

ThermoFisher SCIENTIFIC

The Value of Ion Chromatography to Environmental Analysis Jonathan Beck, Ph.D.

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Topics

- IC-MS analysis of Perchlorate
 - IC with suppressed conductivity detection
- IC-MS/MS analysis of Haloacetic Acids
- IC-MS/MS analysis of Polar Pesticides
- Summary

Ion Chromatography Family - 2016









ICS-5000+

Aquion

Integrion



Ion Chromatography System





New High Efficiency Dionex IonPac 4 µm IC Columns

Dionex IonPac ion-exchange columns with 4 µm particle-size resin

Require high-pressure capable IC systems

Benefits

- Smaller particles provide better performance
- Faster run times with higher flow rates using 150 mm columns
- Better resolution with standard flow rates using 250 mm columns

Applications

- Anions in environmental waters
- Organic acids in foods and beverages
- Amines in chemical process solutions



Improved resolution provides faster runs and better results



SEM Image of 4 μm Supermacroporous Bead



Advantages of Suppressed Conductivity





Dionex Electrolytically Regenerated Suppressors





Ion Chromatography System





Disinfection Byproducts

- Chlorination Processes
 - Generate chlorite, chlorate, halomethanes and haloacetic acids
- Ozonation Processes
 - Generates bromate from bromide, and halomethanes and haloacetic acids
- Highly regulated due to associated health issues
 - Chlorite: nervous system, affect fetal development, anemia
 - Bromate: carcinogenic
 - Chlorate: produce gastritis, a late toxic nephritis, hemolysis, methemoglobinemia, hemoglobinuria, and acute renal failure



Perchlorate General Chemistry

- The perchlorate anion (ClO₄⁻) 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)



- 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)



Legend

- Perchlorate Detections at: ▲ Department of Defense (DOD) Facilities △ Other Federal Agency Facilities: Department of Energy (DOE) National Aeronautics and Space Agency (NASA) Department of the Interior (DOI) ▲ Privately- owned Sites ▲ Unregulated Contaminant Monitoring Rule (UCMR) Detections ▲ Texas Tech University - West Texas Study Detections
- O Point Contains One Site
- A Point Contains Multiple Sites



"Perchlorate interferes with the iodide uptake into the thyroid gland."

- Interferes with thyroid hormone production
- Interferes with thyroid regulation of metabolism
- Interferes with neurological development of fetus and newborn
 - Behavior changes
 - Delayed development
 - Decreased learning capability
- Changes in thyroid hormone levels may result in thyroid gland tumor
 - * U.S. EPA website (www.epa.gov)





Perchlorate EPA Method Summary

Technique	EPA	MDL in Water	LCMRL ppb	Column(s)
IC-Suppressed Conductivity	314.0 9058	1 ppb	n/a	AS16 or AS20
IC-Suppressed Conductivity Matrix Rinse-Elimination Primary and Confirmation Columns	314.1	0.030 ppb	0.150	AS16 + AS20
2-D IC Suppressed Conductivity Matrix Rinse-Elimination Primary and Confirmation Columns	314.2	0.06 0.005	0.19 	4 mm AS16 + 2 mm AS20 with TAC-ULPC 4 mm AS16 + 0.4 mm AS20 with MAC-200
IC-MS SIM 99 and 101 m/z	<u>332.0</u> 6860	0.010 ppb	0.050	AS16 or AS20
IC-MS/MS MRM 99/83 and 101/85 m/z	<u>332.0</u> 6860	0.005 ppb	0.020	AS16 or AS20
LC-MS SIM 99 and 101 m/z	6850	0.010 ppb	0.050	AS21
LC-MS/MS MRM 99/83 and 101/85 m/z	<u>331.0</u> 6850	0.005 ppb	0.020	AS21

Notes: 1. SIM = Single Ion Monitoring

3. LCMRL = Lowest Concentration Method Reporting Limit ThermoF 2. MRM = Multiple Reaction Monitoring 4. n/a = not available SCIENTIFIC

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EPA Method 332.0*–Perchlorate Analysis Using RFIC[™] System with Matrix Diversion and MS Detection

Eluent:	Electrolytically generated hydroxide
Analytical Column:	IonPac [®] AG16, AS16, 2 mm
Inj. Volume:	200 µL/min
Suppressor:	ASRS® ULTRA II, 2 mm
Diversion:	Eluent divert valve before detector to send common anions (e.g., chloride, bicarbonate, sulfate) to waste
Detector:	Single quadrupole MS (MSQ [™])**

* Jointly developed by EPA and Dionex ** Triple quadrupole MS-MS optional Note: MS detection makes the method inherently confirmatory

EPA Method 332.0* IC-MS System with Matrix Elimination





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



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



Example #2: Perchlorate in California Groundwater EPA Method 332.0 (IC-MS)*





EPA Method 332.0 IC and MS-MS





- The *m/z 99/101* area count ratio and relative retention time are used for confirmation of ClO₄⁻
- Quantitation is made by *m/z* 101
- High SO₄ can interfere: (1) an inability to detect the *m/z 99 ion, (Try m/z 101 ion, or (2) an area count ratio (m/z 99/101) that did not meet the QC requirement (Sect. 9.3.). If either of* these effects are observed, the analyst must evaluate the background counts at *m/z 99 in the half minute before* CIO₄⁻ elutes.
- Dilution or SO₄ removal cartridges can be used to remove sulfate interference.



