

# Specific and Selective MS Detection for Environmental Analysis by Ion Chromatography

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## Executive Summary

IC is a commonly used analytical approach in environmental applications. For samples with known analytes, IC-MS provides significant advantages over IC methods that utilize general detectors, including improved sensitivity, enhanced selectivity, and unambiguous identification. Selected ion monitoring (SIM) mode provides the most sensitive and selective detection, allowing accurate determination of low ppb levels of analytes in high matrix samples with minimal sample preparation.

## Key Words

IC-MS, Environmental analysis, MSQ, IonPac

## Abstract

IC is a well-established and routine method for analyzing ionic compounds in environmental samples, and is incorporated into environmental regulatory methods worldwide for quantifying contaminants.

Analysis of environmental samples presents challenges, such as the proposed method's low-level-of-detection (LLOD) requirements and the often complex matrices encountered. General IC detectors may not be capable of achieving the levels required by the method without sample enrichment and/or large-volume injection. Samples may be in high-level matrices and target analytes may coelute with other compounds. General detectors cannot differentiate between coeluting compounds, making detection and quantitation difficult. Identification by retention time alone may not be adequate or meet the method development prerequisites, and requiring confirmation by alternate methods could double analysis time.

MS detection can help address these challenges. With samples of known analytes, SIM can significantly improve detection limits and differentiate between coeluting analytes contained in high matrix samples. Many environmental samples analyzed by IC are of low mass, requiring the MS be capable of efficient low-mass detection. For most regulated methods, the detection at the sample's mass-to-charge ratio ( $m/z$ ) provides additional specificity for acceptable identification.

This study describes the advantages of IC-MS in three environmental applications in which low-mass compounds were analyzed: perchlorate (<0.5 ppb); glyphosate (<1 ppb); and low-mass organic acids (<10 ppb). Samples were injected directly without labor-intensive sample preparation. Combined with rapid chromatographic separation, throughput was significantly improved over other methods. The MS was operated in SIM mode, which enabled minimum sample cleanup while ensuring sensitive and selective quantitation. Isotope-labeled internal standards were used to ensure quantitation accuracy. Method performance parameters such as linearity, calibration range, precision, accuracy, and detection limits are presented for these applications.

## Applications Introduction

### Perchlorate<sup>1</sup>

Perchlorate ( $\text{ClO}_4^-$ ) has been used extensively as an oxidizer in rockets, munitions, and fireworks since the 1950s, and has been found to cause thyroid dysfunction in humans. The California Department of Health Services first reported the determination of perchlorate in drinking water in 1997. The U.S. EPA has stated that defense facilities from Los Angeles to Cape Cod have discharged large amounts of perchlorate onto the ground, contaminating groundwater in many of these sites. In 2001, it was reported that perchlorate was migrating into groundwater in California from a landfill site for civilian and military explosives. In 2002, the U.S. EPA recommended a maximum contaminant level (MCL) for perchlorate of 1 ppb in drinking water. Some states have set their own limits ranging from 4 to 18 ppb. For example, California's current action level is 6 ppb.

### Glyphosate<sup>2</sup>

Glyphosate (*N*-[phosphonomethyl] glycine) is a nonselective herbicide which inhibits the shikimic acid pathway in plants. Glyphosate is the most commonly used agricultural pesticide and second most used pesticide around homes and gardens. It is used to control woody and herbaceous weeds in forestry, both cropped and noncropped sites. Although the bacteria in soil break down glyphosate into aminomethyl phosphonic acid (AMPA), wastewater discharge samples and drinking water samples in United States and Europe have tested positive for glyphosate. Studies have raised global health and environmental concerns about the usage of glyphosate. In 2006, the U.S. EPA set the MCL for glyphosate at 0.7 mg/L. Long-term exposure to glyphosate at levels above the MCL may cause kidney damage and reproductive defects in human biological systems.

