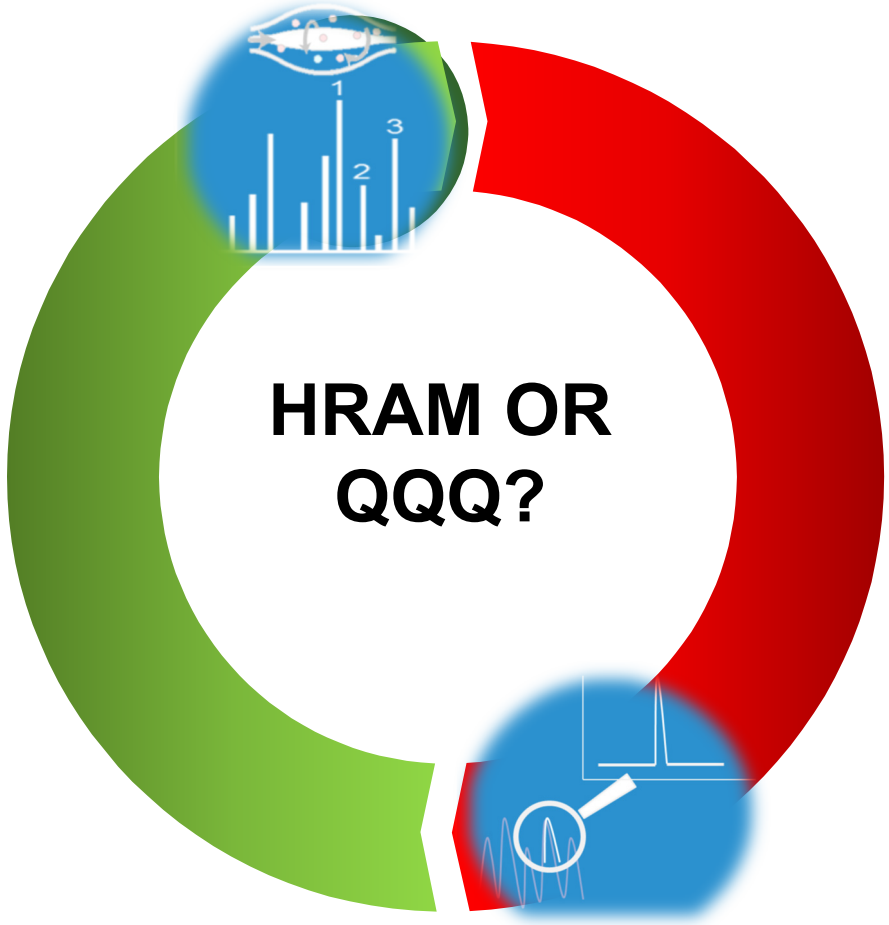
For a host of molecule types

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

Introduction to TSQ Altis MS and TSQ Quantis MS

Performance: Sensitivity, Selectivity (H-SRM)

TSQ Altis MS

TSQ Quantis MS

	TSQ Altis MS *High-end*	TSQ Quantis MS *Mid-tier*
Mass Range	5-2000	5-3000
SRM/sec	600	600
Selectivity (H-SRM)	0.2 Da FWHM	0.4 Da FWHM
Sensitivity (HESI Reserpine 1 pg)	500,000:1	150,000:1
Targeted Market	Omics, Research, Pharma/Biopharma, Clinical Research and Forensic Toxicology	Environmental and Food Safety, Clinical Research, and Forensic Toxicology

