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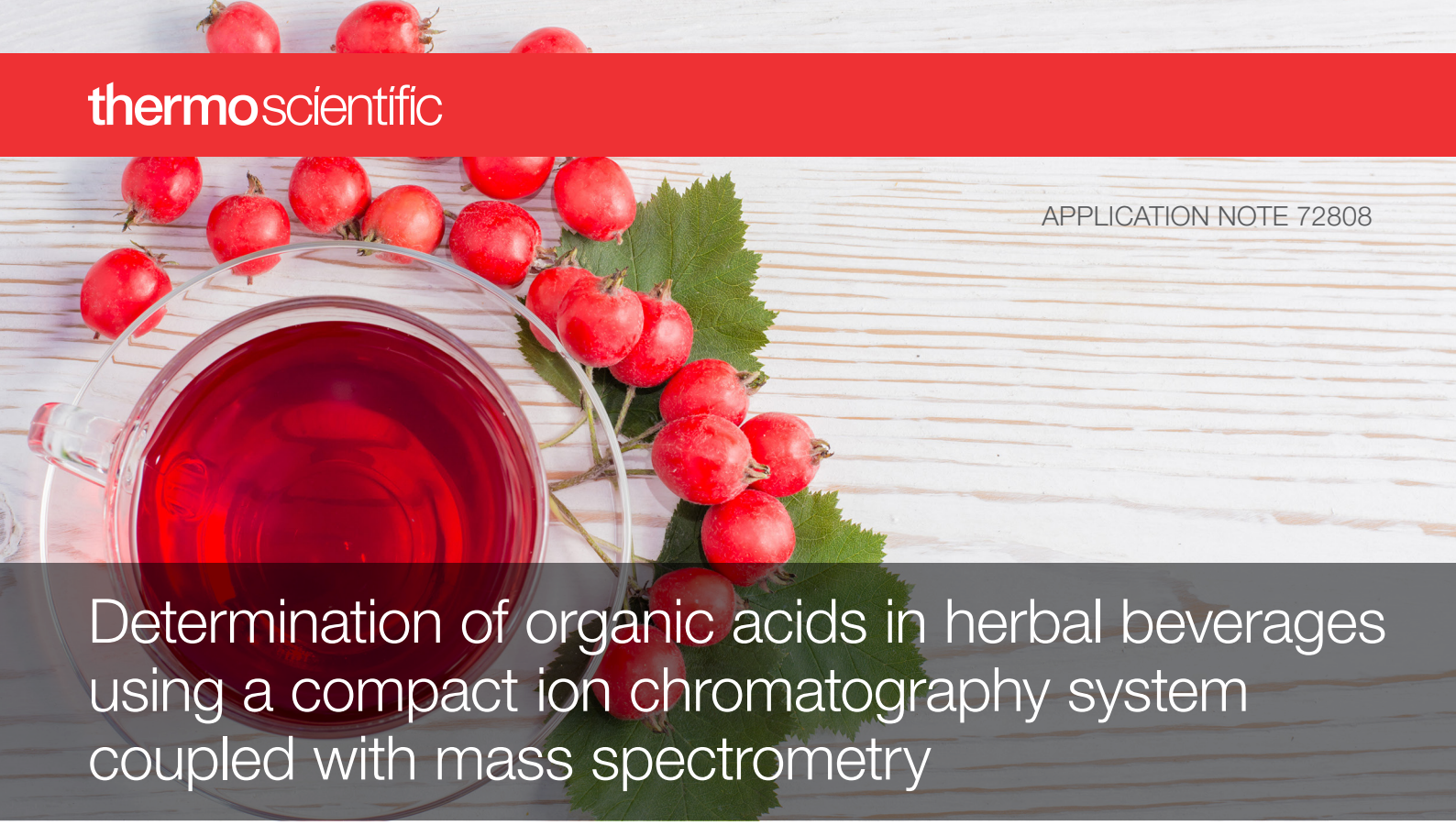

Determination of organic acids in herbal beverages using a compact ion chromatography system coupled with mass spectrometry

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Keywords
Dionex Integrion, RFIC system, Dionex IonPac AS11-HC-4µm column, ISQ EC single quadrupole mass spectrometer, organic acid, ion suppression

Goal

Develop an easy method to identify and determine organic acids in herbal drinks using a compact high-pressure ion chromatography (HPIC) system coupled to a single quadrupole mass spectrometer

Introduction

Herbal beverages are drinks that primarily contain extracts from parts of plants with aromatic properties. In recent years these beverages have become popular among people who are seeking specific health benefits from their diet. Amongst the active components of these beverages, organic acids are of particular interest to consumers.

The determination of organic acids plays an important role in revealing the possible beneficial effects of the herbal beverage. For example, malic acid is believed to promote “detoxification” by chelating aluminum and promoting its excretion.[1] Organic acids affect the flavor and taste of the drink. Therefore, for product quality reasons, it is prudent to monitor the herbal beverage’s organic acid profile.