- Allow quantification of perchlorate at sub-ppb levels, even in high TDS samples
- Ion-exchange separation with eluent suppression provides superior performance compared to LC or nonsuppressed IC separations, particularly in high TDS matrices
- Confirmation provided for perchlorate by mass spectrometry detection does not require a second injection.
- MS detection eliminates any false positives that may co-elute with perchlorate.



Analysis of Haloacetic Acids in drinking water

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!"



Haloacetic acids (HAA5 and HAA9)

Acid	HAA	Formula	рК _а	Boiling Point (°C)
Monochloroacetic Acid	MCAA*	CICH ₂ CO ₂ H	2.86	187.8
Dichloroacetic Acid	DCAA *	Cl ₂ CHCO ₂ H	1.25	194
Trichloroacetic Acid	TCAA *	Cl ₃ CCO ₂ H	0.63	197.5
Monobromoacetic Acid	MBAA *	BrCH ₂ CO ₂ H	2.87	208
Dibromoacetic Acid	DBAA *	Br ₂ CHCO ₂ H	1.47	195
Tribromoacetic Acid	TBAA**	Br ₃ CCO ₂ H	0.66	245
Bromochloroacetic Acid	BCAA**	BrClCHCO₂H	1.39	193.5
Chlorodibromoacetic Acid	CDBAA**	Br ₂ CICCO ₂ H	1.09	NA
Bromodichloroacetic Acid	BDCAA**	Cl ₂ CICCO ₂ H	1.09	NA

Summary of EPA Methods for HAAs (& Bromate, Dalapron)

Technique	EPA Method	Thermo Scientific™ Dionex™ IonPac™ Columns	MDL (ppb)
1) Liquid/Liquid Extraction	552.2		Mono: 0.13–0.20
2) Derivitization	552.3	GC-ECD	Di: 0.02–0.08
3) GC-ECD			Tri: 0.03-0.10
IC-MS, IC-MS/MS	557	Thermo Scientific™ Dionex™ IonPac™	Mono: 0.06–0.20
		Thermo Scientific [™] Dionex [™] IonPac [™]	Di: 0.02–0.11
		AS24 separation column (2 mm i.d.)	Tri: 0.04–0.09
		First dimension: Dionex IonPac AG24A precolumn + Dionex IonPac AS24A concretion	Mono: 0.17–0.45
2D-IC Suppressed Cond. (direct)	Pending	column (4 mm i.d.)	Di: 0.06–0.13
	302.0, 314	Second dimension: Thermo Scientific™ Dionex™ IonPac™ AG26 precolumn + Thermo Scientific™ Dionex™ IonPac™ AS26 separation column(0.4 mm i.d.)	Tri: 0.08–0.27

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
- Just need to add internal standard to samples
- Fully automated





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



IC Conditions

		-	
Retention Time (min)	[KOH] mM	Column	Dionex IonPac AG24 (2×50mm), IonPac AS24 (2×250mm)
		Suppressor	Dionex AERS 500, 2 mm
0.0	7.0	Cuppicsson	
15.1	7.0	Column Temperature	15 °C
30.8	18.0		400
31	60	Volume	
		Flow Rate	0.3 ml /min KOH gradient
46	60		electrolytically generated
47	7.0		
58	7.0		



Introduction to TSQ Altis and TSQ Quantis

Performance: Sensitivity, Selectivity (H-SRM)





	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



Mass Spectrometer Conditions

Parameter	Value
Ion Source Polarity	Negative Ion Mode
Spray Voltage	3200 V
Vaporizer Gas Pressure	45 units N ₂
Auxiliary Gas Pressure	10 units N ₂
Capillary Temperature	200 C
Vaporizer Temperature	200 C
Collision Gas Pressure	1.5 mTorr Argon
Ion Cycle Time	0.5 seconds

Analyte	Q1 (m/z)	Q3 (m/z)	RF lens (V)	CE (V)
MCAA	92.9	35.0	67	10
MBAA	136.9	79.0	60	13
DCAA	126.9	82.9	70	10
DBAA	216.8	172.8	72	12
BCAA	172.9	128.9	70	11
ТСАА	160.9	116.9	45	8
BDCAA	162.9	81.0	60	10
DBCAA	206.9	81.0	90	16
ТВАА	252.8	81.0	70	17
Dalapon	140.9	96.8	56	7
Bromate	126.9	110.9	90	22
MCAA-ISTD	94.0	35.0	67	10
MBAA-ISTD	138.0	79.0	60	13
DCAA-ISTD	128.0	84.0	70	10
TCAA-ISTD	162.0	118	45	8