Robustness, Reproducibility, Speed, Ease-of-Use, Flexibility

TSQ Altis MS: Sensitivity with Robustness, No Compromises

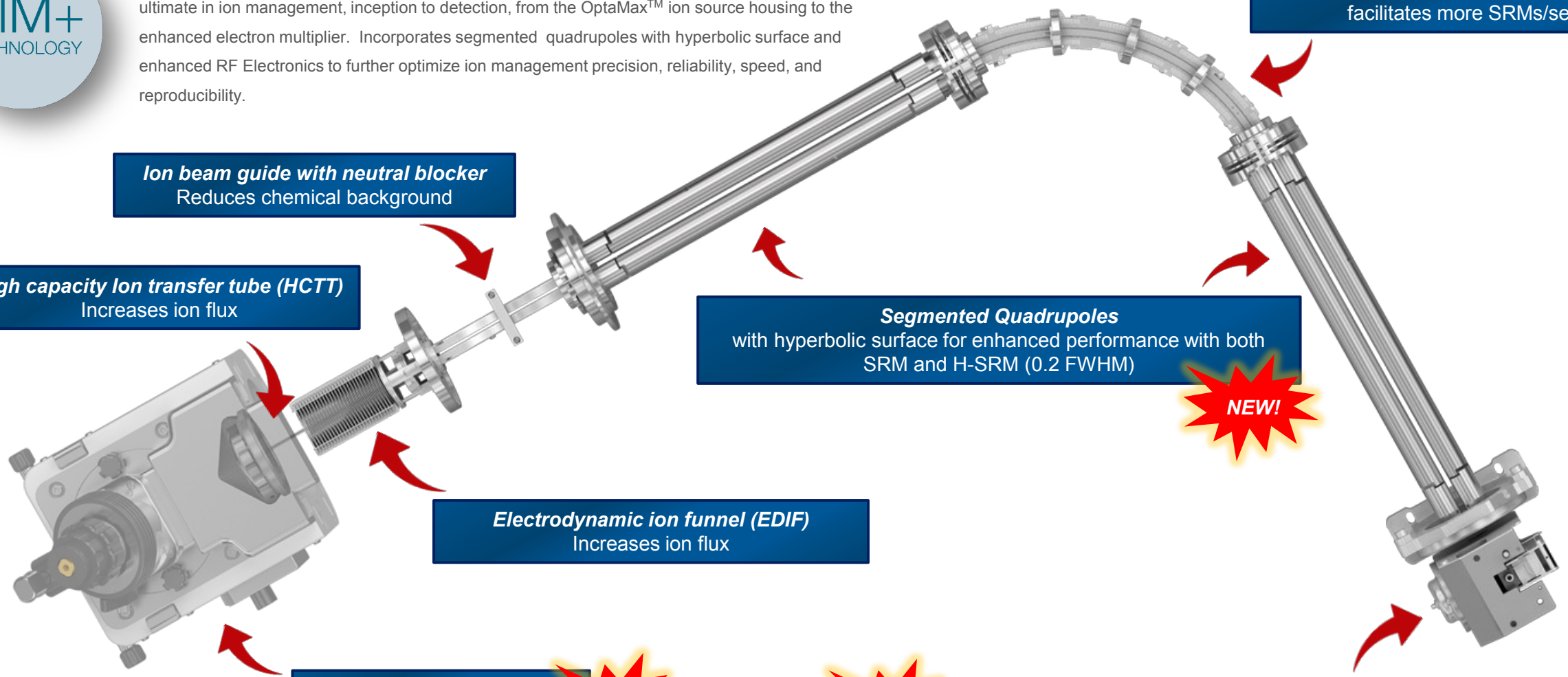
AIM+ TECHNOLOGY

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

Ion beam guide with neutral blocker
Reduces chemical background

High capacity Ion transfer tube (HCTT)
Increases ion flux



Segmented Quadrupoles
with hyperbolic surface for enhanced performance with both SRM and H-SRM (0.2 FWHM)

NEW!

Electrodynamic ion funnel (EDIF)
Increases ion flux

OptaMax™ NG
APCI ready

NEW!

NEW!

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

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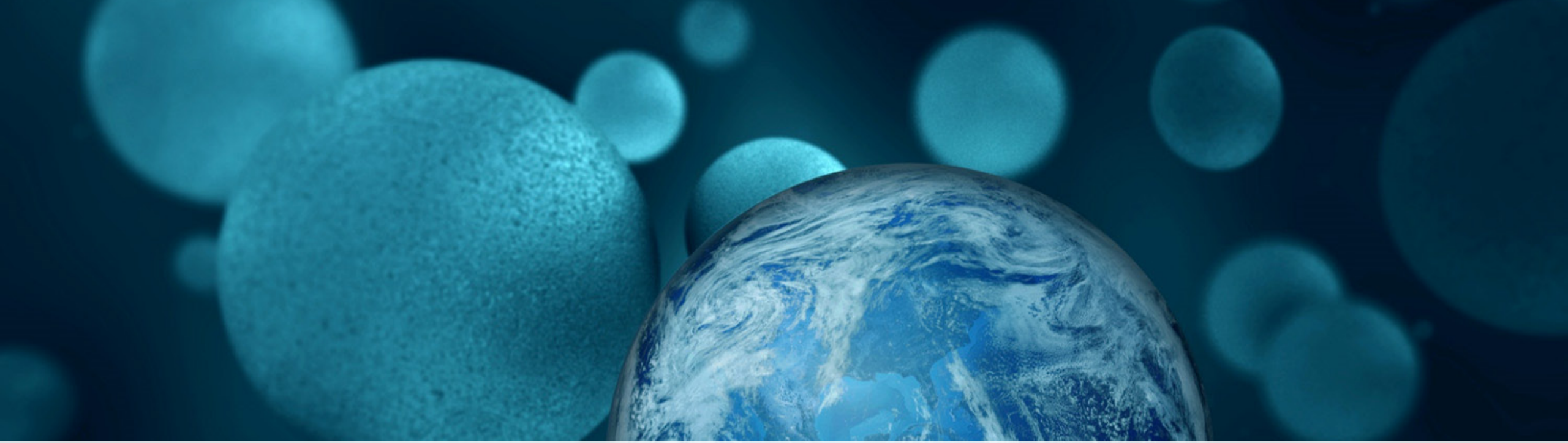

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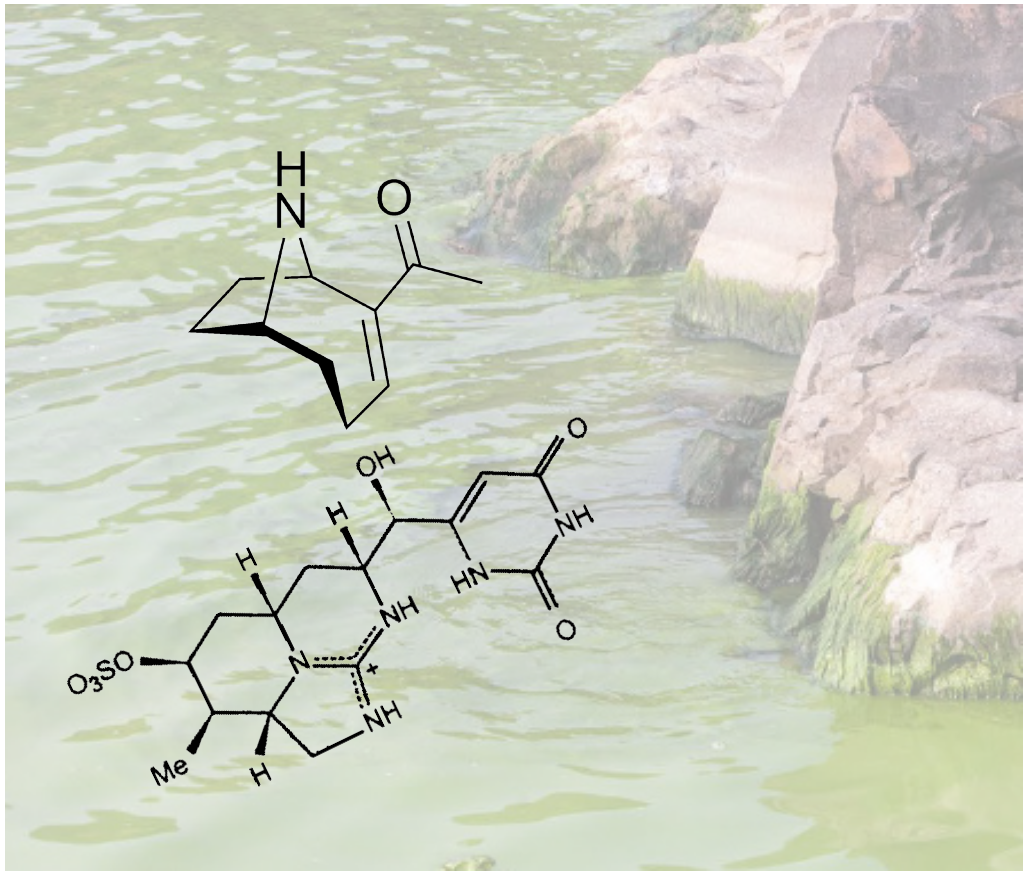
Reduced injection volume applied to the quantitation of cylindrospermopsin and anatoxin-a in drinking water according to EPA Method 545