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Traditionally, organic acids have been analyzed by gas chromatography (GC), liquid chromatography (LC), and ion chromatography (IC). However, several organic acids exhibit poor UV absorption and therefore lack sufficient sensitivity for detection when using LC. Also, herbal beverages contain other components—such as sugars and phenolic compounds—that are either at a much higher concentration or have much stronger UV absorption, and therefore can interfere with the detection of target analytes. Gas chromatography requires sample derivatization for organic acid analysis, which carries additional labor and expertise requirements. Ion chromatography with suppressed conductivity detection is the technique of choice to separate and detect a large variety of organic acids. However, some organic acids like succinate and malate do not separate with typical IC eluents.[2] The addition of a mass spectrometer after the conductivity cell, however, facilitates the identification and accurate quantification of co-eluting organic acids.

The Thermo Scientific™ ISQ™ EC single quadrupole mass spectrometer (MS) increases analytical confidence by providing sensitivity, selectivity, and confirmation of identity.[3] The ISQ EC MS can operate in Full Scan and Single Ion Monitoring (SIM) mode, to either scan a mass range for all detectable analytes or to focus on a specific compound. This work uses IC with suppressed conductivity and mass spectrometry detection for organic acid determinations. This dual detection approach increases the information available from the sample. Samples were separated with a high-resolution Thermo Scientific™ Dionex™ IonPac™ AS11-HC-4µm column set using a Thermo Scientific™ Dionex™ Integrion™ HPIC™ system with suppressed conductivity detection, and an ISQ EC MS. Co-eluting organic acids can be accurately quantified with mass spectrometric detection, eliminating the need for the addition of methanol to the hydroxide eluent to enhance separation. This application note demonstrates an easy IC-MS method for determination of organic acids in aloe, hawthorn/plum, and goji drinks, especially showing selectivity in detection for co-eluting compounds and peak confirmation.

Experimental

Equipment and consumables

- Dionex Integrion HPIC system including:
  - Eluent Generator
  - Pump
  - Degasser
  - Conductivity Detector (CD)
  - Column oven temperature control
  - Detector-suppressor compartment temperature control
  - Tablet control
- Thermo Scientific™ Dionex™ AS-AP Autosampler with Sample Syringe, 250 µL (P/N 074306) and Buffer line, 1.2 mL (P/N 074989)
- ISQ EC single quadrupole mass spectrometer (P/N ISQEC000IC)
- Thermo Scientific™ Dionex™ AXP Auxiliary Pump (P/N 063973)
- Peak™ Scientific Genius 1022 nitrogen generator (P/N 10-6022 (230 v))
- Thermo Scientific™ Dionex™ EGC 500 KOH Eluent Generator Cartridge (P/N 075778)
- Thermo Scientific™ Dionex™ CR-ATC 600 Continuously Regenerated Anion Trap Column (P/N 088662)
- Thermo Scientific™ Dionex™ AERS 500e Anion Electrolytically Regenerated Suppressor for External Water Mode (2 mm) (P/N 302662)
- Thermo Scientific™ Dionex™ IC PEEK Viper™ Fittings Kit (P/N 088798)
- Dionex AS-AP Autosampler Vials 10 mL (P/N 074228)
- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) version 7.2 SR6
- Thermo Scientific™ Nalgene™ Syringe Filters, PES, 0.2 µm (Fisher Scientific P/N 09-740-61A)
- Air-Tite™ All-Plastic Norm-Ject™ Syringes, 5 mL, Sterile (Fisher Scientific P/N 14-817-28)
- Thermo Scientific Nalgene 1000 mL, 0.2 µm Nylon Filter Units (P/N 09-740-46)

Reagents and standards

Reagents

Deionized (DI) water, Type I reagent grade, with 18 MΩ·cm resistivity or better filtered through a 0.2 µm filter immediately before use

Standards

- D(⁻)-Quinic acid, +98%, ACROS Organics™ (Fisher Scientific P/N AC160500250)
- Acetic acid, Glacial (Certified ACS Plus), Fisher BioReagents™ (Fisher Scientific P/N BP 2401-500)
- L(⁺)-Lactic acid, 90% solution in water, ACROS Organics (Fisher Scientific P/N AC189872500)
- Glycolic acid, 99%, ACROS Organics (Fisher Scientific P/N AC154510250)
- Formic acid, 99%, for analysis, ACROS Organics (Fisher Scientific P/N AC 270480250)
- L(⁻)-Malic acid, 99% (Fisher Scientific P/N AC15059)
- Pyruvic acid, Sodium Salt, +99%, ACROS Organics (Fisher Scientific P/N AC 132151000)
- Succinic acid (Crystalline/Certified), Fisher Chemical (Fisher Scientific P/N A294-500)
- Succinic acid ($^{13}C_4$, 99%), Cambridge Isotope Laboratories (CLM P/N CLM-1571-0.1)
- DL-Tartaric acid, 99.5%, ACROS Organics (Fisher Scientific P/N AC137871000)
- Malonic acid, 99%, ACROS Organics (Fisher Scientific P/N AC125262500)
- Oxalic acid dihydrate (Crystalline/Certified ACS), Fisher Chemical (Fisher Scientific, P/N A219-250)
- Fumaric acid, 99+%, ACROS Organics (Fisher Scientific P/N AC119751000)
- Citric acid anhydrous, Crystalline, USP (Fisher Scientific P/N A95)

