Specific and Selective Detection for Food and Beverage Analysis by Ion Chromatography-Mass Spectrometric Detection

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INTRODUCTION

Ion chromatography (IC) is a well-established, routine method for analyzing ionic compounds for the food and beverage market.

Analysis of food and beverage samples can present challenges given the requirement for low-level detection, often in complex matrices. General IC detectors may not be routinely capable of achieving the desired detection levels, samples may be in high-level matrices, and target analytes may coelute with other compounds. Since general detectors rely on identification by retention time alone, they may not be adequate or acceptable in these cases. Confirmation by alternate methods could more than double analysis time, and differentiation between coeluting compounds can make detection and quantitation difficult or impossible. Mass spectrometry (MS) detection can help address these challenges. With samples of known analytes, selected ion monitoring (SIM) can significantly reduce detection levels and differentiate between coeluting analytes contained in high-matrix samples. However, many food and beverage samples analyzed by IC are of low molecular weight, requiring that MS be capable of efficient, low-mass detection. For most methods, the supplementary detection at the sample's mass-to-charge ratio (*m/z*) provides additional specificity for reliability in identification.

Compounds analyzed by IC are generally ionic or highly-polar species, and thus electrospray ionization (ESI) is the preferred interface to couple IC and MS. However, challenges remain due to the inherent composition of the IC eluent.

Eluent generation, an essential part of a Reagent-Free™ IC (RFIC™) system, is used to electrolytically produce high-purity potassium hydroxide (KOH) eluent, which is then separated on column, and finally modified by an electrolytic suppressor (Figure 1). These suppressors employ the electrolytic reactions of water to generate hydronium $[H_3O]^+$ and hydroxide $[OH]^-$ ions, eliminating the need for a separate source of regenerant. The hydronium $[H_3O]^+$ ions replace the eluent potassium cations and neutralize the hydroxide eluent. By using an eluent suppressor, the strongly ionic eluent is converted to water before entering the mass spectrometer. Additionally, an organic postcolumn flow can be introduced to the main stream prior to MS to assist in the ESI desolvation process.

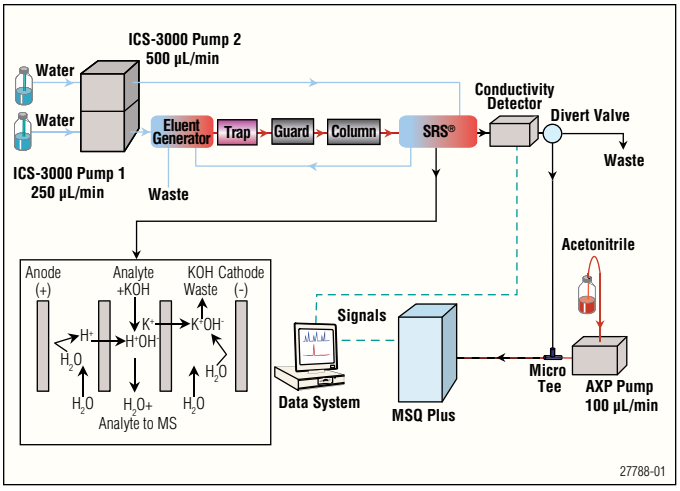

Figure 1. Schematic of an RFIC-MS System.

This study describes the advantages of IC with MS detection (IC-MS) in three food and beverage applications. Four areas are highlighted: low-level-compound detection in a matrix; use of stable-labeled internal standards (ISTDs); compound identification by mass, differentiating coeluting compounds; and detection of low-mass compounds.

APPLICATIONS INTRODUCTION

Application 1—Perchlorate[1]

Perchlorate (ClO_4^-) contamination has been detected in soil, fruits, vegetables, milk, ground water and surface water in the U.S., and recently in other parts of the world. Perchlorate competitively inhibits iodide uptake by the thyroid gland in humans, with possible adverse effects on normal growth and development.

Researchers have suggested that perchlorate accumulates in leafy vegetables by moving with the transpirational water stream of the plant. To test this, three types of lettuce were grown with two environmentally-relevant perchlorate concentrations in two controlled environments in which 2.0–2.7-fold differences in transpiration ratios were achieved: cool, humid, cloudy; and hot, dry, sunny. However, the difference in perchlorate accumulation was only 1.2–2.0-fold. This suggests that although the transpiration rate has an effect in lettuce perchlorate accumulation, the effect is not as great as expected. The differences seen among the genotypes appear to be far more important.