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



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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

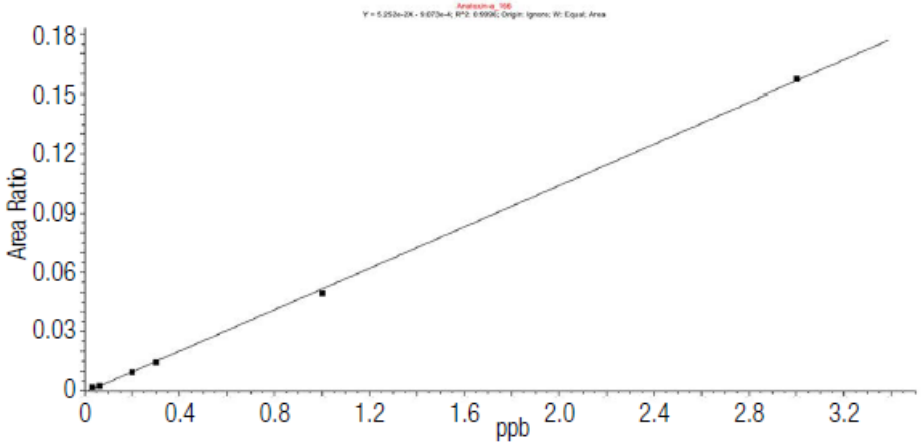
Table 1. MS source conditions.

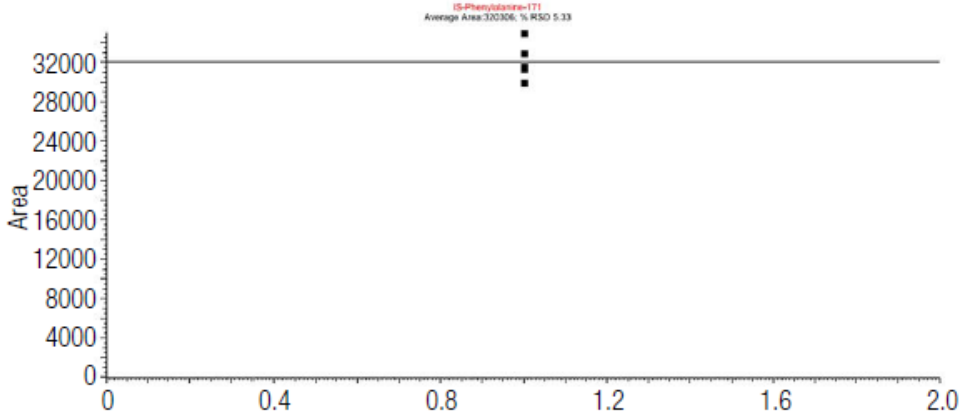
Ion Source Parameter	Value
Spray Voltage	3500 V
Sheath Gas	45 Arb
Aux Gas	10 Arb
Sweep Gas	0 Arb
Ion Transfer Tube Temperature	325 °C
Vaporizer Temperature	275 °C

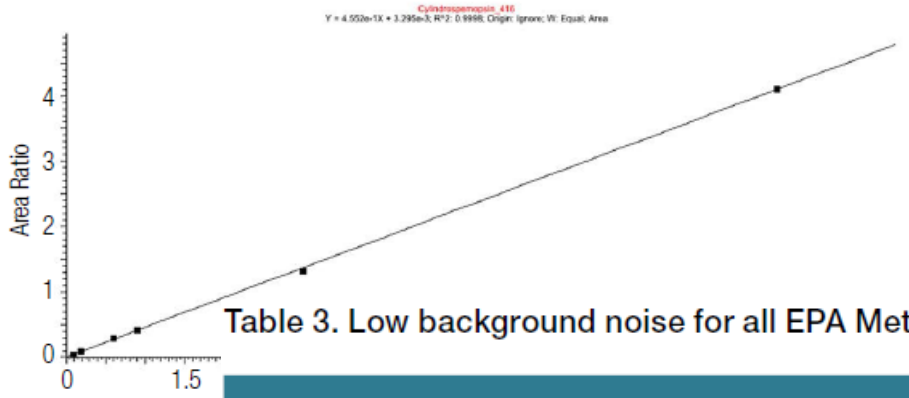
Table 2. Optimized SRM conditions.

Compound	Polarity	Precursor (*m/z*)	Product (*m/z*)	Collision Energy (V)	RF Lens (V)
Anatoxin-a	Positive	166	131	18	100
Cylindrospermopsin	Positive	416	176	35	120
Phenylalanine-d5 IS for Anatoxin-a	Positive	171	125	12	80
Uracil-d4 IS for Cylindrospermopsin	Positive	115	98	10	55

Reduced Injection Volume Applied to the Quantitation of Cylindrospermopsin and Anatoxin-a in Drinking Water According to EPA Method 545







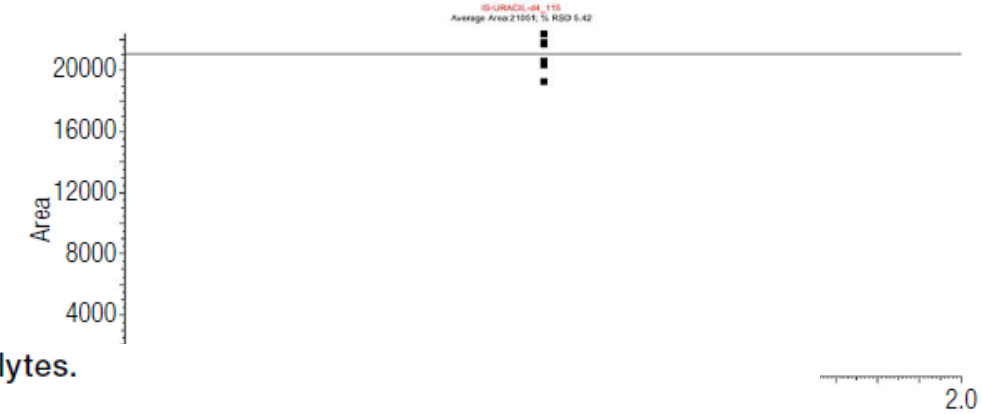


Figure 1A. Calibr
cylindrospermos
of internal

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

Analyte	MRL (µg/L)	1/3 MRL (µg/L)	5 µL	10 µL	25 µL
Anatoxin-a	0.03	0.01	NF	NF	NF
Cylindrospermopsin	0.09	0.03	NF	NF	NF

NF=Not found

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Reduced injection volume applied to the quantitation of cylindrospermopsin and anatoxin-a in drinking water according to EPA Method 545

Table 4. Precision and accuracy at 10× MRL for all EPA Method 545 analytes.