Samples*

- Aloe beverage Sample A
- Hawthorn/plum beverage Sample B
- Goji berries, used to prepare Sample C

*Note: Samples were purchased from a local store.

Conditions

IC System:	Dionex Integrion HPIC system
MS Detector:	ISQ EC single quadrupole mass spectrometer
Columns:	Dionex IonPac AG11-HC-4-µm Guard, 2 × 50 mm (P/N 078036) Dionex IonPac AS11-HC-4-µm Analytical, 2 × 250 mm (P/N 078035)
Eluent Source:	Dionex EGC 500 KOH Eluent Generator Cartridge with Thermo Scientific™ Dionex™ CR-ATC 600
Gradient:	1 mM KOH (0–17 min), 1–15 mM KOH (17–24 min), 15 mM KOH (24–35.3 min), 15–60 mM KOH (35.3–54.6 min), and 1 mM KOH (54.6–60 min)
Flow Rate:	0.38 mL/min
Injection Volume:	2.5 µL
Temperature:	30 °C (column compartment), 20 °C (detector compartment)
System Backpressure:	~3500 psi (100 psi = 0.6894 MPa)
Detection:	Suppressed Conductivity, Dionex AERS 500e Anion Electrolytically Regenerated Suppressor (2 mm), AutoSuppression, 57 mA, external water mode via Dionex AXP Pump, external water flow rate (0.76 mL/min)
Background Conductance:	~ 0.3 µS/cm
Run Time:	60 min

Mass Spectrometric Detection	
Ionization Interface:	Electrospray ionization (ESI), negative mode
Gas Control:	Sheath gas pressure: 50 psi Aux gas pressure: 8 psi Sweep gas pressure: 0.0 psi
Source Voltage:	-2500 V
Vaporizer Temperature:	450 °C
Ion transfer Tube Temperature:	150 °C
SIM Scan:	Table 1
Full Scan:	Mass Range: 20–200 *m/z* Source CID Voltage: 0 V
Groups:	Chrom. Filter Peak Width: 25 s

Preparation of solutions and reagents

Deionized water with 18 MΩ·cm resistivity or better is used for eluent and standard preparation and for diluting samples. Individual stock standard solutions of 1000 mg/L are prepared gravimetrically from the reagents and DI water. A mixed standard solution is prepared by diluting the individual stock standard solutions into a 100 mL volumetric flask with DI water. Calibration standards are prepared similarly by diluting the stock standards in DI water. Thirteen compounds and their masses listed in Table 2 are used to prepare 100 mL of 1000 mg/L stock solutions.

Table 2. Amounts of compounds used to prepare 100 mL of 1000 mg/L stock solutions

Anion	Compound	Mass (mg)
Quinate	Quinic Acid	100.00
Lactate	Lactic Acid	100.00
Acetate	Acetic Acid	100.00
Formate	Formic Acid	100.00
Glycolate	Glycolic Acid	100.00
Pyruvate	Pyruvic Acid, Sodium Salt	124.96
Succinate	Succinic Acid	100.00
Malate	Malic Acid, Disodium Salt	132.78
Tartrate	Tartaric Acid	100.00
Malonate	Malonic Acid, Disodium Salt	142.23
Oxalate	Oxalic Acid Dihydrate	140.03
Fumarate	Fumaric Acid	100.00
Citrate	Citric Acid	100.00

Table 1. Advanced scan mode parameters

Scan Name	Mass List (amu)	Dwell or Scan Times (s)	SIM Widths (amu)	Ion Polarity	Source CID Voltage
Acetate	59	0.8	0.3	Negative	0
Lactate-Oxalate	89	0.2	0.3	Negative	0
Formate	45	0.8	0.3	Negative	0
Glycolate	75	0.2	0.3	Negative	0
Quinate-Citrate	191	0.2	0.3	Negative	0
Malonate	103	0.2	0.3	Negative	0
Bicarbonate (HCO_3)	61	0.2	0.3	Negative	0
Pyruvate	87	0.2	0.3	Negative	0
Succinate	117	0.2	0.3	Negative	0
Succinate ISTD*	121	0.2	0.3	Negative	0
Malate	133	0.2	0.3	Negative	0
Fumarate	115	0.2	0.3	Negative	0
Tartrate	149	0.2	0.3	Negative	0