Perchlorate concentrations in store-bought lettuce and spinach ranged from 0.6 to 6.4 µg/kg. The highest concentrations were found in the butterhead lettuce variety and spinach. The method detection limit (MDL) for determination of perchlorate in plant extracts using this method is 40 ng/L.

Application 2—Low-Molecular-Mass Organic Acids[2]

Low-molecular-mass organic acids (LMMOAs) are present in many plants and function in various capacities. Also present in beverages, many LMMOAs are related to stability and organoleptic influences, such as flavor, color, and aroma. LMMOAs either originate from plants in their natural state or are generated during processes such as fermentation. For commercial and regulatory purposes, it is necessary to monitor LMMOA levels in both raw materials and marketed products.

Among the analytical techniques used for determination of LMMOAs in beverages, chromatographic methods with various modes of detection provide the information to profile and monitor LMMOAs. In this application, IC-MS was used for the simultaneous determination of 32 LMMOAs in different beverages. Calibration curves were generated from 1.0 to 5000 ppb and MDLs ranged from 0.034 to 0.5 ppb.

Application 3—Carbohydrates[3]

A wide variety of carbohydrates present in an extensive range of food and beverages are consumed by people every day. Carbohydrates are a complex class of organic molecules with the formula $C_nH_{2n}O_n$. They include monosaccharides (trioses, tetroses, pentoses, and hexoses), disaccharides, and oligosaccharides. A well-established technique for the determination of underivatized carbohydrates is high-performance anion-exchange chromatography (HPAEC) using alkali-hydroxide-based and alkali-acetate-based eluents. To verify the identity of individual sugars, the retention times of the peaks are compared with those obtained from reference solutions.

MS detection offers the advantage of faster and more reliable identification and peak conformation by using the *m/z* of the saccaride classes (pentoses, hexoses, and oligosaccharides). The analyses of neutral carbohydrates, including oligosaccharide samples, were evaluated in native inulin, chicory coffee, lager beer and honey using SIM MS detection of adducts formed with postseparation addition of lithium. The results obtained included MDL's for glucose of 1.49 pmol, fructose 1.19 pmol, and sucrose 0.36 pmol.

ADVANTAGES OF MS DETECTION

Low-Level Detection in Matrix—SIM

The mass spectrometer can provide lower detection limits in high-ionic strength matrices than conductivity detectors.

Perchlorate

IC is used in conjunction with ESI-MS detection to provide higher sensitivity and selectivity (as compared to general conductivity detectors) for perchlorate detection in water and plants. ESI is considered a soft ionization technique, preserving the analyte's molecular ion. Using optimized ESI-MS parameters, fragmentation of the analyte is insignificant, making spectral data less complicated. When ESI-MS is combined with SIM mode detection, individual molecular ions can be simultaneously monitored and mass chromatograms of several ions for each run can be generated. When analyzing perchlorate in this manner, 99 and 101 *m/z* (from the natural stable isotopes of $^{35}ClO_4^-$ and $^{37}ClO_4^-$) are monitored.

Spike Recoveries of Initially Perchlorate-Free Lettuce

Unspiked extracts of hydroponically-grown butterhead lettuce were analyzed and did not contain detectable perchlorate (data not shown). Spike recoveries of initially perchlorate-free lettuce ranged from 93 to 101% and 91 to 98% for the 37.7 µg/kg and the 10.3 µg/kg FW perchlorate spikes, respectively (Table 1). A *t*-test revealed that there were no differences between the spiked-at-beginning and spiked-at-end values ($P > .05$) for both sets of spikes. These data indicate that no appreciable loss of perchlorate occurs during the sample extraction and preparation steps, including centrifugation, filtration, and sample transfer.

Store-Bought Lettuce and Spinach

The concentrations of perchlorate in the five types of lettuce and spinach ranged from 0.6 to 6.4 µg/kg FW (Table 1). The highest concentrations of perchlorate were found in the butterhead variety and in spinach, whereas the lowest was found in the red leaf lettuce. After accounting for perchlorate in the samples initially, spike recoveries of perchlorate in these extracts ranged from 89 to 100% (Table 1). These excellent spike recoveries at low concentrations give further indication that this method is very sensitive and ideally suited to the analysis of perchlorate at low concentrations in leafy vegetation.