Analyte	Actual (µg/L)	LFB1 (µg/L)	LFB2 (µg/L)	LFB3 (µg/L)	LFB4 (µg/L)	%Rec	%RSD
5 µL Injection							
Anatoxin-a	0.3	0.315	0.326	0.286	0.29	101%	6%
Cylindrospermopsin	0.9	1.011	0.952	1.182	1.12	118%	10%
IS-Phenylalanine-d5		90%	96%	123%	117%		
IS-Uracil-d4		122%	119%	127%	119%		
10 µL Injection							
Anatoxin-a	0.3	0.297	0.306	0.309	0.309	102%	2%
Cylindrospermopsin	0.9	1.09	0.994	0.992	1.02	114%	4%
IS-Phenylalanine-d5		90%	99%	100%	98%		
IS-Uracil-d4		89%	101%	93%	83%		
25 µL Injection							
Anatoxin-a	0.3	0.347	0.319	0.324	0.315	109%	4%
Cylindrospermopsin	0.9	0.956	0.899	1.072	1.055	111%	8%
IS-Phenylalanine-d5		88%	94%	96%	94%		
IS-Uracil-d4		93%	101%	80%	78%		
IS criteria	50–150%						
%Recovery	70–130%						
%RSD	<20						

Reduced injection volume applied to the quantitation of cylindrospermopsin and anatoxin-a in drinking water according to EPA Method 545

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

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

FS stands for Field Sample. LFSM stands for Laboratory Fortified Sample Matrix. LFSMD stands for Laboratory Fortified Sample Matrix Duplicate.

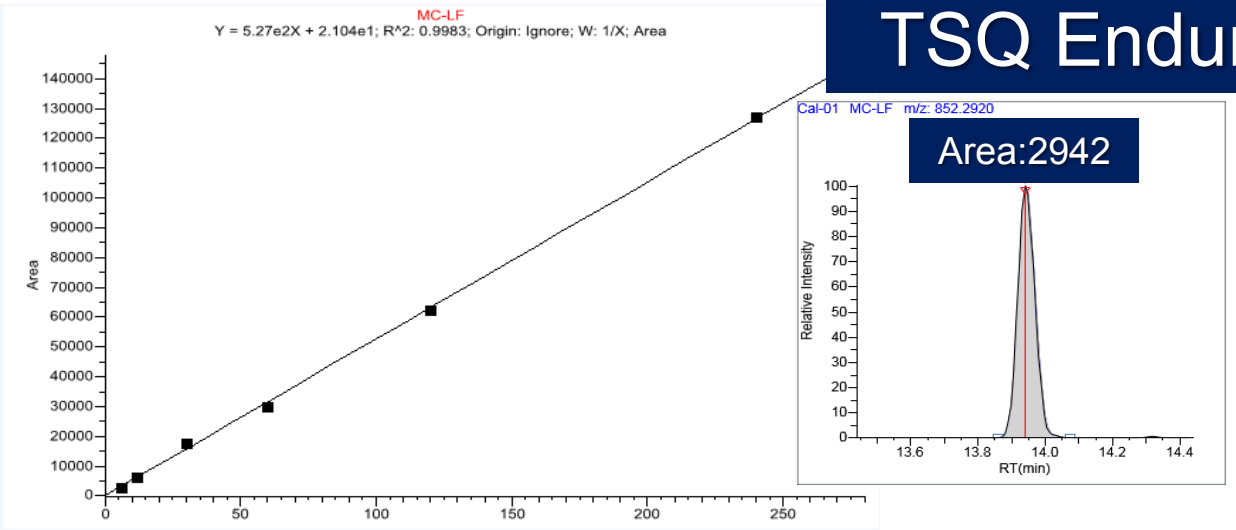
Conclusions

- 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

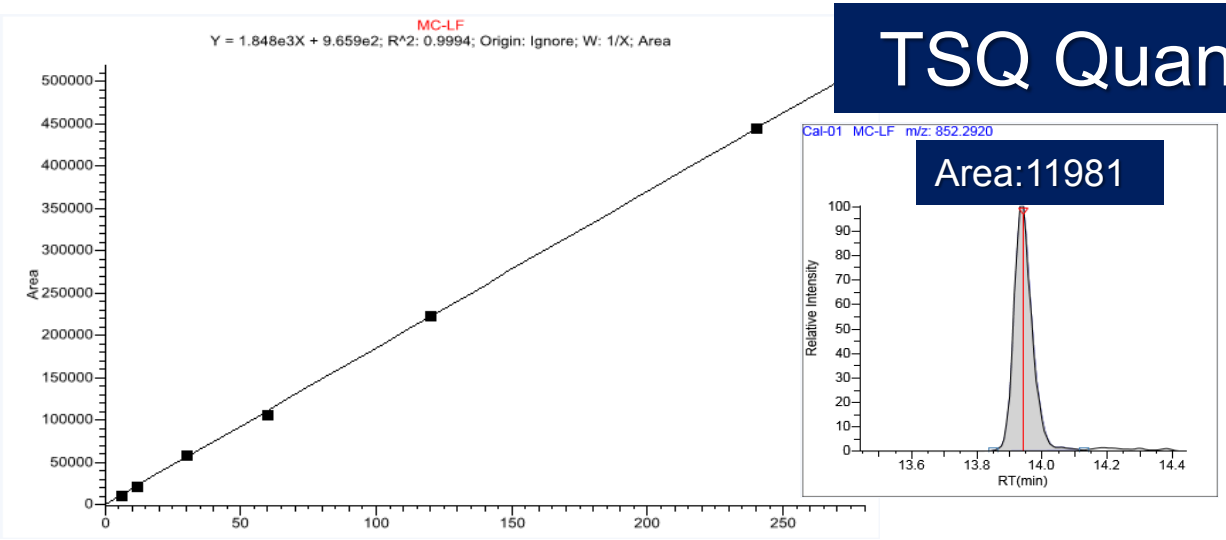
= Enhanced sensitivity
&
Reduced maintenance intervals

