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S C I E N T I F I C

The Value of Ion Chromatography to Environmental Analysis

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Thermo Fisher Scientific

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Outline

- Introduction to IC-MS/MS
- IC-MS analysis of Perchlorate
- IC-MS/MS analysis of Haloacetic Acids
- IC-MS/MS analysis of Polar Pesticides

Ion Chromatography Family - 2016



Aquion



Integrion



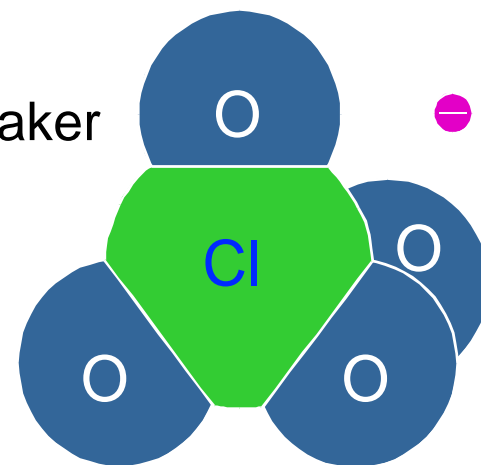
ICS-4000



ICS-5000+

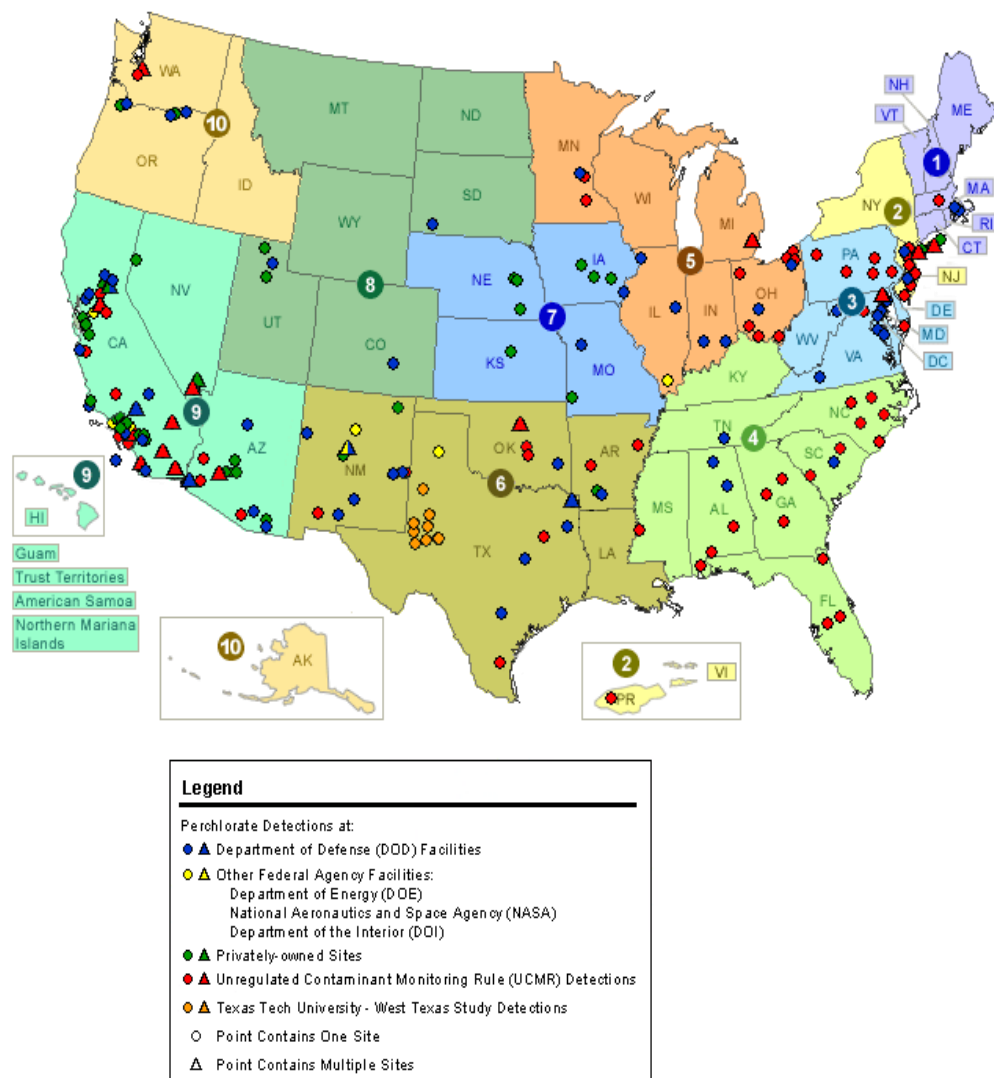
Perchlorate General Chemistry

- The perchlorate anion (ClO_4^-) is a tetrahedral array of oxygen atoms around a central chlorine atom
- The oxidation state of the chlorine is +7
- Perchlorate is a strong oxidizing agent (slightly weaker than dichromate or permanganate)
- ***Perchlorate reduction is extremely nonlabile (slow) and “rarely” observed in chemical systems***
- Perchlorate is not reduced in 0.1- 4.0 M acid;
- Other than some bacterial systems, perchlorate reduction is not observed
- ***Perchlorate is very stable in the chromatographic conditions encountered in IC applications (i.e., sample matrix and eluents)***



Where Perchlorate Has Been Found in Significant Concentrations (>1 ppb)

- Drinking and ground water
 - Thirty-plus states in the U.S.
- Aquifers associated with disposal sites
- Lakes and rivers associated with contaminated aquifers
 - Lake Mead (NV)
 - Colorado River (NV, CA, AZ)
- Crops irrigated by contaminated water
- Other foods (e.g., milk)