Table 1. Perchlorate Content of Store-Bought Lettuce and Spinach with Spike Recovery Data (n = 3)						
Vegetation Type	**Initial ClO_4^- Content**	**Amount of ClO_4^- Spike**	**Expected Recovery**	**Measured ClO_4^- Recovery**	**Standard Deviation**	**Percent Recovery**
Crisphead	2.3	1.8	4.1	4.0	0.30	99
Butterhead	5.4	7.1	12.5	11.2	0.23	90
Romaine	0.7	0.8	1.5	1.4	0.06	90
Green Leaf	2.1	2.0	4.1	3.8	0.03	93
Red Leaf	0.6	1.2	1.7	1.7	0.06	100
Spinach	6.4	6.5	12.9	11.5	0.20	89

All values are in µg/kg FW.

ISTD—Overview

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

Perchlorate

The ISTD used in this study was $Cl^{18}O_4^-$ (P/N 062923, Dionex® Corporation, Sunnyvale, CA) with ion masses at 107 (used for quantitation) and 109 *m/z*. This ISTD was ideal because it is chemically, and thus chromatographically, very similar to SIM 99 perchlorate ($^{35}Cl^{16}O_4^-$), yet it is distinguishable by its mass.

To determine whether water standards were acceptable for accurately quantifying perchlorate in plant matrices, five-point standard curves (1–20 µg/L, spiked $^{35}Cl^{16}O_4^-$) in both water and perchlorate-free lettuce extract matrices were compared. The plant matrix yielded consistently low recoveries as compared to the standards in water (Figure 2a). The slopes of the lines were compared and found to be significantly different. Because the standard solutions were prepared simultaneously using the same calibrated pipettes, some factor, i.e., ion suppression, occurred when perchlorate was analyzed in the lettuce extracts.

Ion suppression is the process by which coeluting ions either prevent the ionization of analytes during ESI nebulization or inhibit the effective transfer of analyte ions. It is most likely to happen if another ion coelutes and is present at a much higher concentration than that of the analyte of interest. Because the Thermo Scientific Dionex IonPac® AS16 Anion-Exchange Column is designed to elute most ions quickly and preferentially retains perchlorate longer, it seems unlikely that early eluting common ions present at high concentrations (i.e., chloride, sulfate, or nitrate) would cause ion suppression. Because the electrical conductivity detector in line with the IC-ESI-MS showed no other coeluting ions in the range of perchlorate elution, the substance causing suppression is likely not an ionic species, but perhaps a molecule that is not detected by electrical conductivity.

A full scan on the MS during routine analysis ranged from 20 to 150 *m/z* and did not show any evidence of a coeluting species in that mass range. The dissolved organic carbon (DOC) analysis indicated that a substantial amount of organic carbon was in the water-clear extracts, and thus present during analysis.[1] Thus, it seemed likely that if a coeluting species was present and was causing ion suppression, it likely was an organic ion with a molecular weight >150. To confirm the presence of perchlorate-suppressing compounds in plant matrices, prepared butterhead lettuce extracts were run through the IC and directed to a PDA-100 photodiode array detector. Some organic compounds were found with one overlapping the perchlorate peak at 13 min; this is likely the cause of the observed ion suppression in Figure 2a.

Internal standards are useful because they provide a correction for MS fluctuation with time as well as for any ion suppression that may occur. Compensation for the observed ion suppression was successfully achieved with the use of the $Cl^{18}O_4^-$ ISTD. Figure 2b shows the same set of water and plant matrix standards fortified with 1 µg/L ISTD. The slopes for these two standard curves are not significantly different when the ISTD is used. This result indicates that a water matrix standard curve can be used when plant samples are analyzed, as long as the ISTD is utilized in all of the samples and standards.

Identification by Mass

The mass spectrometer is a more selective detector than conductivity in that it monitors the analyte's *m/z*. Mass and mass ratios, in conjunction with a compounds retention time, can help provide confidence in the analyte's identification.

