Direct Determination of Small Organic Acids in Sea Water by IC-MS

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Key Words

MSQ Plus Single Quadrupole Mass Spectrometer, Organic Acids, Environmental Application, 2D Analysis, Ultra Trace Anion Concentrator

Introduction

Studies show that low molecular weight species in aquatic environments are important sources of carbon for marine bacteria.[1] Short-chain organic acids in the marine environment play important roles in adjusting the pH value, forming a variety of complexes and then increasing the solubility of trace metals in sea water. In light of the significant growth in aquaculture for farmed fish and shellfish, several feed additives—including acidifiers consisting of organic acids and their salts—may provide promising alternatives to the use of in-feed antibiotics in aquaculture.[2]

Previously, analyses for organic acids in aqueous matrices have been accomplished using gas chromatography (GC) methods with derivatization; this allows fisheries to monitor the water environment and control its impact on the health of the fish. However, direct analysis by ion chromatography-mass spectrometry (IC-MS) provides a faster, more direct approach that does not require sample pretreatment.

Goal

To demonstrate a 2D-IC-MS system that allows low levels of small organic acids (C1–5) to be analyzed in a high-ionic-strength matrix. The first dimension of the system separates the low-level analytes from the matrix while the second dimension resolves each of the target analytes. The mass spectrometer confirms identity and achieves low detection limits for a sensitive and selective assay.

Equipment

- Thermo Scientific Dionex ICS-3000 or ICS-5000 Reagent-Free analytical scale IC (RFIC) system, including:
  – DP Dual Pump
  – EG Eluent Generator
  – EGC III KOH Eluent Generator Cartridges, quantity two
  – DC Detector/Chromatography Compartment with AM Automation Manager
  – CR-ATC Continuously Regenerated Anion Trap Column, quantity two
  – ASRS 300 Anion Self-Regenerating Suppressors, quantity two
  – CD Conductivity Detector, quantity two
  – AS-AP Autosampler
- Thermo Scientific MSQ Plus Single Quadrupole Mass Spectrometer
  – Dionex AXP-MS Auxiliary Pump
- Thermo Scientific Dionex Chromeleon Chromatography Data System software version 6.8, SR10

Chromatographic Conditions

Columns: First Dimension
- Thermo Scientific Dionex IonPac AG24 Guard Column (2 × 50 mm)
- Dionex IonPac™ AS24 Analytical Column (2 × 250 mm)

Second Dimension
- Dionex IonPac AG11 Guard Column (2 × 50 mm)
- Dionex IonPac AS11-HC Analytical Column (2 × 250 mm)
- Dionex IonPac UTAC-ULP1 Ultra Trace Anion Concentrator Ultralow Pressure (5 × 23 mm)

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Mobile Phase: Gradient 1:	Two Electrolytically Time (min)	Generated KOH Gradients Conc (mM)
	-5.0	10
	0.1	10
	10.0	15
	10.1	60
	20.9	60
	21.0	10
	33.1	10

Gradient 2:	Time (min)	Conc (mM)
	-5.0	1
	13.0	1
	23.0	4
	28.0	60
	33.0	60
	33.1	1

Flow Rate:	0.4 mL/min for both 1st and 2nd dimensions
Inj. Volume:	25 µL
Temperature:	35 °C
Detection:	Suppressed conductivity: Dionex ASRS™ 300 Anion Self-Regenerating Suppressor™, 2 mm, external water mode (2 mL/min)

Suppressor 1:	Time (min)	Current mA
	-5.0	15
	10.0	15
	10.1	60
	28.0	15

Suppressor 2:	Time (min)	Current mA
	-5.0	2
	23.0	10
	28.0	60
	36.0	4

MS Parameters

ESI:	Negative ion
SIM Scans:	0.7 m/z span, dwell time 0.2 s/scan
N_2 Pressure:	80 psi
Probe Temperature:	500 °C
Needle Voltage:	4 kV

Scan Parameters:	Name	Mass (m/z)	Cone Voltage (V)
	Acetate	44.9	50
	Formate	58.9	45
	Propionate	73.0	45
	Butyrate/pyruvate	86.85	45
	Valerate	100.85	45

Plumb the two divert valves in the Automation Manager after the CD detectors. One valve enables the 2D system to operate; the other valve directs unwanted compounds away from the mass spectrometer. Add a flow of 0.4 mL/min of acetonitrile via a low-volume mixing tee prior to the mass spectrometer (see Figure 1) to aid with the thermally assisted pneumatic nebulization of the electrospray ionization (ESI) source.