### Low-Molecular-Weight Organic Acids<sup>3</sup>

Organic acids are present in many media and play various crucial roles. Low-molecular-mass organic acids (LMMOAs) have been researched extensively in areas such as:

- Ecosystems and environmental science, as these acids act as key components in mechanisms that some plants use to cope with environmental stress and fertilizer release
- Biotechnology, to better understand and optimize fermentation processes
- Biomedical research

Recently, LMMOAs were found to have inhibitory effects on the bioconversion efficiencies of lignocelluloses to ethanol. Among the analytical techniques for determination of LMMOAs, chromatographic methods with various modes of detection provide the most thorough information to profile and monitor LMMOAs.

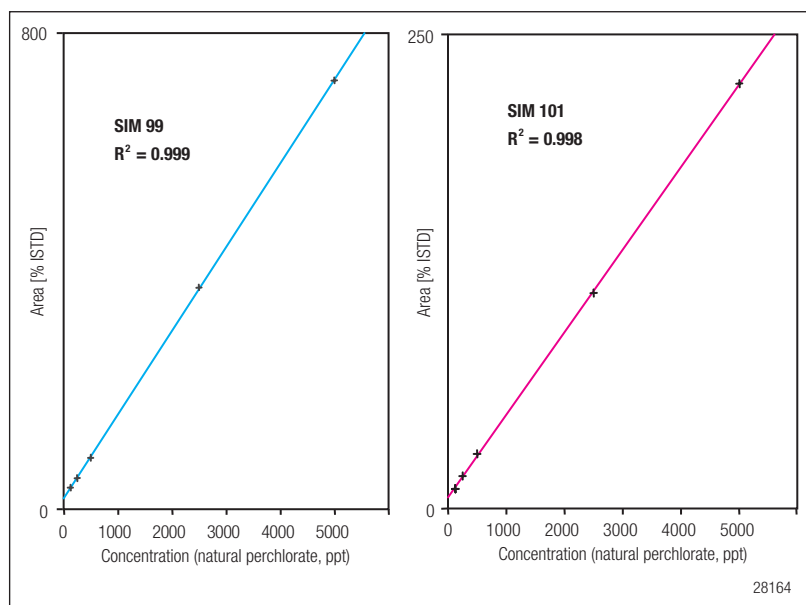


Figure 1. Linear calibration of perchlorate spiked into 1000 CCS matrix using neg ESI/MS and internal standard ( $\text{Cl}^{18}\text{O}_4$ ).

## Advantages of MS Detection

### Low-Level Detection in Matrix—SIM

The MS can provide lower detection limits in high-ionic-strength matrices than suppressed conductivity detection.

#### Perchlorate

The selectivity of the single quad MS allows the quantification of perchlorate at  $m/z$  99/101 in high-ionic-strength matrices at well below the California 6 ppb action level. Recoveries must be determined in the appropriate matrices.

Figure 1 shows the linear calibration curves for perchlorate in a matrix of 1000 mg/L each of chloride, carbonate, and sulfate (1000 CCS) at  $m/z$  99 and  $m/z$  101 over the range of 125–5000 ng/L using an internal standard method. The sodium chloride used to prepare the simulated matrix contributed a small perchlorate peak to the matrix, as seen by the elevated y-intercept. These data show that quantification can be performed at either  $m/z$  with good linearity and sensitivity.

#### Glyphosate

Using a single quadrupole MS in SIM mode configured with matrix diversion, the MS detects glyphosate and AMPA at  $m/z$  168 and 110, respectively, well below the low ppb levels required by current U.S. EPA guidelines found in Method 547.