*ISTD: Internal Standard

The succinate ISTD is enriched with ^{13}C, and the base mass peak is *m/z* 121. The 10,000 mg/L stock solution of succinate ISTD is prepared by dissolving 100 mg succinate ISTD into 10 mL DI water. The working solution of succinate ISTD at 100 mg/L is prepared gravimetrically by diluting the 1 mL stock standard solution into a 100 mL volumetric flask with DI water. The recommended internal standard concentration in each standard and sample is 100 µg/L, as indicated below.

Sample preparation

For goji samples, immerse 50 g dried goji berries in 1000 mL hot water overnight and allow to cool to room temperature. Centrifuge the goji solution at 6500–7500 g for 20 min, and pass the supernatant through a Nalgene syringe filter (0.2 µm). For the aloe and hawthorn/plum bottled beverages, centrifuge for 20 min, and then pass the supernatant through a Nalgene syringe filter (0.2 µm) and dilute 1:20 with DI water.

Internal standard method of use

1. Prepare 10 mL each of:
   - Organic acid standards in water blank (no ISTD)
   - Sample dilution
2. Add 10 µL 100 mg/L succinate ISTD to each 10 mL solution of standard and sample.

System configuration

Install and configure the Dionex AS-AP Autosampler in Push Mode. Follow the instructions in the Dionex AS-AP Autosampler Operator's Manual (Document No. 065361) to calibrate the sample transfer line to ensure accurate and precise sample injections.

Install and hydrate the Dionex EGC 500 KOH cartridge, Dionex CR-ATC 600 Continuously Regenerated Anion Trap Column, and Dionex AERS 500e suppressor according to the product manual instructions.[4-6]
Note: The system pressure needs to be above 2000 psi for effective degassing of the KOH produced by the eluent generator. The Dionex AERS 500e suppressor is recommended for all applications where external water mode is employed, in particular, IC-MS applications. The ISQ EC mass spectrometer is installed according to the ISQ EC Operating Manual.[7]

Flow goes into the MS detector after passing through the electrolytically regenerated anion suppressor and the conductivity cell. Ionization improvements to the ISQ EC mass spectrometer's electrospray source (HESI-II electrospray) eliminate the need for adding organic solvents to assist ionization and thus simplify operation.

Results and discussion

Separation

The Dionex IonPac AS11-HC-4µm column is a high-resolution, high-capacity anion exchange column, providing analyte separation that allows for the peak quantification of a large number of inorganic anions and organic acid anions from a single sample injection. The column is operated in the gradient mode using a hydroxide eluent. Certain organic solvents can be added to the hydroxide eluent to modify the ion exchange process, and thereby column selectivity, or improve sample solubility. Under aqueous eluent conditions, succinate and malate, acetate and glycolate, and malonate and tartrate form co-eluting pairs. The separation could be improved by adding methanol to the eluent. The organic solvent in the eluent, however, can reduce the sensitivity of conductivity measurements by up to half due to increased eluent viscosity, decreased ionization of organic acids, and, in some cases, lower peak efficiencies. Moreover, it increases the difficulty of operation by adding solvent to the aqueous eluent.

In this study, an easy IC-CD/MS method was developed to separate 13 common organic acids on a Dionex IonPac AS11-HC-4µm column set (Figure 1). An IC separation could resolve the majority of organic acids. The separation was enhanced with mass spectrometric detection in SIM mode. Co-eluting analytes were fully resolved in different SIM channels (Figure 1). The hydroxide eluent started with a low concentration (1 mM KOH) to separate the weakly retained anions, such as quinate, lactate, acetate/glycolate, and formate. After maintaining this concentration for 17 min, the eluent concentration was gradually increased to elute more strongly retained anions. The KOH concentration was increased to 15 mM at 24 min, and remained at 15 mM from 24 to 35.3 min, during which pyruvate, succinate/malate, bicarbonate, and malonate/tartrate eluted. After 35.3 min, a KOH gradient was executed from 15 mM to 60 mM at 54.6 min to elute fumarate, oxalate, and citrate.