2D-IC Valve (Eluent Flow)

Time (min)	Position
-5	Trap column connected to Dionex IonPac AS11-HC column
0	Trap column connected to Dionex IonPac AS24 column
8	Trap column connected to Dionex IonPac AS11-HC column

MS Divert Valve (Eluent Flow)

Time (min)	Position
-5	To waste
8	To mass spectrometer
28	To waste

Chemicals and Reagents

- Sodium acetate (Fluka P/N 71179)
- Sodium formate (Sigma-Aldrich® P/N 71541)
- Propionic acid (Sigma-Aldrich P/N P1386)
- Sodium butyrate (Sigma-Aldrich P/N B5887)
- Valeric acid (Sigma-Aldrich P/N 240370)
- Pyruvic acid sodium salt (Kodak P/N 8719)
- Sodium propionate-d5 (CDN Isotopes P/N D-5378)
- Acetonitrile, HPLC grade (Honeywell Burdick & Jackson P/N AH015-4)
- Deionized (DI) water, 18.2 MΩ-cm resistivity
- Synthetic sea water (SW): prepare from a commercially available synthetic sea salt (Instant Ocean® Sea Salt) by dissolving 30 g of salt into 1 L of DI water

Prepare a primary stock solution of each of the six organic acids at 1000 µg/mL (ppm) in DI water. Prepare a mixture of the six acids at 100 ppm in DI water. Prepare working stock solutions by diluting the primary stock mixture to 10 ppm and 100 ppb concentrations in both DI and SW. Prepare calibration standards by using the DI water mixture stocks. Prepare SW samples by dilution of the SW stock.

Table 1. Summary of results for organic acids analysis

| Compound | Corr. Coeff. | MDL (ppb) | % Recovery in DI Water | |
			100 ppb	1 ppm
Acetate	99.993	100	—	88
Formate	99.995	200	—	103
Propionate	99.991	15.5	102	99
Butyrate	99.998	7.8	90	96
Pyruvate	99.991	5.6	105	107
Valerate	99.987	3.8	93	108

Calibration

Prepare the mixture of six organic acids in synthetic SW at 10 concentrations: 5, 10, 20, 50, 100, 200, and 500 ppb; and 1, 2, and 5 ppm. Spike an internal standard of propionate-d[5] at 100 ppb into each of the standards. Full-loop injections of 25 µL will yield a total amount of 125 pg to 125 ng loaded on column. Prepare samples in DI water spiked at 100 ppb and 1 ppm to evaluate the recovery efficiency of the system (Table 1).

Chromatography

The SW matrix necessitates use of a 2D-IC analysis. In the first dimension, the Dionex IonPac AS24 column weakly retains the organic acids but retains the inorganic components more strongly. Thus, the early eluting organic acids can be trapped onto a Dionex IonPac UTAC-ULP1 trap column. A primary divert valve (Divert Valve 1 in Figure 1) controls flow to the Dionex IonPac UTAC-ULP1 trap column, allowing trapping from the Dionex IonPac AS24 column initially, and then connecting the trap cartridge to the Dionex IonPac AS11-HC column flow path at a later time to allow for separation and analysis of just the organic acids. A secondary divert valve (Divert Valve 2 in Figure 1) allows analytes eluting from the Dionex IonPac AS11-HC column to be directed to the mass spectrometer for detection but directs flow to waste at all other times to reduce the chance of background or matrix affecting the ESI spray interface.
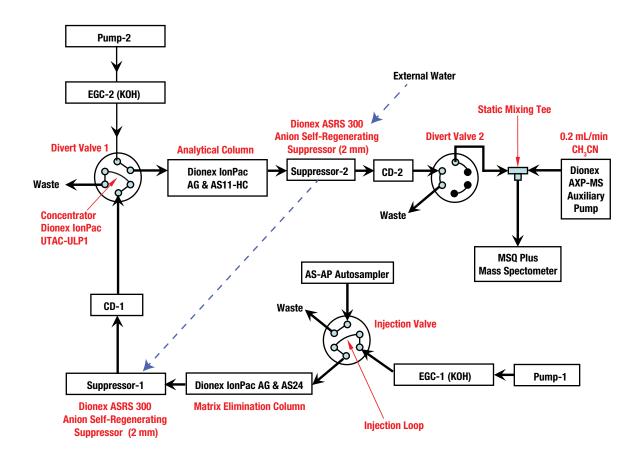

Figure 1. Schematics for 2D-IC-MS system

Set the two IC channels so that while one column is used for separation of the analytes, the other column is being cleaned and equilibrated for the next sample. After the analytes elute off the Dionex IonPac AS24 column and the primary divert valve changes position so that the Dionex IonPac AS24 column is then connected directly to waste, rapidly increase the eluent strength and hold it at high concentration to quickly wash off inorganic components. Simultaneously raise the suppressor current to compensate for increased eluent concentration and maintain suppressed conditions. Then return the eluent strength to starting conditions and allow the column to equilibrate while completing the analysis on the Dionex IonPac AS11-HC column.