The minimum detection limit (MDL) in matrix was calculated by seven replicate injections of 5 ppb in a simulated matrix with high concentrations of chloride, carbonate, nitrate, and sulfate (250 ppm chloride and sulfate, 150 ppm sodium bicarbonate, 20 ppm nitrate). Using the equation  $MDL = t_{99\%} \times S_{(n-1)}$  where  $t$  equals the Student's  $t$  test at 99% confidence intervals ( $t_{99\%,6} = 3.143$ ) and  $S$  is the standard deviation, the MDLs for both compounds were calculated. For the two-dimensional method, the estimated MDL for AMPA in matrix was 0.877 ppb, and the MDL for glyphosate was 0.542 ppb. The linearity of the standards was calculated from 0.75 to 100 ppb for AMPA, and 0.50 to 100 ppb for glyphosate (Figure 2).

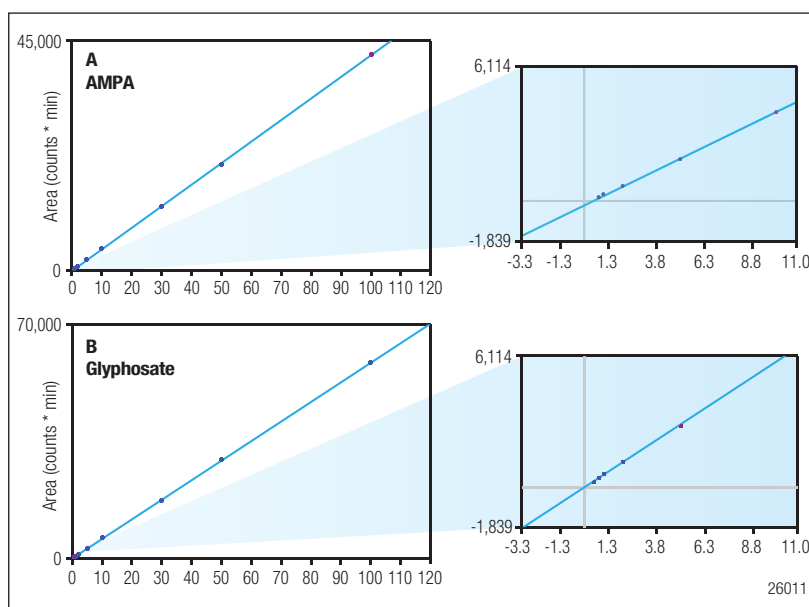


Figure 2. AMPA was calibrated with a range of 0.75–100 ppb. The correlation coefficient ( $r$ ) was calculated at 0.9999 (top trace). The calibration curve of glyphosate ranged from 0.50 to 100 ppb with an  $r = 0.9999$  (bottom trace).

## Advantages of MS Detection

### Internal Standards

Use of a stable-labeled internal standard (ISTD) is a well-accepted methodology for accurate, long-term quantification in chromatography-MS methods. Because the internal standard and analyte are chemically indistinguishable, the two species exhibit the same behavior in the analytical method and are affected in the same way by chemical and instrumental variations. The analyte and the internal standard coelute, and each has a unique SIM channel used in the MS for selective detection. A ratio of the response for the internal standard of known concentration and the analyte can give very accurate and sensitive quantification.

### Perchlorate

In this method,  $m/z$  99 or 101 is used to detect the indigenous perchlorate. Sodium perchlorate ISTD is enriched (>99.9%) with  $^{18}\text{O}$ , and the base mass peak of 107  $m/z$  is used as the unique mass for the internal standard. Figure 3 shows low-level quantification using separate SIM channels for the analyte perchlorate and the internal standard. The recommended concentration of the ISTD in each standard and sample is 1  $\mu\text{g/L}$ , as indicated in Figure 3.