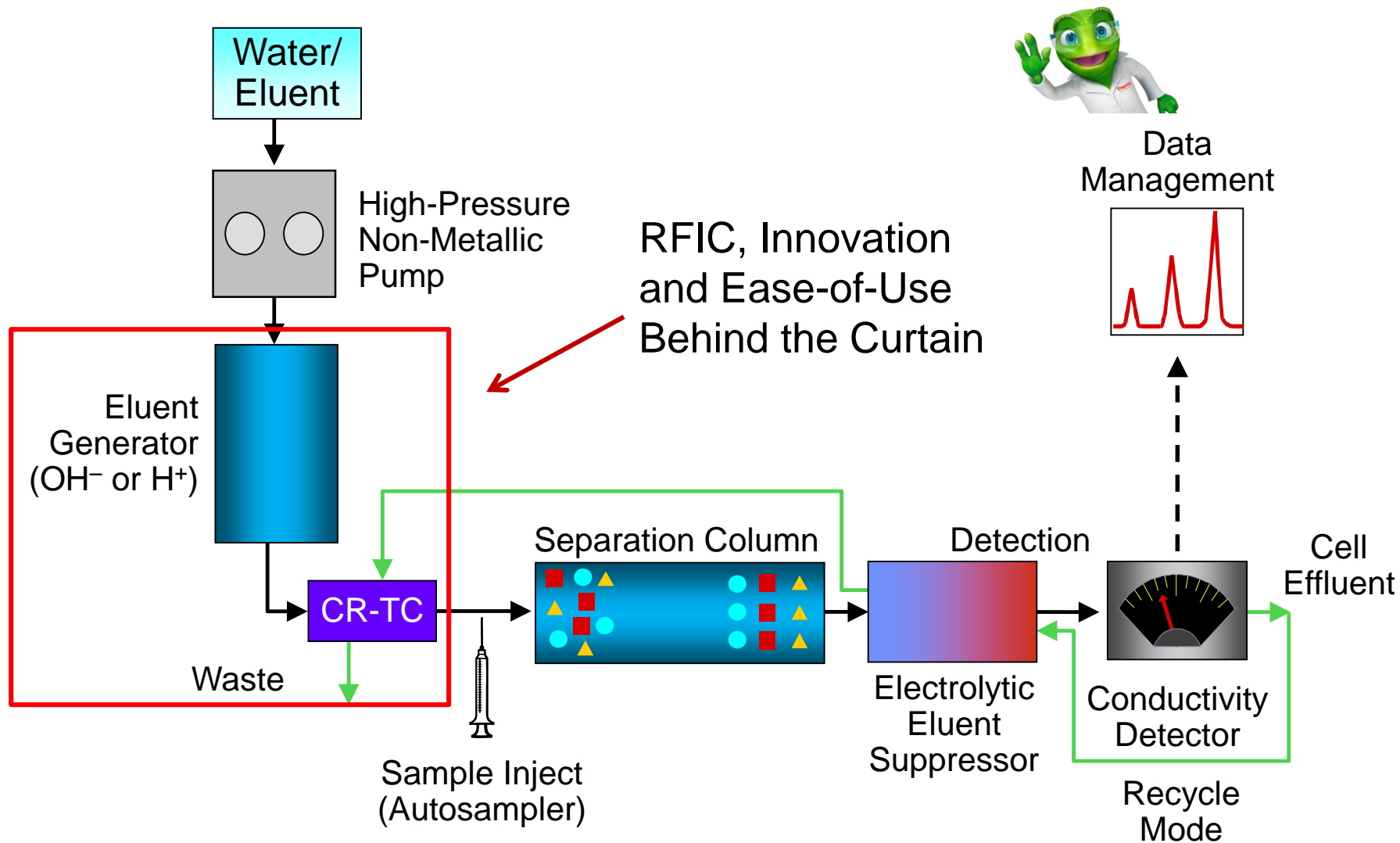
Benefits of Combining Suppressed IC with Mass Spectrometry Detection



Dionex ICS-5000 with ISQ™ EC

- Separate ionic analytes using standard IC conditions
- Suppressor permits use of high ionic strength eluents to get the benefits of high capacity columns
- Detect and identify analytes with high specificity
 - Avoid co eluting interferences to ensure accurate identification
 - Avoid background interferences to ensure highest analyte sensitivity
 - Identify analytes by mass and isotope ratios for added confirmation
 - Internal standard adds to method robustness
- Identify unknowns

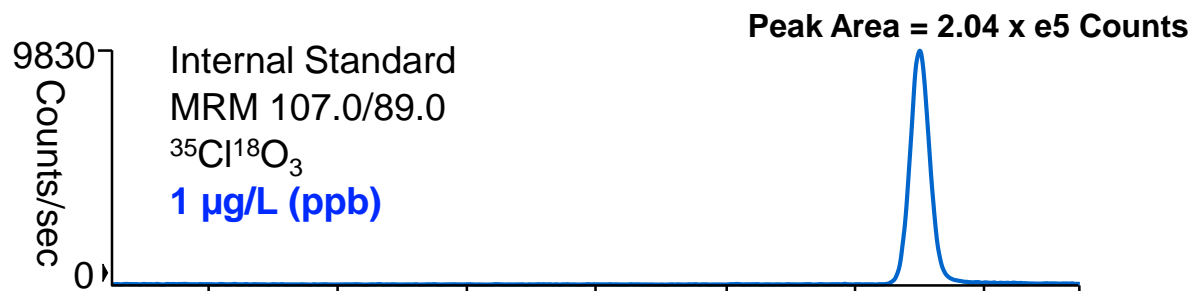
Ion Chromatography System



Advantages of MS Detection vs Conductivity Detection for Perchlorate

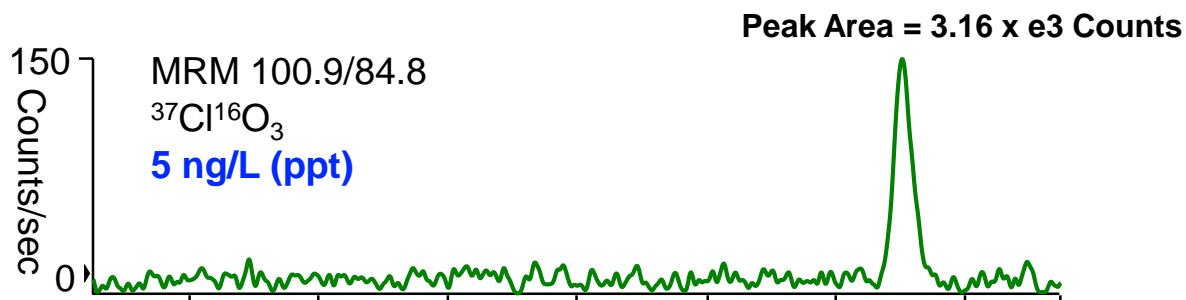
- Much greater sensitivity—MRL on order of 5–50 ppt
- Specific determination of two perchlorate isotopes
- Unique perchlorate isotope ratios
- Oxygen-18 Perchlorate isotope can be used as an internal standard for improved method robustness
- Avoids inaccurate identification due to co eluting interferences
- Sensitivity maintained even in high TDS matrices
- MS detection is inherently confirmatory