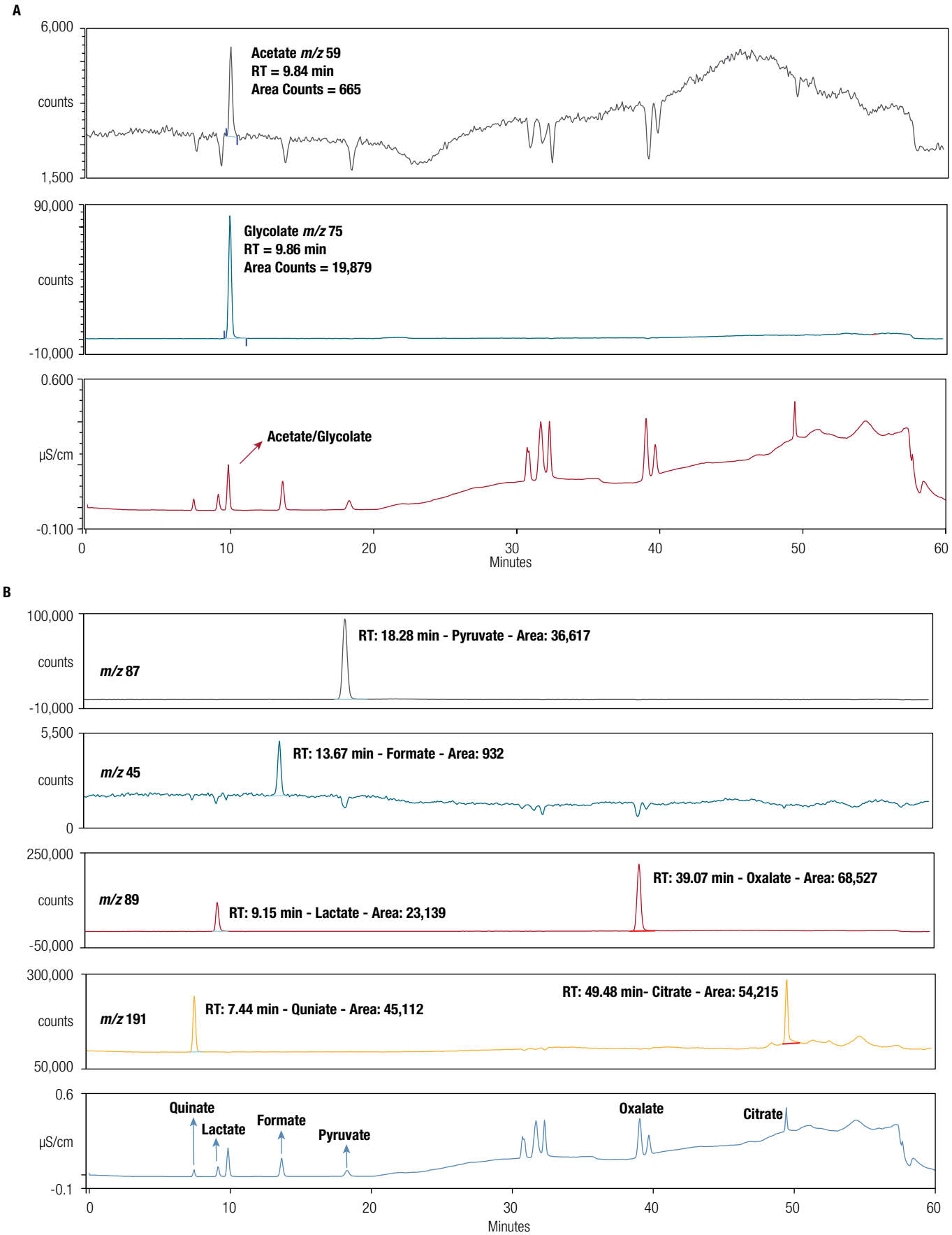

Figure 1 (A and B). Conductivity and SIM chromatograms of 13 common organic acids (0.5 mg/L each). A) acetate and glycolate; B) quinate, lactate, formate, pyruvate, oxalate, and citrate.