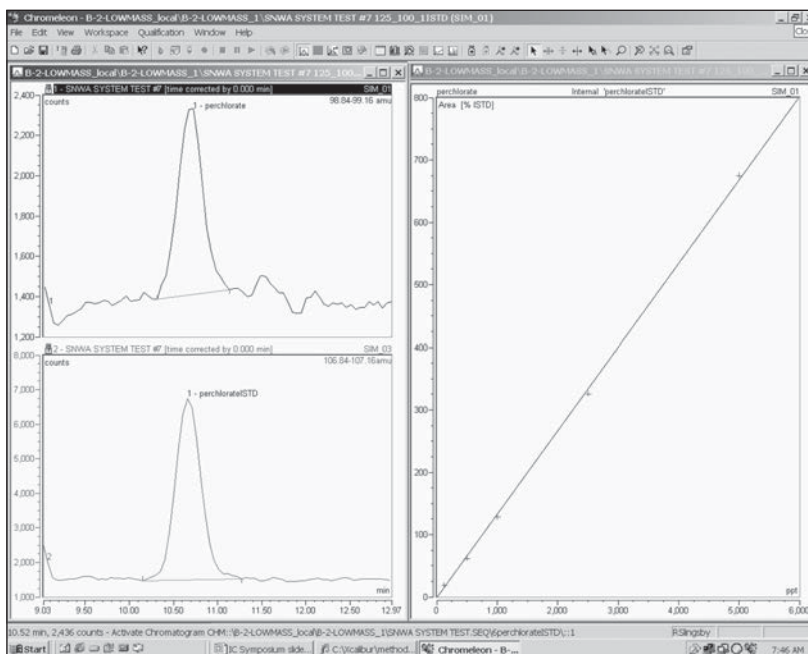


Figure 3. Quantification of perchlorate with stable-labeled internal standard.

### Low-Molecular-Mass Organic Acids Linearity

Calibration curves were generated from calibrators at 12 levels: 0, 1, 5, 10, 20, 50, 100, 200, 500, 1000, 2000, and 5000 ppb with three isotope-labeled internal standards (valerate- $d_5$ , glutarate- $d_6$ , citrate- $d_4$ ) at 100 ppb for each level. Correlation coefficients ( $R^2$ ) greater than 0.99 were achieved for most analytes over three orders of magnitude, and details are listed in Table 1.

### Precision and MDLs

Seven replicate injections of the level 5 calibrator (20 ppb) were performed, the result was used for precision (shown in Table 1 as %RSD), and MDLs were calculated based on the equation:  $\text{MDL} = t_{99\%} \times S_{(n-1)}$ . The MDL of formate and acetate was defined as the lowest injected amount to consistently give a signal-to-noise ratio (S/N) greater than 3 ( $S/N > 3$ ).

## Advantages of MS Detection

### Identification by Mass

The MS is a more selective detector than conductivity in that it monitors the  $m/z$  of the analyte. Mass and mass ratios, in conjunction with a compound's retention time, can help provide more confidence in its identity.

## Perchlorate

The  $m/z$  ratio provides additional peak identification information for perchlorate by the presence of both  $m/z$  99 and 101 due to the relative isotopic abundance of  $^{35}\text{Cl}$  and  $^{37}\text{Cl}$ .

Figure 4 shows an expected result from the injection of 1000 CCS high salt matrix spiked with 125 ng/L perchlorate in an IC-MS system, including the use of matrix diversion and the internal standard. A comparison of the MS response between SIM 99 and SIM 101 results in a 3:1 ratio which is characteristic of a mono-chlorine containing compound. The presence of  $m/z$  99 and 101 and their ratio are indicative that this compound is perchlorate. SIM 107 ( $m/z$  107) is the  $(^{35}\text{Cl}^{18}\text{O}_4)^-$  enriched stable-labeled isotope of perchlorate.

Table 1. Linearity and method detection limits.