EPA Method 332.0 IC and MS-MS



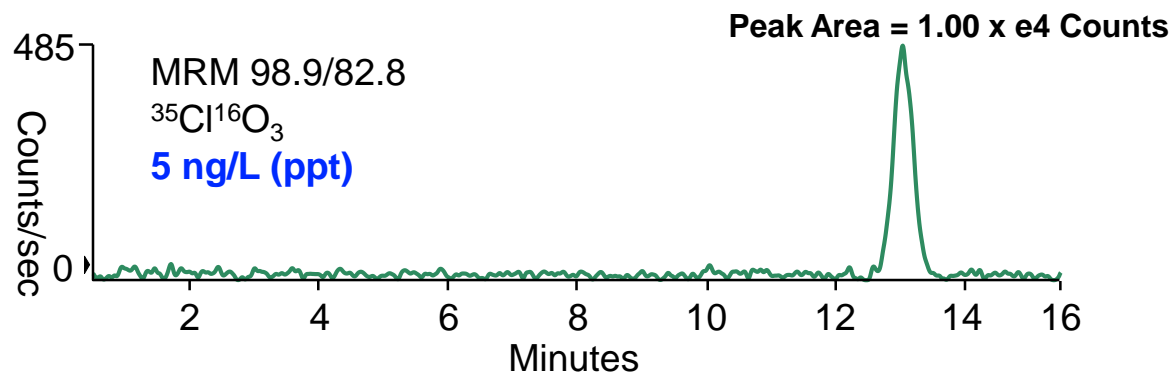
Internal Standard:
 ^{18}O perchlorate: 1 $\mu\text{g/L}$ *

Inject Volume:
100 μL



Perchlorate Isotope Ratio:
 $10,000/3160 = 3.16$

* Prepared from 1 mg/L stock
standard (Dionex Corp)



EPA method 557

Disinfection Byproducts in Drinking Water

- Disinfection treatment is essential to eliminate waterborne disease-causing microorganisms
- Ozonation – bromate
- Chlorination (chlorine or chloramine)
 - Chlorite, chlorate
 - Trihalomethanes (THM) and haloacetic acids (HAAs)
- Highly regulated due to associated health issues
 - Chlorite: nervous system, affects fetal development, anemia
 - Bromate: carcinogenic
 - Chlorate: produce gastritis, blood diseases, and acute renal failure.
 - THM & HAAs: chronic exposure could increase risk of cancer
- Regulated under Safe Drinking Water Act
- EPA promulgated to the states
- UCMR4 HAA9
- Who is interested in HAAs? “Anyone who drinks water!”



Suppressed ion chromatography with MS or MS-MS detection

- Direct injection method with matrix diversion
- Eliminates liquid-liquid extraction and labor intensive derivatization
- Eliminates co-elution issues because MS is a selective detector
- MS/MS provides molecular information assuring confirmation of analyte
- Fully automated
- Recovery > 90%

Dionex ICS-5000+ HPIC

Highly Versatile Modular Design

- Dual Reagent-Free IC system
- Improved performance in sensitivity, noise reduction, stable, and ease of use
- Increased temperature control for HAA applications
- Supports smaller particle separation columns and all column formats
- Supports multiple detection techniques





Performance: Sensitivity, Selectivity (H-SRM)

TSQ Altis



TSQ Quantis



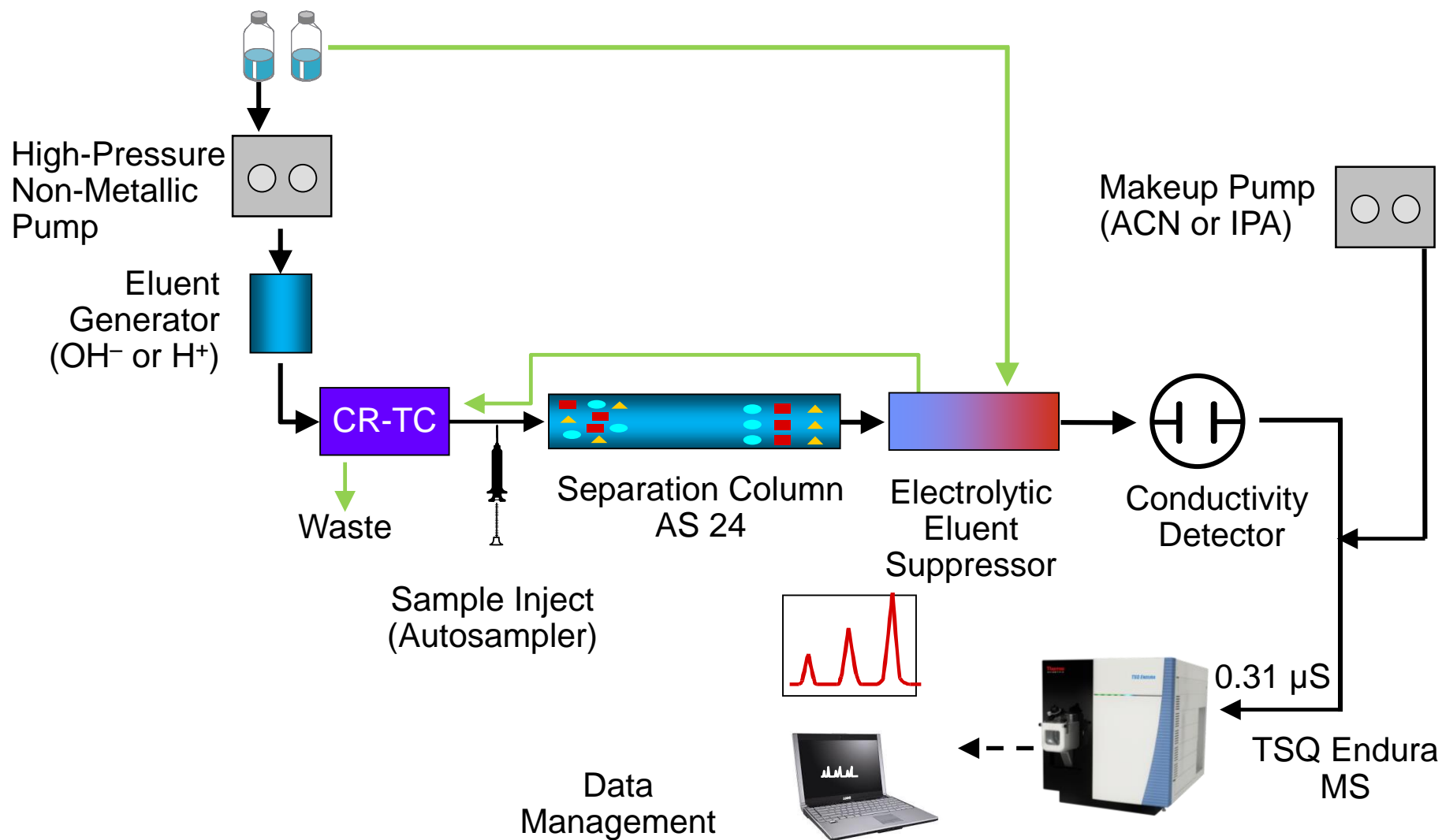
	TSQ Altis <i>High-end</i>	TSQ Quantis <i>Mid-tier</i>
Mass Range	5-2000	5-3000
SRM/sec	600	600
Selectivity (H-SRM)	0.2 Da FWHM	0.4 Da FWHM
Sensitivity (HESI Reserpine 1 pg)	500,000:1	150,000:1
Targeted Market	Omics, Research, Pharma/Biopharma, Clinical Research and Forensic Toxicology	Environmental and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