TSQ Quantis: Sensitivity in Regulatory Methodology (EPA 544)

TSQ Endura

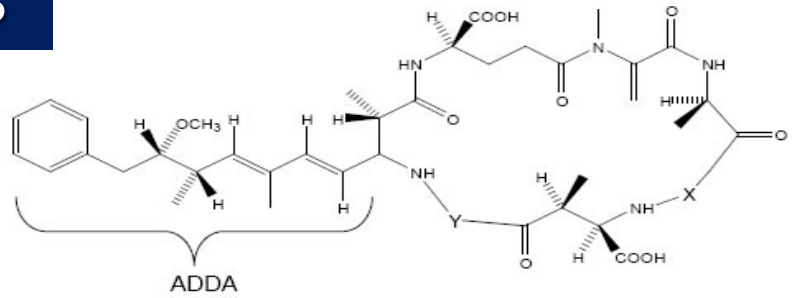


TSQ Quantis



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



Algae blooms in Western Lake Erie

~ 4 x average sensitivity improvement over TSQ Endura

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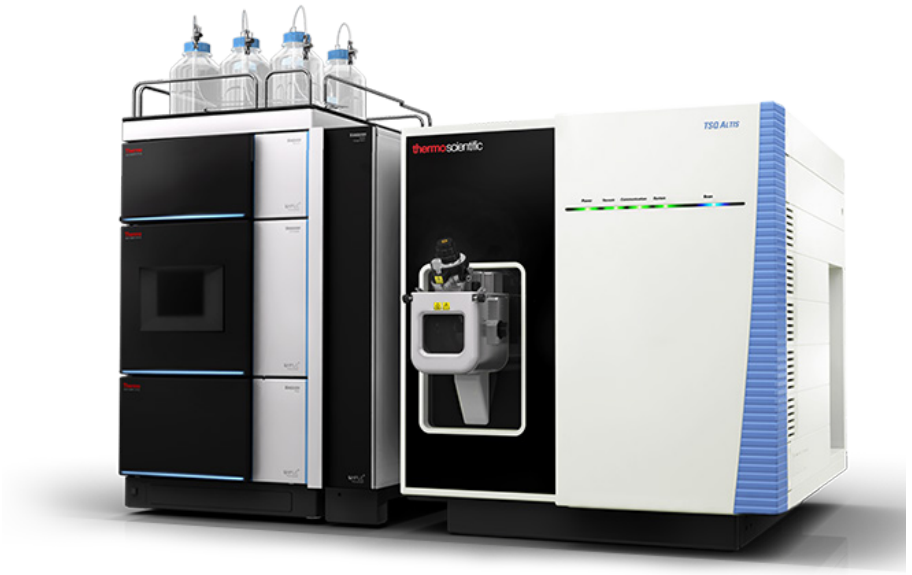
Soil Analysis for Organic Contaminants: LC-MS