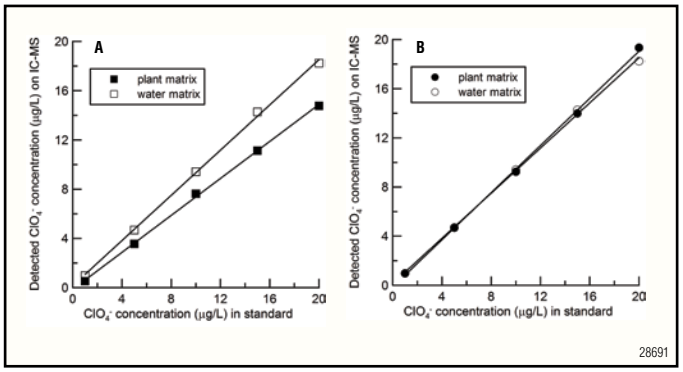

Figure 2. Standard curves for perchlorate using IC-ESI-MS in water matrix vs plant matrix: (A) without ISTD; and (B) with $Cl^{18}O_4^-$ ISTD.[1]

Perchlorate

ESI-MS detection has been used in conjunction with IC in order to provide higher sensitivity and selectivity for perchlorate detection in water and plants. The soft ESI ionization process predominantly produces the molecular ion with insignificant fragmentation of the analyte. SIM of individual molecular ions can be simultaneously monitored and ion mass chromatograms for each run can be generated. When analyzing perchlorate in this way, identification is based on the similar retention times of the 99 and 101 *m/z* species to the ISTD, and the 3:1 natural isotopic ratio of ^{35}Cl to ^{37}Cl is used for confirmation.

Carbohydrate

A chicory coffee, a lager beer and a honey were bought off-the-shelf. Samples were filtered and diluted appropriately before injection.

Neutral carbohydrates were detected in the positive ion mode in the MS after formation of lithium quasi-molecular ions by the addition of trace amounts of lithium chloride. For efficient ionization of the eluted compounds, a solution of 0.5 mM LiCl was pumped into the eluent stream (lithium chloride forms charged complexes with carbohydrates). A typical separation of sugar alcohols, monosaccharides, and disaccharides is presented in Figure 3.

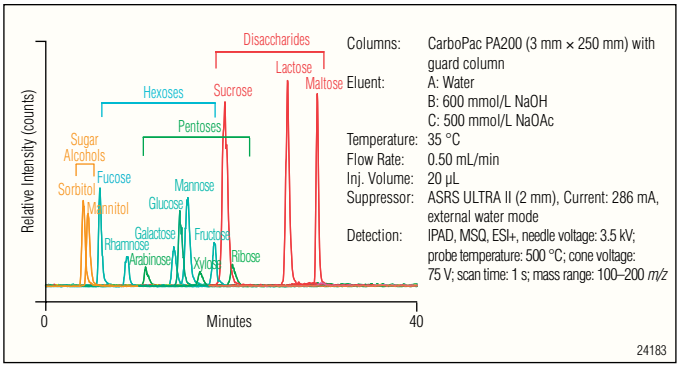

Figure 3. Mass chromatograms of sugar alcohols, monosaccharides, and disaccharides in the presence of LiCl; ESI positive, cone voltage 70 V.

The carbohydrates are detected as the lithium adducts $[M+7]^+$ in ESI positive mode. In-source collision-induced dissociation (CID) of carbohydrates after ESI can be achieved by accelerating the ions into the radio frequency (RF)-only focusing lens region of the MS with enough energy to fragment ions. CID can be used to form characteristic fragment ions, as shown in Figure 4, which illustrates the mass spectrum of maltose. The quasi-molecular ion at 349 *m/z* is clearly the base peak of maltose. In addition, fragment ions from glycosidic cleavages were observed. The mass loss of 162 (Y fragment at 187 *m/z*) is a clear indication for a hexose. The fragment ion at 205 *m/z* is a water adduct of the Y fragment. B fragment ion at 169 *m/z* is a glycosidic cleavage on the other side of the oxygen linkage.