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

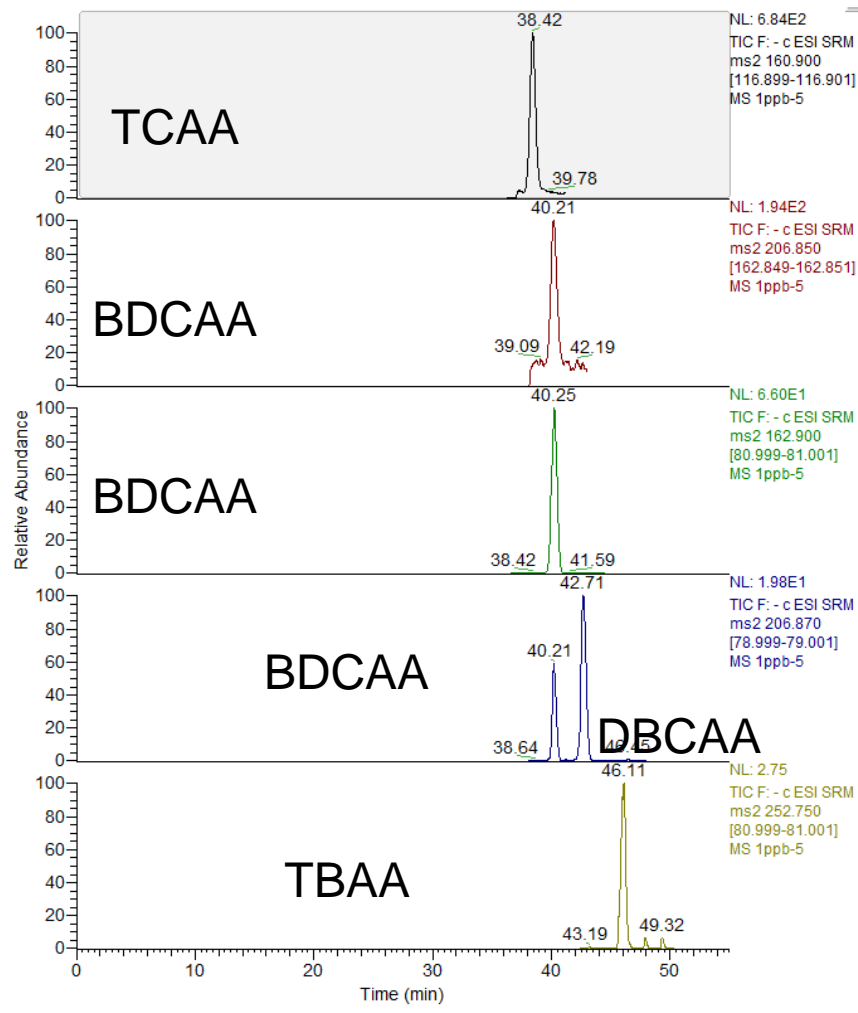
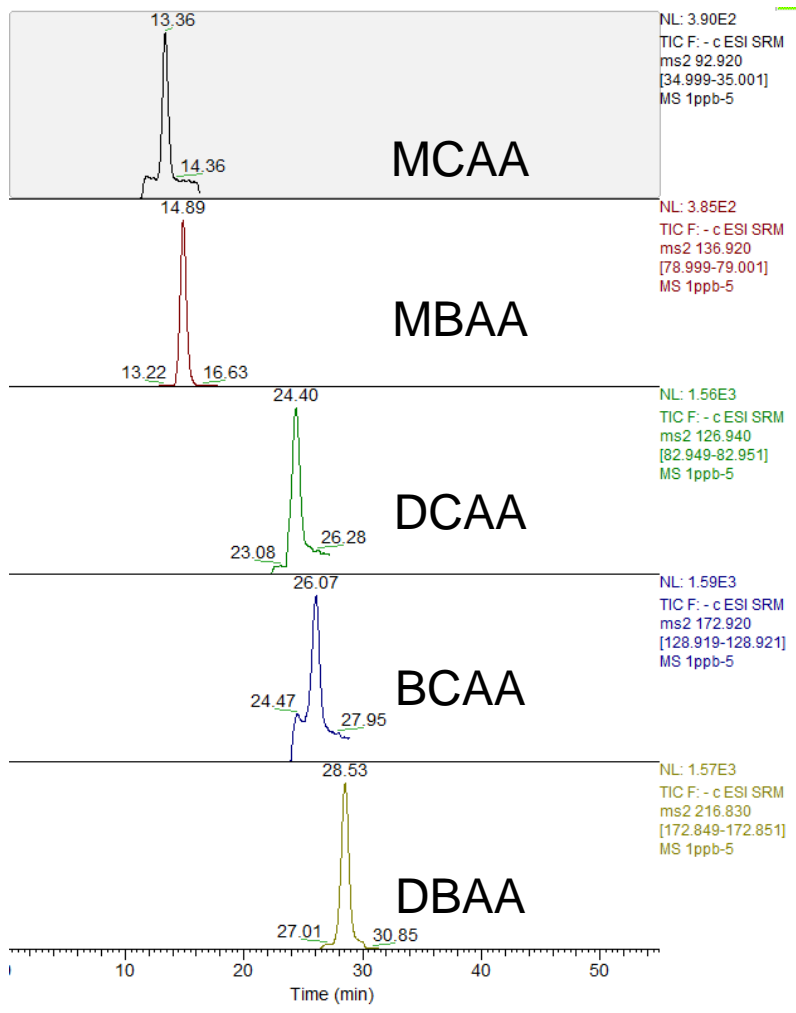
Experimental Details

- Dionex ICS 5000+ HPIC coupled to a Thermo Scientific™ TSQ Endura™ MS
- Assay – Halo Acetic Acids
 - EPA method 557
 - Regulated compounds, disinfection byproducts
 - Calibration curve ranged from 0.25-20ppb
- EPA regulates 5 HAAs*, but there are currently 9 total that are of interest. This analysis contains all 9.
 - MCAA Monochloro AA*
 - DCAA Dichloro AA*
 - TCAA Trichloro AA*
 - MBAA Monobromo AA*
 - DBAA Dibromo AA*
 - TBAA Tribromo AA
 - BCAA Bromochloro AA
 - DBCAA Dibromochloro AA
 - DCBAA Dichlorobromo AA