Return suppressor current to its lower level for better baseline stability while eluent concentration is low during analyte elution. Likewise, while the Dionex IonPac AS11-HC column is equilibrating for the next sample, the Dionex IonPac AS24 column will be loading the trap cartridge (Figure 2). By overlapping functionality in this way, the total run time for multiple samples is reduced.

Mass Spectrometry

The aim of the study was to develop a selective and sensitive method for direct analysis of trace levels of small organic acids in sea water samples. The mass spectrometer provides inherent selectivity based on *m/z,* and operation in selected ion monitoring (SIM) mode provides increased sensitivity and specificity. All of the acids ionized easily in the ESI source and ionization parameter optimization was straightforward. Each individual analyte was evaluated with respect to cone voltage, whereas a single needle voltage and probe temperature were chosen to give the best compromise in overall results. Optimal parameters are recorded in the Chromatographic Conditions section.

The SIM dwell times were chosen to provide sufficient acquisition rates to yield good peak shape given the chromatographic width of the peaks. Dwell time is a compromise: longer times result in greater signal accumulation and better signal-to-noise ratio (S/N), but reduce the number of points across the chromatographic peak. Narrow chromatographic peaks require shorter dwell times for sampling to sample enough data points to accurately characterize the peak. Optimal parameters for MS analysis are instrument- and compound-dependent; therefore, chemists wishing to repeat these experiments are advised to evaluate all acquisition parameters to determine optimal values for different systems and analytes.

Results and Discussion

The result of the optimized analysis was sufficient separation of the analytes from the background matrix, thus allowing low-level sensitivity by mass spectrometric detection while maintaining a rugged overall analysis (Figure 3). Calibration curves and limits of detection are enumerated in Table 1.
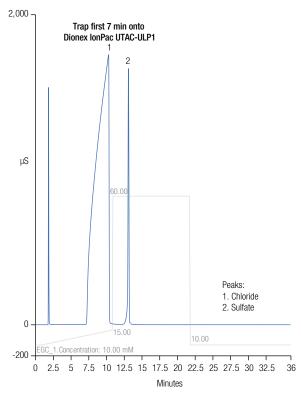

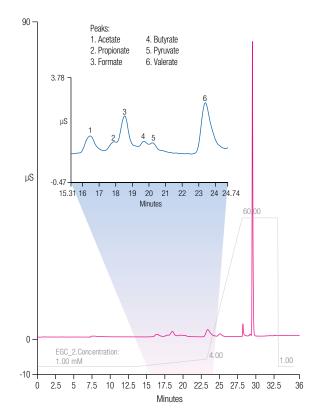Figure 2. First dimension (Dionex IonPac AS24 column)

Figure 3. Second dimension (Dionex IonPac AS11-HC column)

Method Performance

Selectivity for the six organic acids was established through use of SIM scans on the pseudo-molecular ion (M-H)⁻ (Figure 4). When combined with chromatographic retention times, this ensured accurate identification of the analytes. Run-to-run precision and accuracy was evaluated by nine replicate injections of a low-level standard at 20 ppb. Method detection limits (MDLs) for propionate, butyrate, pyruvate, and valerate were estimated using the standard deviation obtained and the following equation:

$$MDL = S \times t_{99\%}, n - 1 = 8$$

where S is the standard deviation and t is the Student's t at 99% confidence interval.

MDLs for acetate and formate were based on the minimum level needed to achieve a S/N ≥3. Carryover was evaluated by injecting sample blanks (DI water) after a 5 ppm standard chromatographic run. All analytes in the carryover analysis were below the MDLs.

This method illustrates that good separation can be achieved with use of the Dionex IonPac AS24 and AS11-HC columns. When combined with the selectivity and sensitivity of the MSQ Plus™ Mass Spectrometer, this approach provides accurate identification and quantification of each compound.

Conclusion

This analysis for small organic acids, using a 2D-IC system, allows low levels of target analytes to be detected in a high-background matrix. The MSQ Plus Mass Spectrometer provides additional selectivity and sensitivity through use of the SIM function to achieve low detection limits. No special sample preparation was required and the results show good precision and reproducibility.

References

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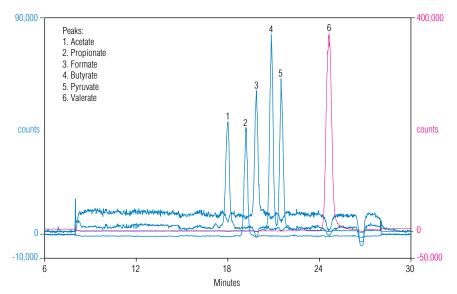
Figure 4. SIM traces from MS

Peaks:
1. Acetate
2. Propionate
3. Formate
4. Butyrate
5. Pyruvate
6. Valerate

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