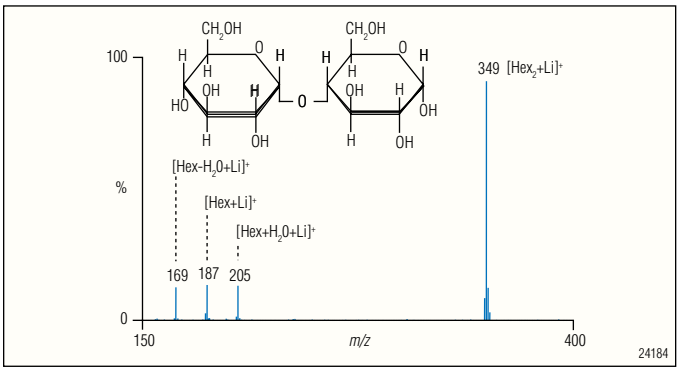

Figure 4. Mass spectrum of maltose in the presence of LiCl; ESI positive, cone voltage 70 V.

For the application of this method to the analysis of carbohydrates in lager beer, the chromatograms obtained by integrated pulsed amperometric detection (IPAD) are very complex, showing a large number of unresolved peaks. MS can be helpful in identifying oligosaccharides by extracting mass selective chromatograms. Beer contains a large variety of oligosaccharides with up to 10 degrees of polymerization. Figure 5 shows an overlay of mass extracted chromatograms of a lager beer sample according to different degrees of polymerization.

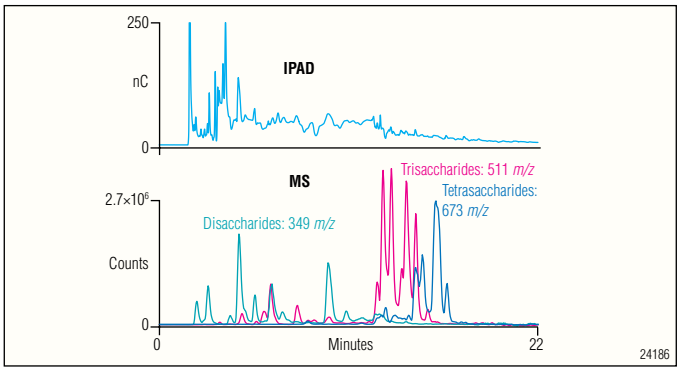

Figure 5. Comparison between IPAD and extracted mass chromatograms of degassed lager beer.

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.

Low-Molecular-Weight Organic Acids

In the analysis of 32 LMMOAs, baseline resolution was not achieved when using a conductivity detector and coelution was present with several compounds. Comparison of the conductivity and SIM chromatograms (Figure 6) shows the determination of 32 LMMOAs in matrix (green tea). The SIM chromatograms easily differentiate the LMMOAs, but there are certain areas were chromatographic separation is essential, especially for analytes with identical or close molecular masses, i.e., butyrate and pyruvate (m/z = 87.05 and 87.02, respectively), maleate and fumarate (m/z = 115.01).

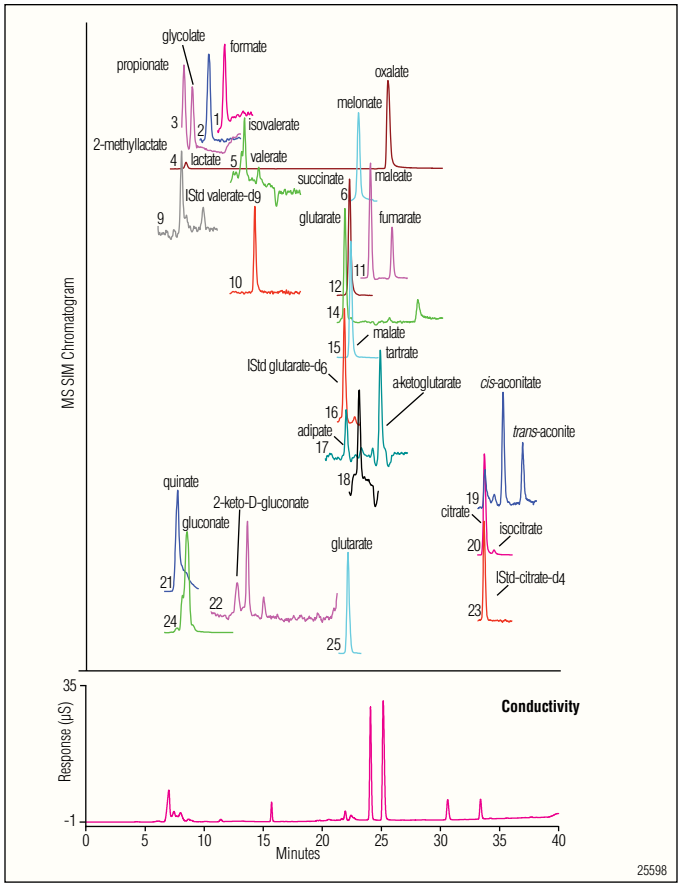

Figure 6. Comparison of 32 LMMOAs using conductivity and MS detection.