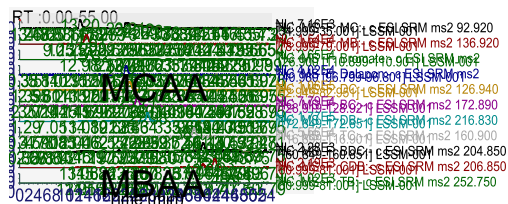
IC-MS Flow Diagram



1ppb HAA standard, mixture of 9 HAAs



LSSM of HAA, Dalapon and Bromate 20ppb spike



Bromate

Dalapon

DCAA

BCAA

DBAA

TCAA

DCBAA

DBCAA

TBAA

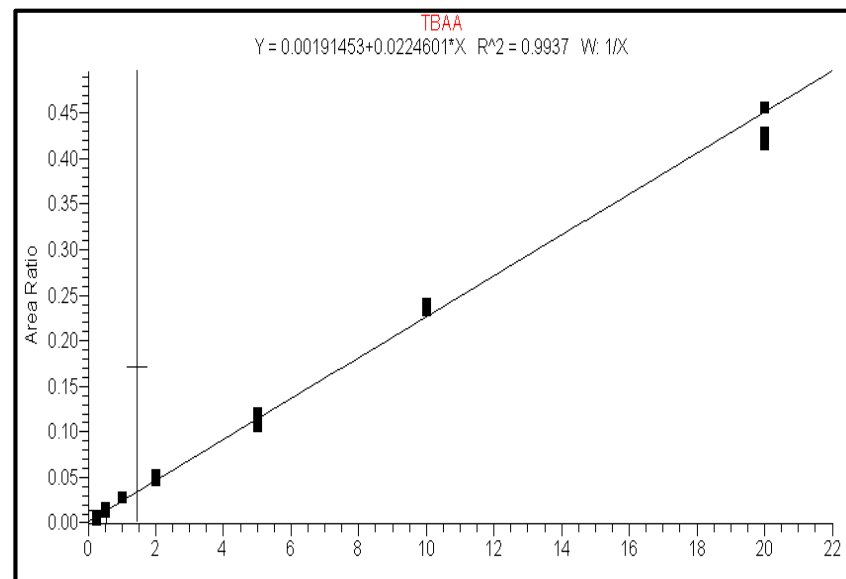
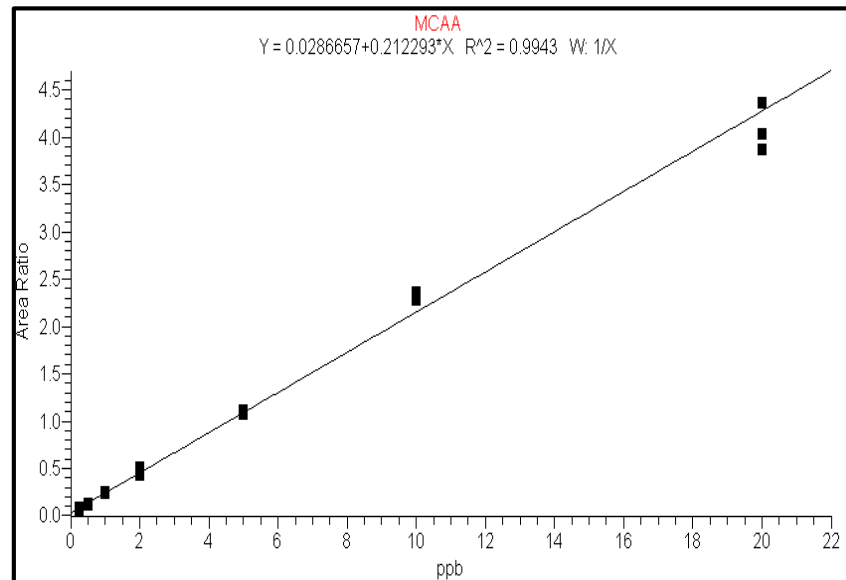
IC-MS for the Quantitation of Haloacetic Acids in Environmental Samples

Experimental Details

ICS 5000+
Flow rate: 0.3 mL/min
Eluent Source: Eluent Generator
Mobile Phase: KOH

MS: TSQ Quantis

Software: TraceFinder Software 4.1



HAA-Conclusions

- Demonstrated the analysis of 9 HAAs, Bromate and Dalapon using IC coupled to MS/MS
- Detection limits exceed the requirements of the EPA method
- No derivitization steps required prior to analysis
- Low chemical noise with suppressor to increase sensitivity, eliminate ion suppression, and enable compatibility with MS
- Ion Chromatography offers excellent separations and selectivity for HAAs
- Dionex ICS-5000+ offers temperature control which is critical for this method
- No sample prep, besides addition of internal standards for analysis
- MS/MS offers specificity and sensitivity over single quadrupole methods
- TSQ Endura offers excellent performance at an attractive price point

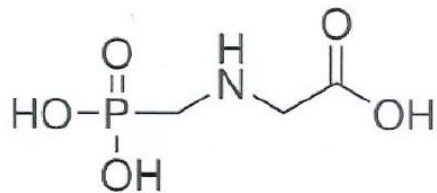
Glyphosate, AMPA, and Glufosinate

- Glyphosate (Roundup – Monsanto) and its metabolite AMPA, as well as the similar herbicide Glufosinate, are becoming increasingly monitored worldwide
- Drinking Water
 - U.S. EPA Method 547, HPLC with Post-Column Derivatization and Fluorescence detection, LOD ~5 ppb (5 µg/L)
 - U.S. EPA Maximum Contaminant Level Goal (MCLG) for drinking water is 700 ppb (700 µg/L)
 - European Drinking Water Regulations 100 ppt or 0.1 µg/L
 - European Customers (esp. Denmark) want a LOD of 10 ppt or 0.01 µg/L
- Food
 - USDA may start to test for this. <http://www.reuters.com/article/2015/04/20/us-food-agriculture-glyphosate-idUSKBN0NB1N020150420>
- Other matrices of interest
 - Breast Milk, Urine, Blood

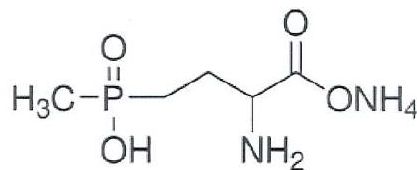
The Analytes of Interest



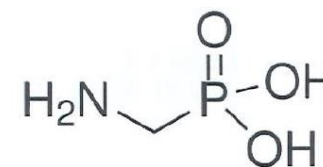
Glyphosate



Glufosinate
(glufosinate ammonium)