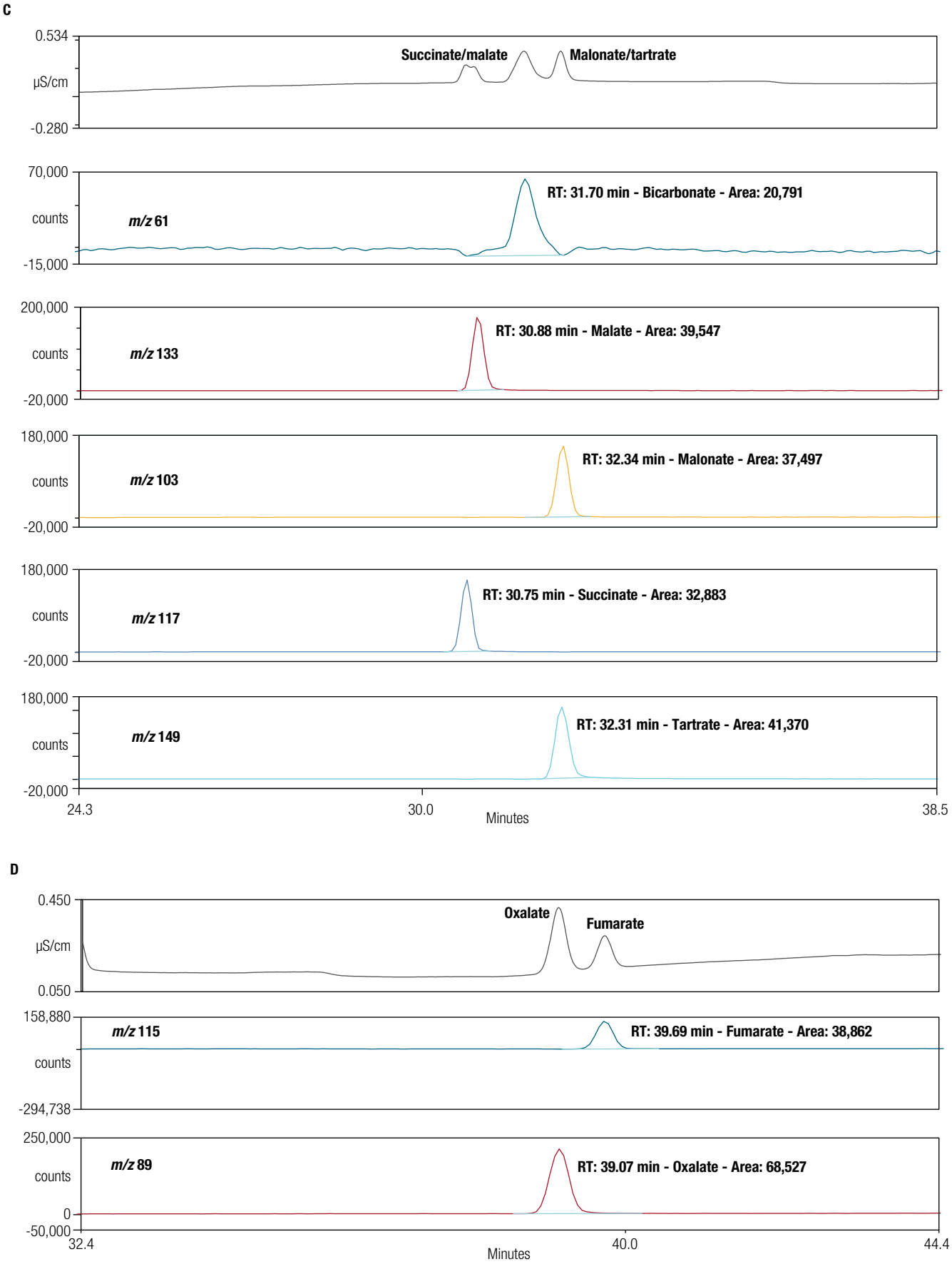

Figure 1 (C and D). Conductivity and SIM chromatograms of 13 common organic acids (0.5 mg/L each). C) succinate, malate, malonate, and tartrate; D) oxalate and fumarate

The eluent condition was restored to the initial condition at 54.6 min to re-equilibrate the column prior to the next injection. The total run time was 60 min. All SIM channels are listed in Table 1. Acetate and glycolate were fully resolved in different SIM channels, *m/z* 59 for acetate and *m/z* 75 for glycolate. Likewise, succinate/malate can be fully resolved using SIM channels *m/z* 117 and *m/z* 133, while malonate/tartrate can be resolved by SIM channels *m/z* 103 and *m/z* 149.

Mass spectra of organic acids

Full Scan mode generally results in the detection of the analytes present and provides their mass information. It facilitates confirmation of peak identity and detection of peak purity. Here we show mass spectra of 14 organic acids including quinate, lactate, acetate, glycolate, formate, pyruvate, succinate, $^{13}C_4$ succinate, malate, malonate, tartrate, oxalate, fumarate, and citrate. (Figures 2–15). Mass-to-charge ratios of deprotonated organic acids $[M-H]^-$ based on the theoretical mass-to-charge ratio of each were used for detection and quantification.