Low-Mass Detection

For most mass spectrometers, low-mass detection (<~100 m/z) is considered to be in the mass range of HPLC solvents and is of little interest. An MS detector capable of efficient low-mass detection is ideally suited for many food and beverage compounds analyzed by IC due to their low molecular weight.

Methods presented here required MS detection of low mass analytes at low detection levels (Table 2 below). For perchlorate, moderately low-mass MS detection is an advantage to achieving low-level detection. Very low-mass MS detection capability is required for many low-molecular-weight organic acid compounds.

Table 2. MS Detection Methods		
	Perchlorate	**Low-Molecular-Weight Organic Acids**
Masses Analyzed	99 and 101 *m/z*	Range 45–209 *m/z*
Detection Level	<0.5 ppb	<10 ppb

SUMMARY

Three methods have been presented, demonstrating specific and selective MS detection for food and beverage analysis by IC. The advantages of MS detection have been demonstrated, and in particular the use of an MS detector with low-level compound detection in matrix, the use of ISTDs, compound identification by mass to differentiate coeluting compounds, and detection of low-mass compounds.

Table 3. Summary of Instruments, Columns and Accessories, Standards			
	Perchlorate	**Lmmoa**	**Carbohydrate**
Chromatography	DX500 IC System consisting of: GP50 Gradient Pump and AS50 Autosampler AXP-MS Auxiliary Pump	ICS-2000 IC System AS Autosampler AXP-MS Auxiliary Pump x 2	BioLC consisting of: AS Autosampler ED50A Electrochemical Detector AS50 Autosampler with sample cooling and TC AXP-MS Auxiliary Pump
Columns	IonPac AG16 (2 × 50 mm) IonPac AS16 (2 × 250 mm)	IonPac AG11 HC (2.1 × 50 mm) IonPac AS11 HC (2.1 × 250 mm)	Thermo Scientific Dionex CarboPac® PA200 (3 × 250 mm) CarboPac PA200 guard (3 × 50 mm)
Accessories	EGC-KOH Cartridge Anion Self-Regenerating Suppressor® (ASRS®) 300, 2 mm suppressor CR-ATC	EGC II-KOH Cartridge ASRS 300, 2 mm suppressor CR-ATC	Flow splitter (Upchurch Microtee Assembly P-775) ASRS ULTRA II 2 mm suppressor (for desalting)
Mass Spectrometer	Thermo Scientific MSQ Elmo Single Quadrupole	Thermo Scientific MSQ Plus™ Single Quadrupole	MSQ Elmo Single Quadrupole
Ionization Mode	Electrospray	Electrospray	Electrospray
Scan Mode	Negative Ion SIM	Negative Ion SIM	Positive Ion SIM
Probe Temperature	450 °C	450 °C	525 °C
Needle Voltage	3.5 kV	3 kV	
Postcolumn Additions	Acetonitrile at 0.2 mL/min	Acetonitrile at 0.2 mL/min	0.5mM LiCl at 0.05 mL/min
Software	Thermo Scientific Dionex Chromeleon® 6.8 Chromatography Data System		
Standards	Ammonium Nitrate (EMD Chemicals) Sodium Chloride (J.T. Baker) Sodium Sulfate (EMD Chemicals) Sodium Carbonate (EMD Chemicals) Glyphosate (Sigma-Aldrich®) AMPA (Sigma-Aldrich)	Deionized water (17.8 MΩ) LMMOA (Sigma-Aldrich) Acetonitrile (Honeywell Burdick & Jackson®)	Deionized water (17.8 MΩ) Sodium hydroxide (J.T. Baker) Sodium acetate (J.T. Baker)
ISTDs	$Cl^{18}O_4^-$ (P/N 062923, Dionex Corporation)	valerate-d9, glutarate-d6, citrate-d4, C/D/N Isotopes	

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