AMPA



Current Capabilities

- HPLC
 - LOD is very high, not sensitive enough for EU regulations
- GC-MS
 - Requires derivitization prior to analysis, slow process
- LC-MS
 - Requires derivitization prior to analysis, good sensitivity ($\sim 0.1 \mu\text{g/L}$ drinking water), can be automated
- IC-MS
 - Direct injection of water, best sensitivity

Consumer and Health Concerns

- Hot topic debate. USDA, U.S. EPA have stated that glyphosate is safe at much higher concentrations than the EU.
- Public perception issue –
 - GMO crop usage (Roundup-ready corn, soybeans, etc.)
 - Used to desiccate grains prior to harvest in U.S., Canada, UK, South America
- Faulty science stating that glyphosate causes:
 - Autism http://www.naturalnews.com/049065_glyphosate_autism_gmos.html
 - <http://www.snopes.com/medical/toxins/glyphosate.asp>
 - Gluten intolerance <http://www.biotech-now.org/food-and-agriculture/2014/03/a-lack-of-correlation-between-herbicide-glyphosate-and-celiac-disease>
 - Cancer (maybe if you bathe in it daily!) <http://www.npr.org/sections/thesalt/2015/03/24/394912399/a-top-weedkiller-probably-causes-cancer-should-we-be-scared>

Glyphosate in the News

- **In 2016**
 - The Munich Environmental Institute group - found glyphosate in 14 of Germany's most popular beers (0.46 – 29.74 $\mu\text{g/L}$)
 - Alliance for Natural Health USA tested 24 popular breakfast foods, 10 of 24 goods had detectable levels of glyphosate (86 – 1,327 $\mu\text{g/kg}$) (www.anh-usa.org)



IC-MS Conditions

Column: Thermo Scientific™ Dionex™ IonPac™ AS24 column (2 x 250 mm)

Guard Column: Thermo Scientific™ Dionex™ IonPac™ AG24 column (2 x 50 mm)

Eluent: KOH

Column Temperature: 30 °C

Flow rate: 0.3 ml/min

Make-up flow: 0.1 ml/min

Make-up solvent: IPA, 0.1 mL/min

Duration: 22 min

Injection volume: 100 µL

Injection Mode: PushFull

Loop Overfill: 2.000

Ion Source Type

H-ESI

Spray Voltage (Neg)

2800 V

Sheath Gas (Arb)

30

Aux Gas (Arb)

12

Sweep Gas (Arb)

1

Ion Transfer Tube

340 °C

Vaporizer Temperature

360 °C

Cycle time (s)

0.5

Q1/Q3 Resolution (FWHM)

0.7

CID gas (mTorr)

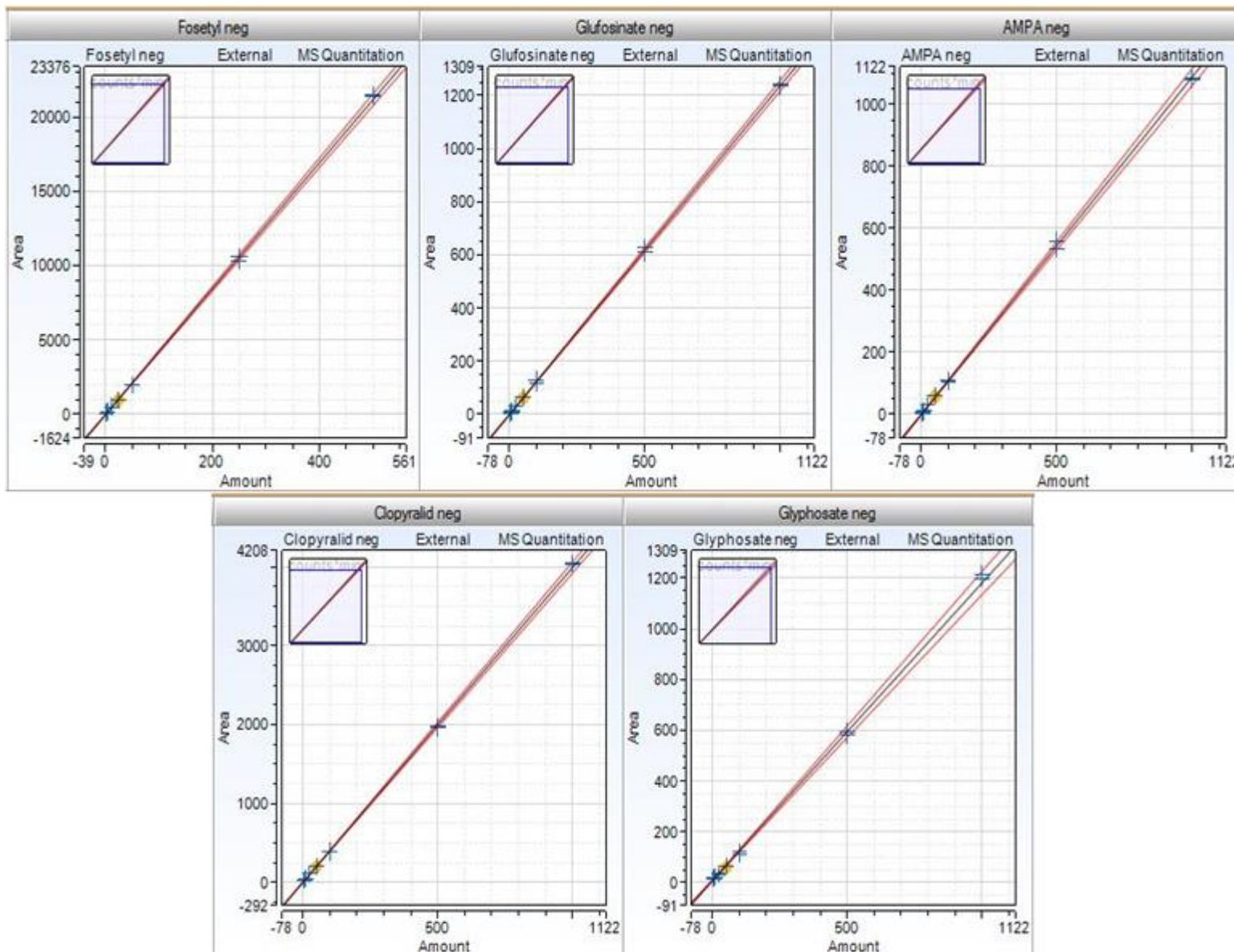
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Source Fragmentation (V)