Peak No.	Analyte	R.T. (min)	SIM ( $m/z$ )	Calibration Range			MDL (ng)	%RSD
				From/ppb	To/ppb	R <sup>2</sup>		
1	quinatate	6.9	191.1	1	5000	0.997	0.062	3.92%
2	gluconate	7.7	195.1	1	5000	0.999	0.091	5.77%
3	lactate	8.2	89.0	20	5000	0.999 <sup>a</sup>	0.129	8.18%
4	2-methylactate	8.2	103.1	1	5000	1.000 <sup>b</sup>	0.061	3.86%
5	acetate	8.8	59.0	200	5000	0.999	5.00	N/A
6	glycolate	8.8	75.0	5	5000	0.999 <sup>a</sup>	0.071	4.50%
7	propionate	10.2	73.0	5	5000	0.999	0.065	4.15%
8	formate	11.5	45.0	20	5000	0.999	0.500	N/A
9	butyrate	12.2	87.0	5	5000	0.998 <sup>a</sup>	0.034	2.15%
10	2-keto-D-gluconate	12.2	193.0	1	5000	0.994	0.102	6.48%
11	2-hydroxyvalerate	12.5	117.1	1	5000	0.999 <sup>b</sup>	0.050	3.20%
12	pyruvate	12.9	87.0	1	5000	0.999 <sup>b</sup>	0.056	3.58%
13	isovalerate	13.1	101.1	5	1000	0.997	0.063	7.16%
14	valerate	14.3	101.1	5	5000	0.997	0.059	4.01%
15	galacturonate	14.4	193.0	1	5000	1.000	0.082	5.22%
16	5-keto-D-gluconate	19.1	193.0	1	5000	0.998	0.109	6.92%
17	glutarate	21.6	131.0	1	5000	1.000	0.043	2.72%
18	mucate	21.6	209.0	1	5000	1.000	0.085	5.41%
19	adipate	21.7	145.1	5	5000	0.995	0.047	2.99%
20	succinate	21.9	117.0	1	5000	0.994	0.060	3.79%
21	malate	22.1	133.0	1	5000	1.000 <sup>b</sup>	0.117	7.44%
22	methylmalonate	22.4	117.0	1	5000	0.999 <sup>b</sup>	0.095	6.03%
23	malonate	22.8	103.0	1	5000	0.996 <sup>c</sup>	0.078	4.96%
24	tartarate	22.8	149.0	5	5000	0.997	0.090	5.75%
25	maleate	23.7	115.0	1	5000	0.998 <sup>b</sup>	0.123	3.57%
26	$\alpha$ -ketoglutarate	24.6	145.1	1	5000	0.999	0.135	8.57%
27	oxalate	25.3	89.0	5	5000	0.998	0.112	7.12%
28	fumarate	25.6	115.0	1	5000	0.999	0.074	4.69%
29	oxalacetate	28.5	131.0	N/A	N/A	N/A	N/A	N/A
30	citrate	33.5	191.0	1	5000	0.999	0.076	4.83%
31	isocitrate	34.3	191.0	1	5000	0.996	0.073	4.64%
32	<i>cis</i> -aconitate	35.1	173.0	1	5000	1.000 <sup>b</sup>	0.034	4.31%
33	<i>trans</i> -aconitate	36.7	173.0	5	5000	1.000	0.051	6.45%

For calibration function, linear fit was used unless noted. <sup>a</sup>Quadratic fit; <sup>b</sup>cubic fit; <sup>c</sup>exponential fit.

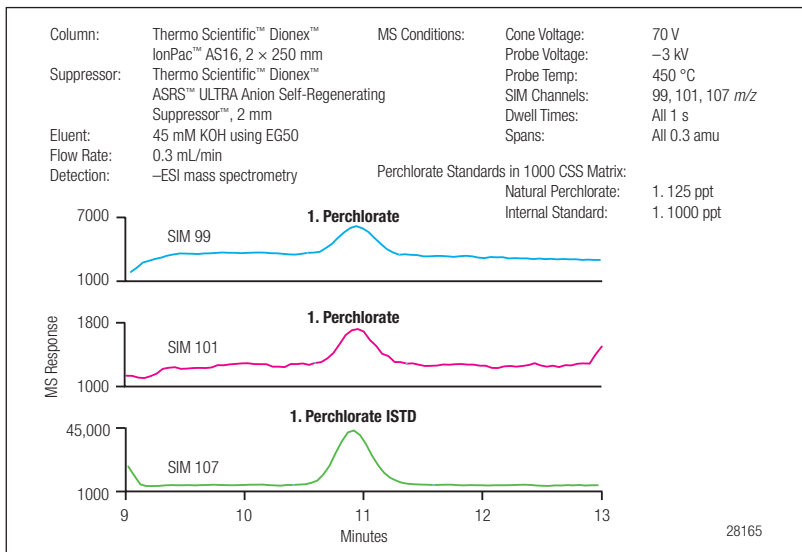


Figure 4. Utilization of  $m/z$  to identify perchlorate.