Targeted Screening and Quantitation



Thermo Scientific TSQ Altis/Quantis Triple Quadrupole MS

Unknown / Known Screening and Targeted Quantitation

Orbitrap LC-MS

ThermoFisher SCIENTIFIC

Thermo Scientific™ Exactive™ Series Portfolio

PERFORMANCE

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

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*

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

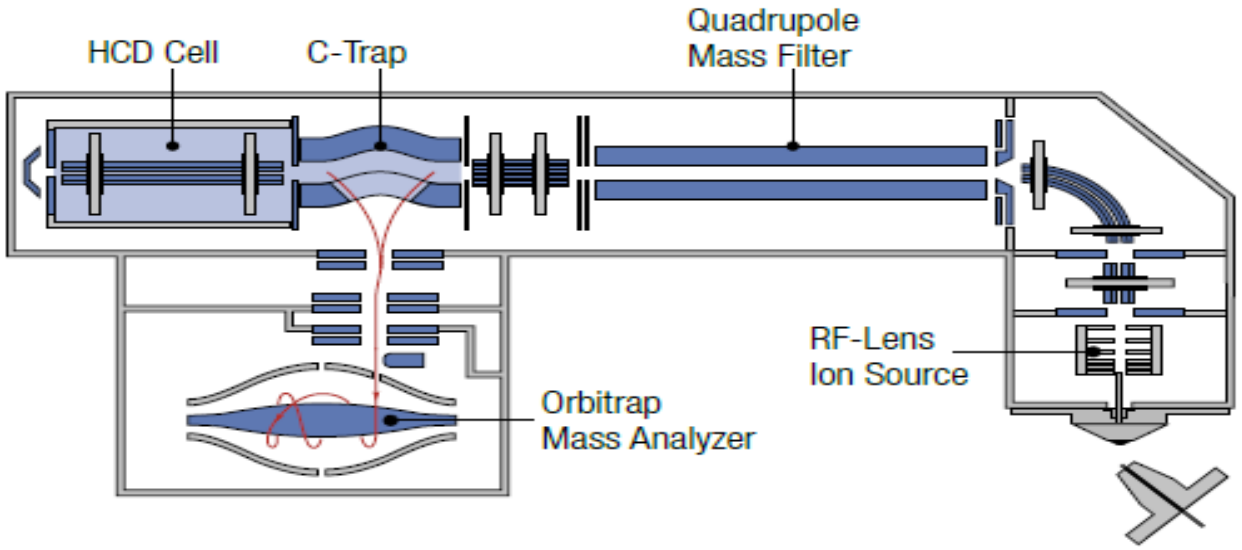
Q Exactive HF

- 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

VALUE

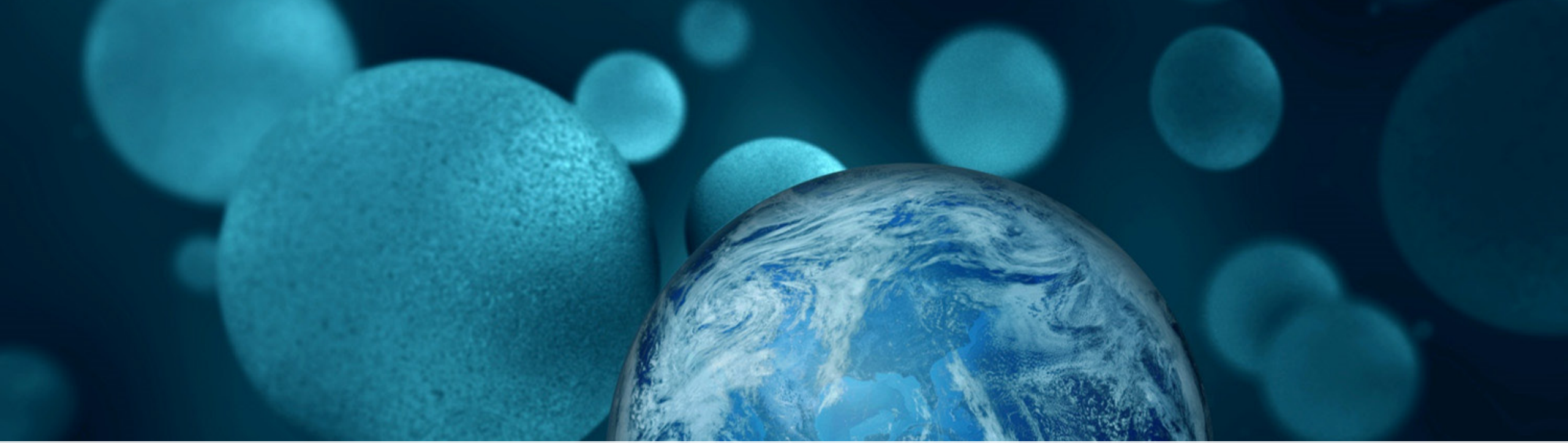
ThermoFisher SCIENTIFIC

Thermo Scientific™ Q Exactive™ Focus Hybrid Quadrupole-Orbitrap™ Mass Spectrometer



Ideal for labs performing routine analysis in food safety, environmental analysis, forensic toxicology, sports doping, clinical research, metabolomics, and pharmaceutical analyses

- 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



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Screening and Quantitation of Micro-pollutants from Sewage Water in the Process of Bank Filtration Using UHPLC-HRAM

Berliner Wasserbetriebe

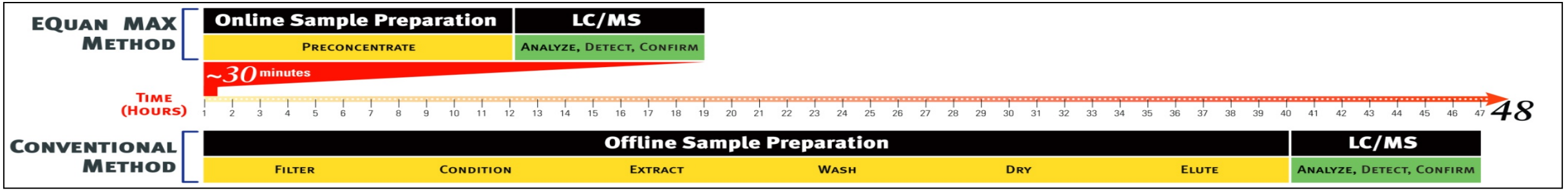
Overview

- 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

Liquid Chromatography with Thermo Scientific™ EQuan MAX Plus™ On-Line SPE

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



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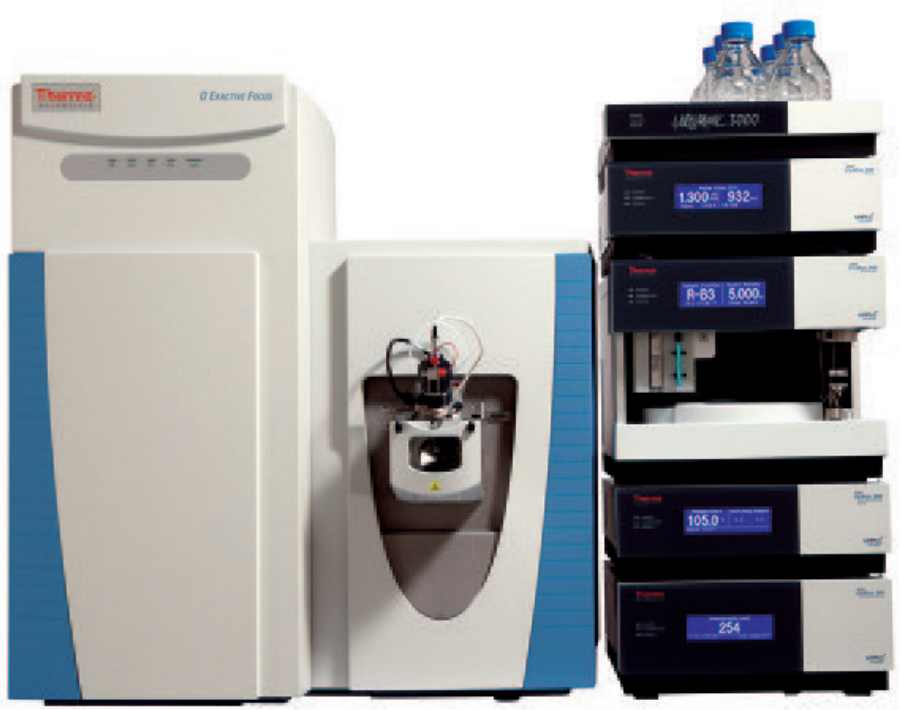
MS HRAM Instrumentation- QE Focus