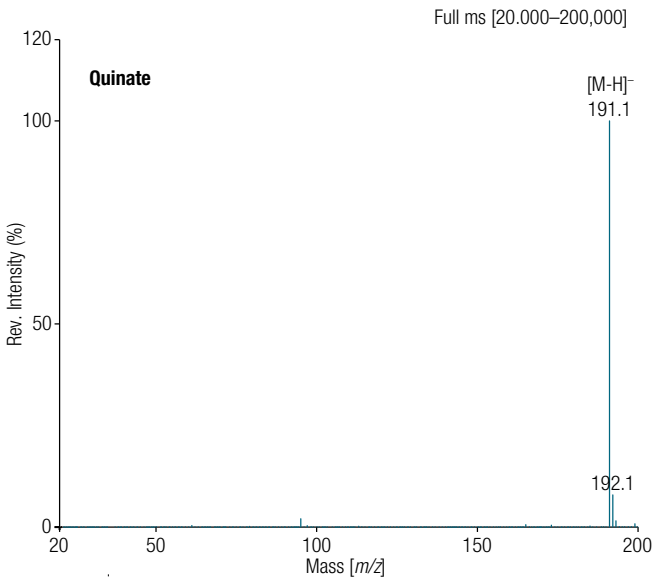

Figure 2. Mass spectrum of quinate

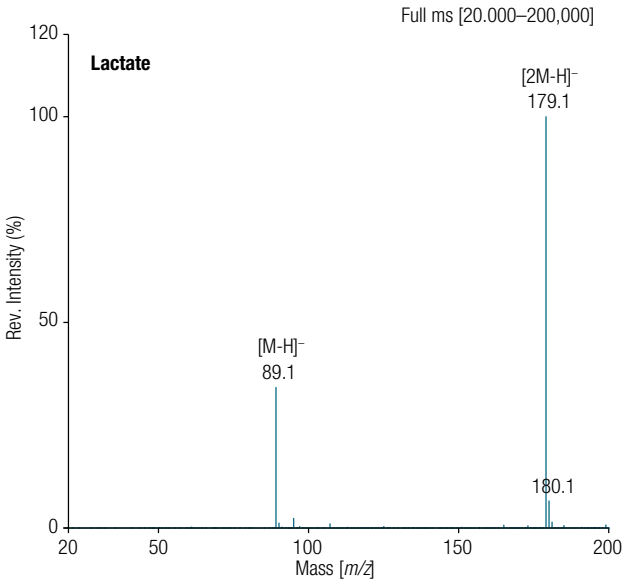

Figure 3. Mass spectrum of lactate

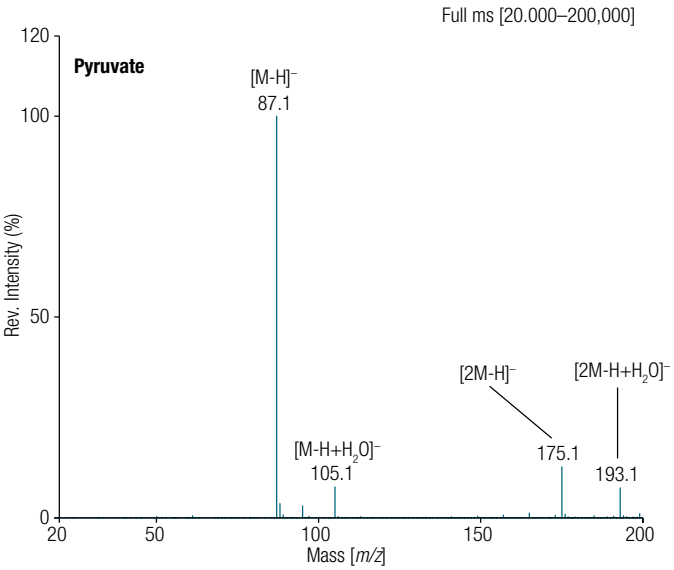

Figure 4. Mass spectrum of pyruvate

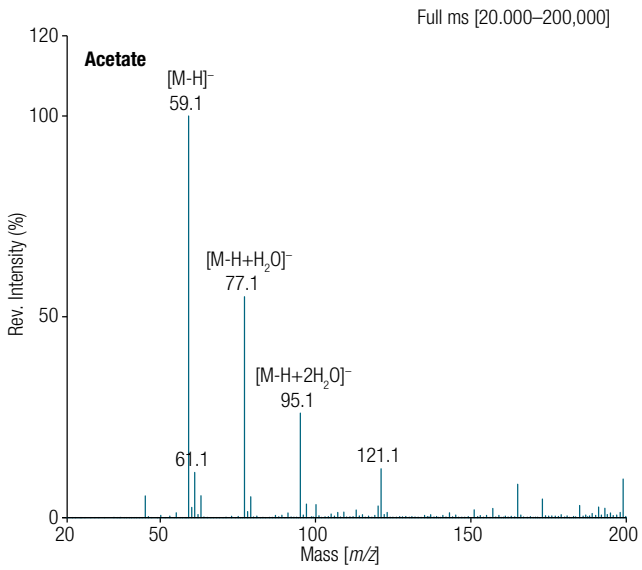

Figure 5. Mass spectrum of acetate