## Advantages of MS Detection

### Differentiation Between Coeluting Compounds

Through the use of SIM, MS detection can help differentiate between coeluting compounds, including high-ionic-strength matrix ions. SIM mode provides the most sensitive and selective detection.

### Glyphosate

The conductivity chromatograph (Figure 5) shows AMPA and glyphosate completely obscured by baseline noise or perhaps the leading and tailing edges of the matrix. The shaded areas of this chromatogram indicate where the matrix was diverted to waste. Multiple SIM chromatograms are overlaid showing strong specific signals enabling the detection of these analytes at  $m/z$  110 (AMPA with 5.05% RSD) and  $m/z$  168 (glyphosate with 3.0% RSD) in this complex matrix.

### Low-Molecular-Weight Organic Acids

In the analysis of the 32 LMMOAs, baseline resolution was not achieved and coelution was present with several compounds using conductivity detection. The conductivity and SIM chromatograms (Figure 6) show determination of 32 LMMOAs in matrix (biomass). The SIM chromatograms easily differentiate the LMMOAs, but there are certain areas where chromatographic separation is essential, especially for analytes with identical or close molecular masses such as butyrate and pyruvate ( $m/z = 87.05$  and  $87.02$ , respectively), and maleate and fumarate ( $m/z = 115.01$ ).

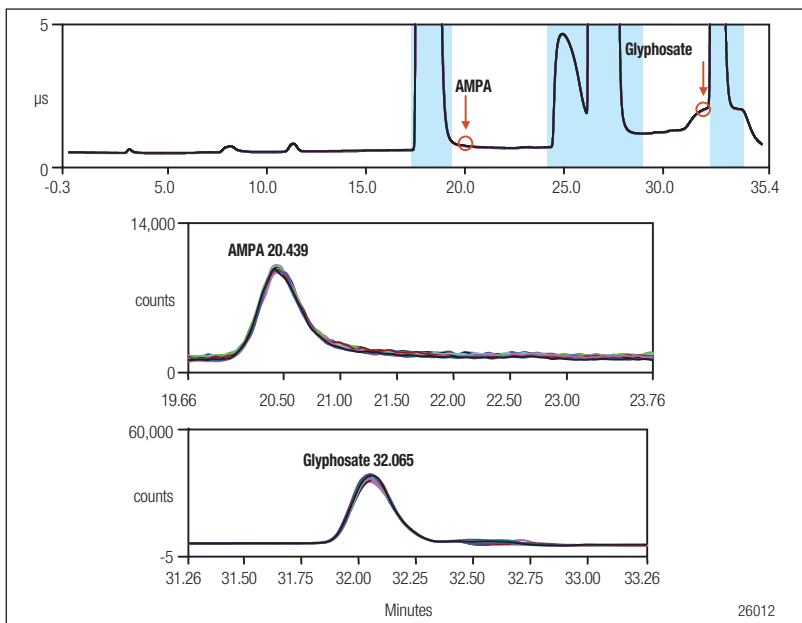


Figure 5. Comparison of AMPA and glyphosate detection in high matrix using conductivity (top trace) and MS SIM detection.