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

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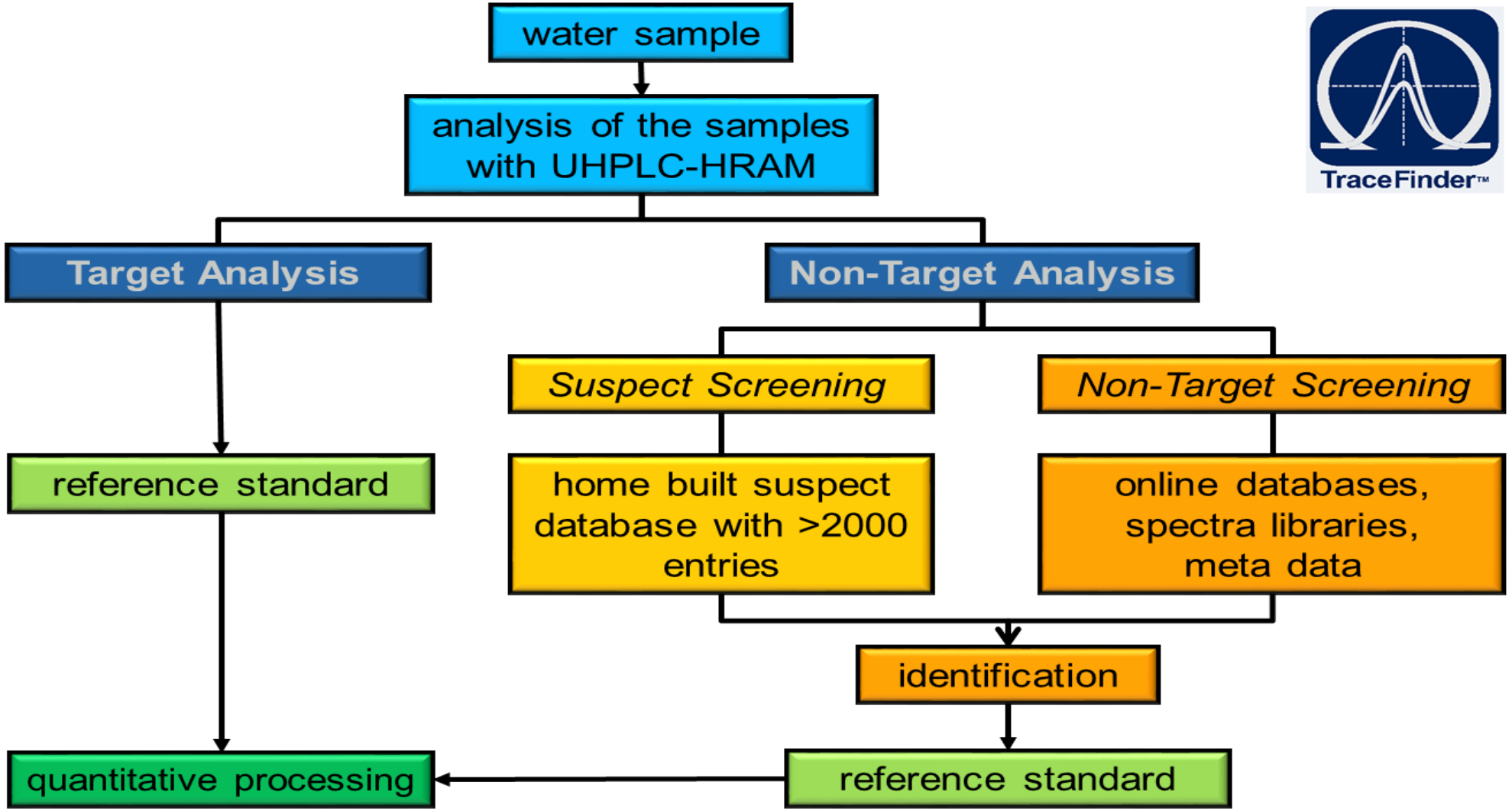
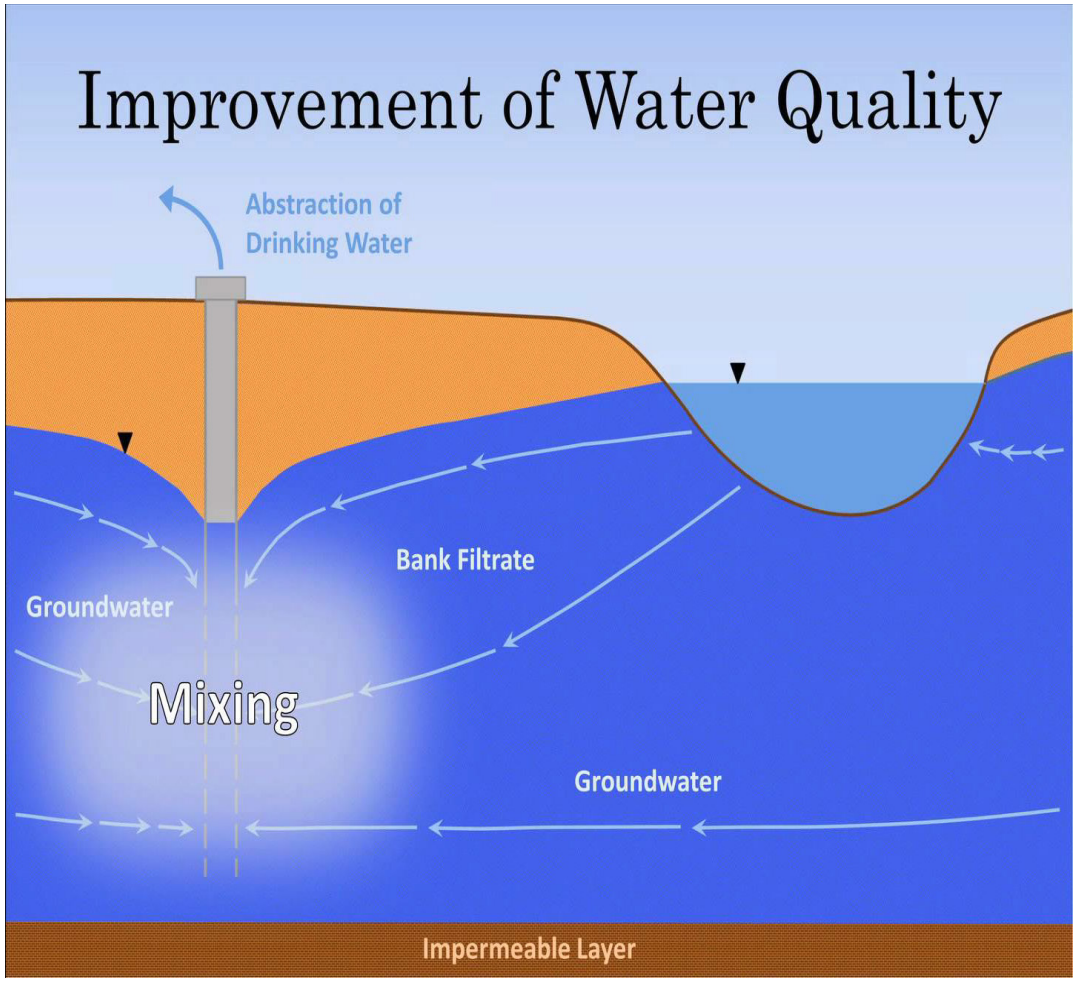
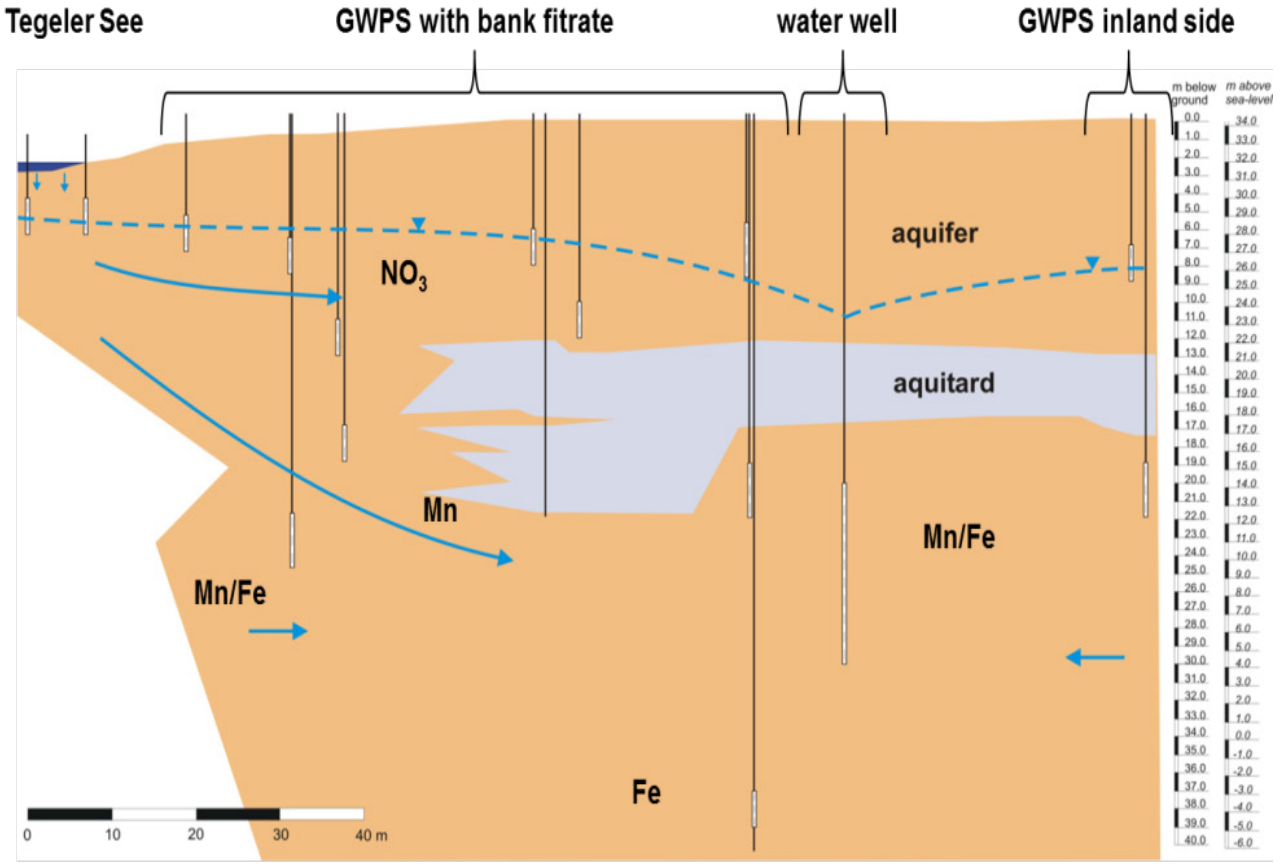
Target and Non-target Analysis Workflows