1ppb HAA standard, mixture of 9 HAAs





LSSM of HAA, Dalapon and Bromate 20ppb spike

RT :0.00-55.00	NIA 7 4659
326625555627622222223333338210969357.	35 (99535:00) LSSALOHM ms2 92.920
	ANG MOTH Brandaten of SI SRM ms2
	The 988 Feb Pelapereon Fisis Sir Mons2
2350602135160 V4 V47 C VE VE V66 66 691	6 M 5 P 9 9 1 5 5 6 6 M ms2 126.940
2320/2010/00/00/00/00/00/00/00/2007/09/20	28,9995128,9219 ESSNE0011152 172.890
	2 16 199511 2 60 19 ESS 680 ms2 160.900
	7460 869 T68851 2559458W ms2 204.850
13418 4 4 0 8 45 1 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	7 10 999 287 (000 H SS 50 00 KM ms2 206.850
02468 024022	708.9999-281:004]-E8564-B044 1132 202.700

Bromate

Dalapon

DCAA

BCAA

DBAA

TCAA

DCBAA

DBCAA

TBAA



Overlaid Chromatograms with Divert Windows





Divert to waste, eliminates salts from matrix











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







Method Detection Limits for HAAs by ICMS

Analyte	Calculated MDL (ppb)	EPA Method 557 MDL (ppb)	EPA Method 552.2 MDL (ppb)
MCAA	0.105	0.20	0.273
MBAA	0.104	0.064	0.204
DCAA	0.044	0.055	0.242
DBAA	0.021	0.015	0.066
BCAA	0.059	0.11	0.251
ТСАА	0.033	0.090	0.079
BDCAA	0.141	0.050	0.091
DBCAA	0.214	0.041	0.468
ТВАА	0.159	0.067	0.820
Dalapon	0.050	0.038	N/A
Bromate	0.059	0.020	N/A



- 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 (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 Derivitization and Fluorescence detection, LOD ~5 ppb (5 μg/L)
 - U.S. EPA Maximum Contaminant Level Goal (MCLG) for drinking water is 700 ppb (700 $\mu g/L)$
 - 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. <u>http://www.reuters.com/article/2015/04/20/us-food-agriculture-glyphosate-idUSKBN0NB1N020150420</u>
- Other matrices of interest
 - Breast Milk, Urine, Blood



The Analytes of Interest







What is Glyphosate?

 Glyphosate (*N*-(phosphonomethyl)glycine) is a broad spectrum systemic herbicide commonly used as weed control



- It is rapidly degraded to aminomethylphosphonic acid (AMPA) metabolite frequently found in plants, water, and soil
- Very polar, zwitterionic compound
- Difficult to retain on C18 LC column







- 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 (~0.1 μ g/L drinking water), can be automated
- IC-MS
 - Direct injection of water, best sensitivity



- 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 <u>http://www.naturalnews.com/049065_glyphosate_autism_gmos.html</u>
 - 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-weedkiiller-probably-causes-cancer-should-we-be-scared



• In 2016

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







IC-MS Conditions

Column: Thermo Scientific™ Dionex™ IonPac™		Ion Source Type	H-ESI
AS24 column (2 x 250 mr	m)	Spray Voltage (Neg)	2800 V
Guard Column: Thermo S	Scientific™ Dionex™	Sheath Gas (Arb)	30
	2 X 30 mm)	Aux Gas (Arb)	12
Eluent: KOH		Sweep Gas (Arb)	1
Column Temperature:	30 °C	Ion Transfer Tube	340 ⁰C
Flow rate:	0.3 ml/min	Vanarizar Tomporatura	
Make-up flow:	0.1 ml/min		300 °C
Make-un solvent:	IPA 0.1 ml /min	Cycle time (s)	0.5
		Q1/Q3 Resolution (FWHM)	0.7
Duration:	22 min	CID gas (mTorr)	1.5
Injection volume:	100 µL	Source Fragmentation (V)	0
Injection Mode:PushFullLoop Overfill:2.000			0
		Use calibrated RF Lens:	



Calibration Drinking Water





Calibration Bottled Evian Water







Comparison of Calibration Curves in Different Matrices





Glyphosate on TSQ Altis



Thermo Fisher SCIENTIFIC

IC/HPLC-MS System

Direzione Laboratorio Veritas







- 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 Quantiva is the recommended MS/MS for water analysis @ ppt levels
- IC-TSQ Endura is suitable for food sample analysis @ ppb levels (LC-TSQ Quantiva an option if more sensitivity or more difficult matrix is analyzed)
- HRAM instrumentation, Q Exactive MS also shows the required detection limits in water samples
- Application notes are being prepared and planned to be out in September/October. Please see thermoscientific.com.



Thanks for Your Attention!