## Advantages of MS Detection

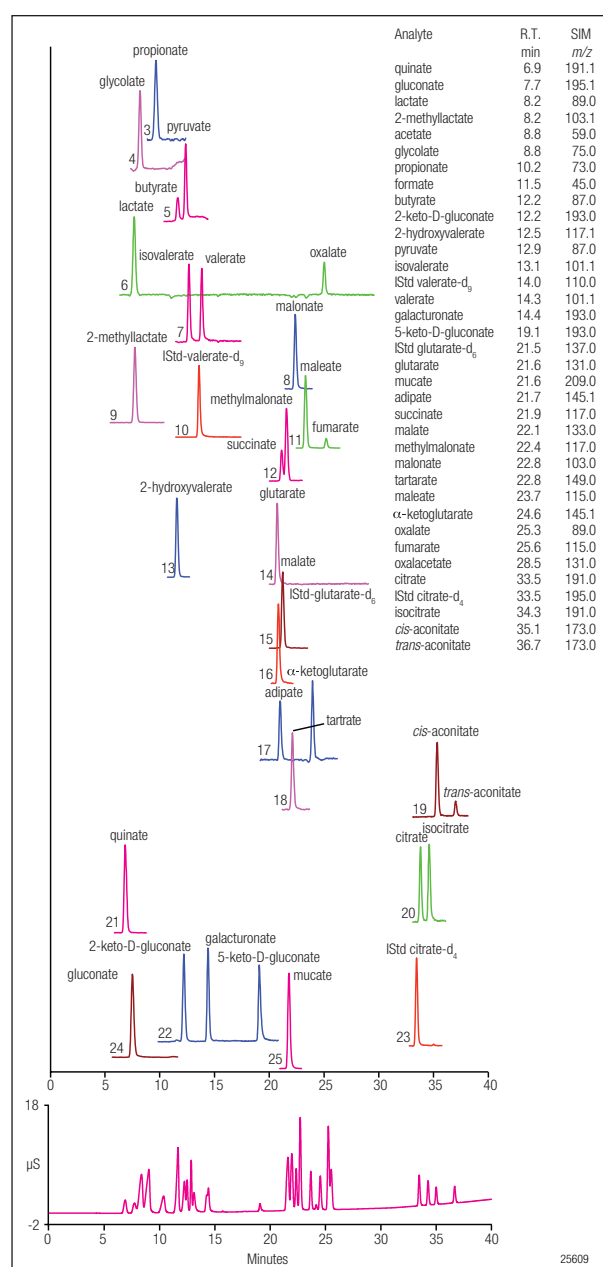
### Low-Mass Detection

For most mass spectrometers, low-mass detection ( $\sim <100$   $m/z$ ) is considered to be in the mass range of HPLC solvents and is of little interest. MS detectors capable of efficient low-mass detection are ideally suited for many environmental compounds analyzed by IC due to the low molecular weight of these analytes.

All methods presented here required MS detection of low-mass analytes at low detection levels (Table 2).

Table 2. Low-mass analytes and detection limits.

	Perchlorate	Glyphosate	Low-Molecular-Weight Organic Acids
Masses analyzed	$m/z$ 99 and $m/z$ 101	$m/z$ 110 (AMPA) and $m/z$ 168 (glyphosate)	Range $m/z$ 45 to $m/z$ 209
Detection level	<0.5 ppb	<1 ppb	<10 ppb



### Conclusion

Three methods have been presented, demonstrating specific and selective MS detection for environmental analysis by IC. The advantages of MS detection—and in particular the use of an MS detector with unique low-mass detection—was shown in the identification by mass and low-level quantitation using SIM in conjunction with internal standards.

Figure 6. Comparison of 32 LMMOAs using conductivity (bottom trace) and MS SIM detection.

Table 3. Thermo Scientific Dionex instrumentation and consumables for the three IC/MS methods referenced: perchlorate, glyphosate, and LMMAA.