Overview-Bank Filtration of Surface Water





GWPS = ground water probing site

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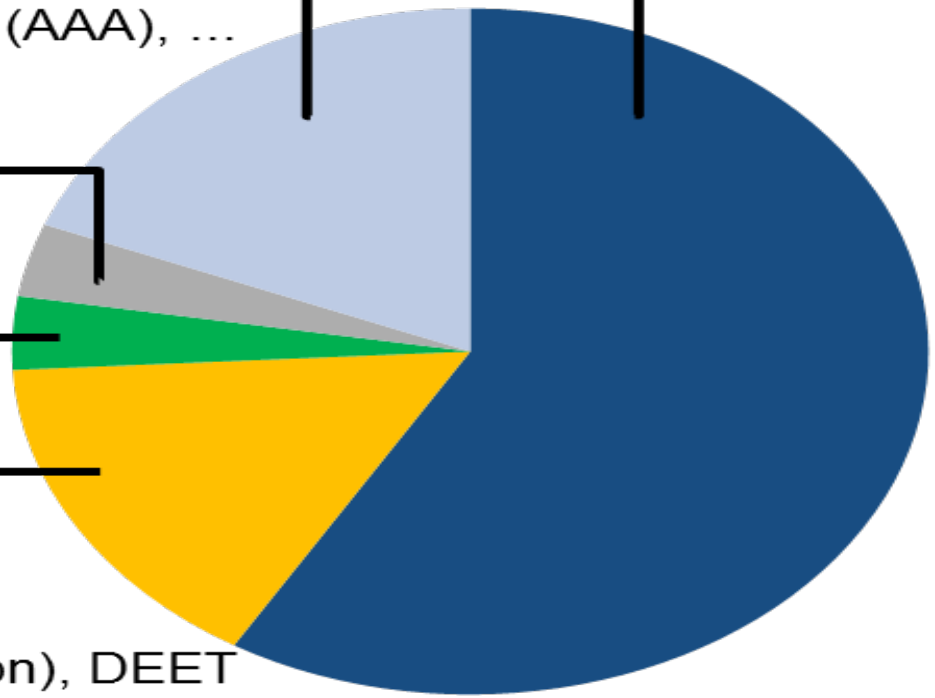
Results: Compound Types Identified and Quantitated

58 compounds were quantified



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

sweteners (2)
sucralose, acesulfame

perflourinated 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* (phenazone, tramadol,…)

Results-Comparison of Samples

- 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_3^- -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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thermofisher/ environmental community

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@ChromSolutions

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http://www.youtube.com/ChromSolutions

Facebook
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Pinterest
http://pinterest.com/chromsolutions/

Questions?


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