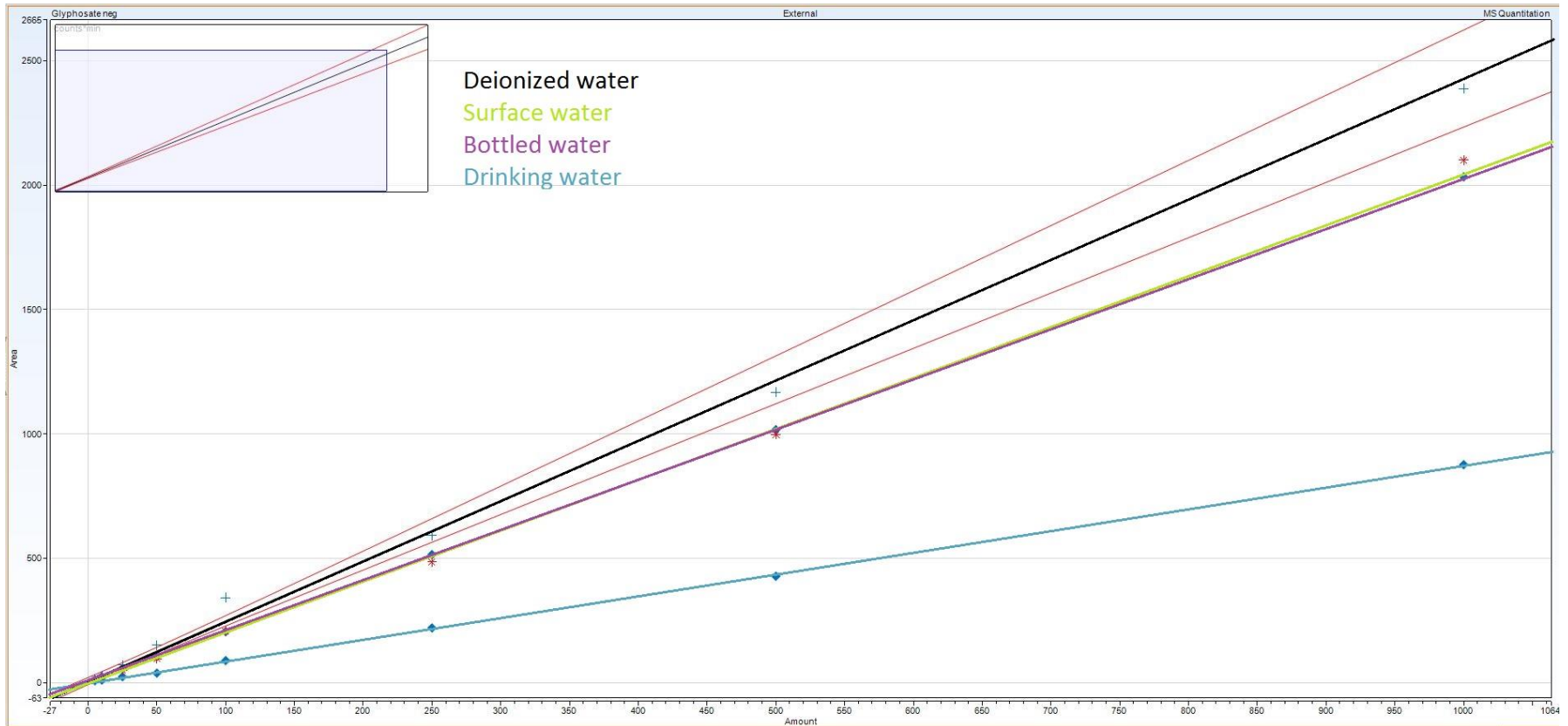
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Use calibrated RF Lens:

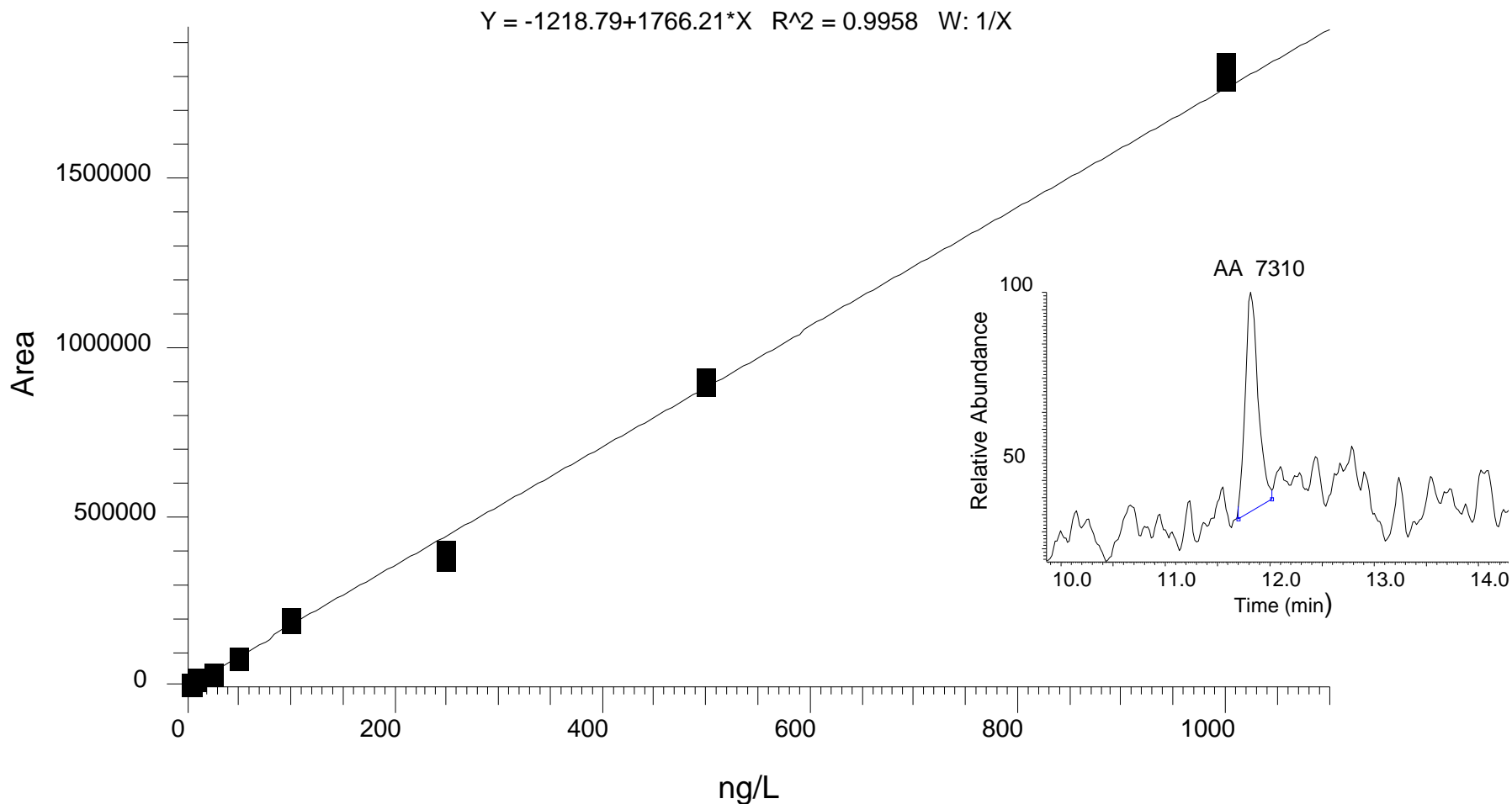
Calibration Drinking Water



Comparison of Calibration Curves in Different Matrices



Glyphosate on TSQ Altis



IC-MS/MS Analysis of Glyphosate at 5 ng/L
3 % RSD for 3 replicate injections



ThermoFisher
S C I E N T I F I C

The analysis of Polar Pesticides by IC-HRMS

With permission of Direzione Laboratorio Veritas



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Direzione Laboratorio Veritas



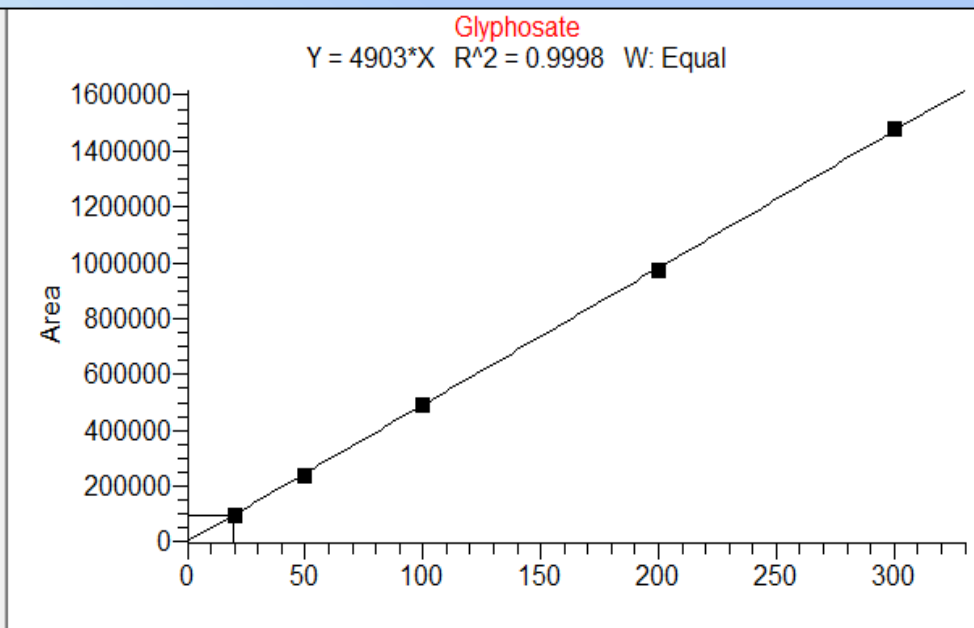
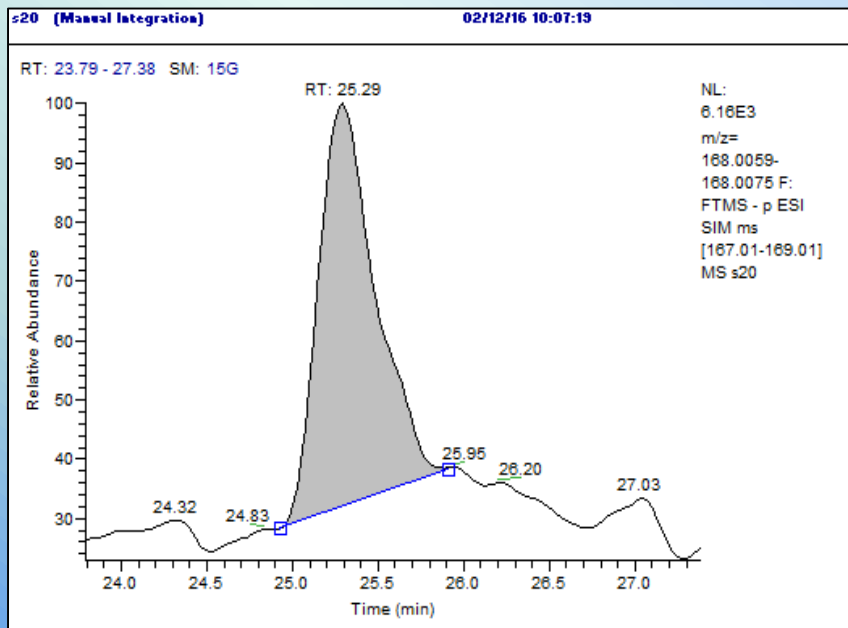


Preparazione Campione

- 8 mL Sample
- Addition of NaOH, conc. in vial 10 mM

Condizioni operative

- ✓ **System:** IC-HRMS Orbitrap Q Exactive Focus, Injection 100 μ L, SIM + Data Dependent Scan
- ✓ **Eluent:** Gradient KOH 11-45 mM
- ✓ **Column:** As11-HC, 40 $^{\circ}$ C, 250 μ L/min
- ✓ **Ionization:** HESI -



Method performance

Prove Validazione Glifosate			
	Campione alto range (ng/L)	Campione medio range (ng/L)	Campione basso range (ng/L)
	78	38	15
	84	40	18
	79	36	22
	78	37	17
	76	43	19
	70	38	20
	78	43	19
	76	39	21
	77	40	23
	73	41	19
Test di normalita Shapiro-Wilk	superato	superato	superato
Test di anomalia di Wilson	superato	superato	superato
media	76,9	39,4	19,4
std.Dev	3,5	2,4	2,3
CV%	4,6	6,1	11,8

Polar Pesticides - Conclusions

- IC-MS/MS portfolio allows determination of polar pesticides in both food and environmental samples well below regulatory limits
- Simple sample preparation for IC separation – no FMOC needed!
- Good separation efficiency of IC makes it a suitable method for most polar pesticides
- TSQ Altiva is the recommended MS/MS for water analysis @ ppt levels
- HRAM instrumentation, Q Exactive MS also shows the required detection limits in water samples
- Application notes are available. Please see thermoscientific.com.

Thanks for Your Attention!