	Perchlorate	Glyphosate	LMMAA
<b>Chromatography</b>	Thermo Scientific Dionex Ion Chromatography Systems		
	Thermo Scientific Dionex AXP-MS Auxiliary Pump		Dionex AXP-MS Auxiliary Pump × 2
<b>Columns</b>	Dionex IonPac AG16, 2.1 × 50 mm	Two-Dimensional Analysis:	Dionex IonPac AG11 HC, 2.1 × 50 mm
	Dionex IonPac AS16, 2.1 × 250 mm	Dionex IonPac AG19, 2.1 × 50 mm	Dionex IonPac AS11 HC, 2.1 × 250 mm
		Dionex IonPac AS19, 2.1 × 250 mm	
		Dionex IonPac UltraTrace Anion Concentrator, 3 × 50 mm	
		Dionex IonPac AG21, 2.1 × 50 mm	
		One-Dimensional Analysis:	
		Dionex IonPac AG24, 2.1 × 50 mm	
	Dionex IonPac AS24, 2.1 × 250 mm		
<b>Accessories</b>	Thermo Scientific Dionex EGC KOH Cartridge	Dionex EGC KOH Cartridge	Dionex EGC II KOH Cartridge
	Dionex ASRS ULTRA II Anion Self-Regenerating Suppressor, 2 mm Suppressor	Dionex ASRS 300 Anion Self-Regenerating Suppressor, 2 mm Desalter	Dionex ASRS 300 Anion Self-Regenerating Suppressor, 2 mm Suppressor
	Thermo Scientific Dionex CR-ATC Continuously Regenerated Anion Trap Column		Dionex CR-ATC Continuously Regenerated Anion Trap Column
<b>Mass Spectrometer</b>	Thermo Scientific™ MSQ™ Series Single Quadrupole		
<b>Ionization Mode</b>	Electrospray	Electrospray	Electrospray
<b>Scan Mode</b>	Negative Ion SIM	Negative Ion SIM	Negative Ion SIM
<b>Probe Temp.</b>	450 °C	400 °C	450 °C
<b>Needle Voltage</b>	3 kV	3 kV	3 kV
<b>Postcolumn Additions</b>	Acetonitrile/water at 0.2 mL/min		Acetonitrile at 0.2 mL/min
<b>Software</b>	Thermo Scientific™ Dionex™ Chromeleon™ 6.8 Chromatography Data System		
<b>Standards</b>	Deionized Water (17.8 mΩ-cm)	Ammonium Nitrate (EM Science)	Deionized Water (17.8 mΩ-cm)
	Sodium Perchlorate (Aldrich)	Sodium Chloride (J.T. Baker)	LMMAA (Sigma Aldrich)
		Sodium Sulfate (EM Science)	Acetonitrile (Burdick & Jackson)
		Sodium Carbonate (EM Science)	
		Glyphosate (Supelco)	
		AMPA (Sigma)	
<b>Internal Standards</b>	Sodium Perchlorate		Valerate-d <sub>9</sub> , Glutarate-d <sub>6</sub> , Citrate-d <sub>4</sub> , C/D/N Isotopes

## References

1. Thermo Scientific. *Determination of Perchlorate in Environmental Waters by Ion Chromatography Coupled with Electrospray Mass Spectrometry (IC-MS)* describes the use of single quad IC-MS to determine perchlorate in environmental waters. Dionex (now part of Thermo Scientific) Application Note 151 (LPN 1496-01, May 2007), Sunnyvale, CA.
2. Thermo Scientific. *Analysis of Glyphosate and AMPA in Environmental Samples by Ion Chromatography Mass Spectrometry (IC-ESI-MS)* describes the use of single quad IC-MS with matrix diversion to determine glyphosate and AMPA in high matrix samples. Dionex (now part of Thermo Scientific) Poster. (LPN 2260, May 2009), Sunnyvale, CA.
3. Thermo Scientific. *Determination of 32 Low Molecular Mass Organic Acids in Biomass Using IC/MS* describes an ion chromatography method using single quad mass spectrometric detection (IC-MS) for the simultaneous determination of 32 LMMAAs in matrix (biomass). Dionex (now part of Thermo Scientific) Poster. (LPN 2262, May 2009), Sunnyvale, CA.

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