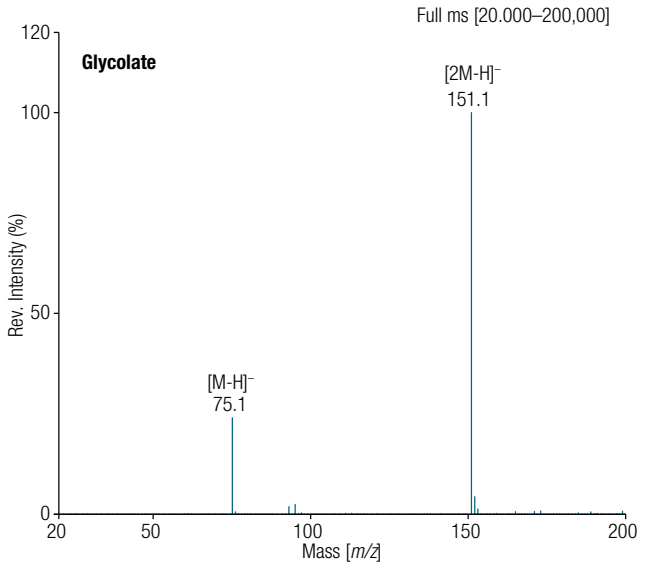

Figure 6. Mass spectrum of glycolate

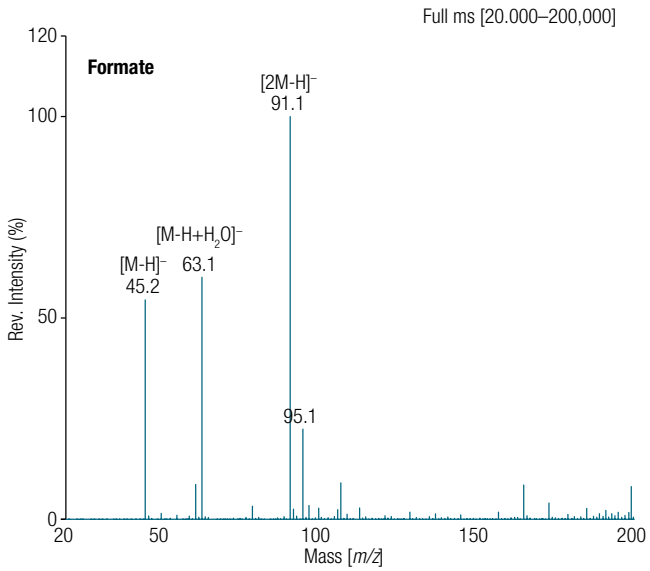

Figure 7. Mass spectrum of formate

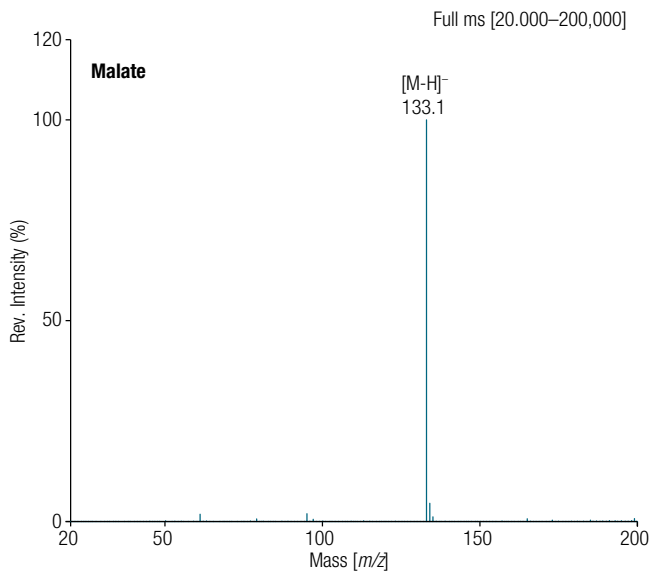

Figure 8. Mass spectrum of malate

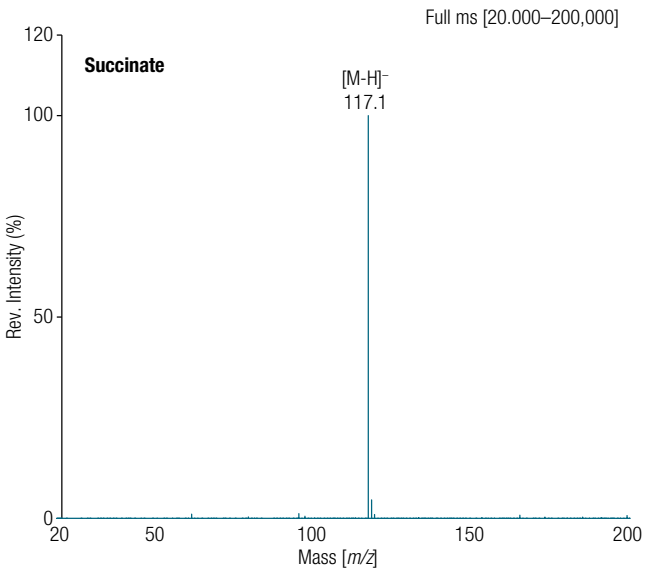

Figure 9. Mass spectrum of succinate

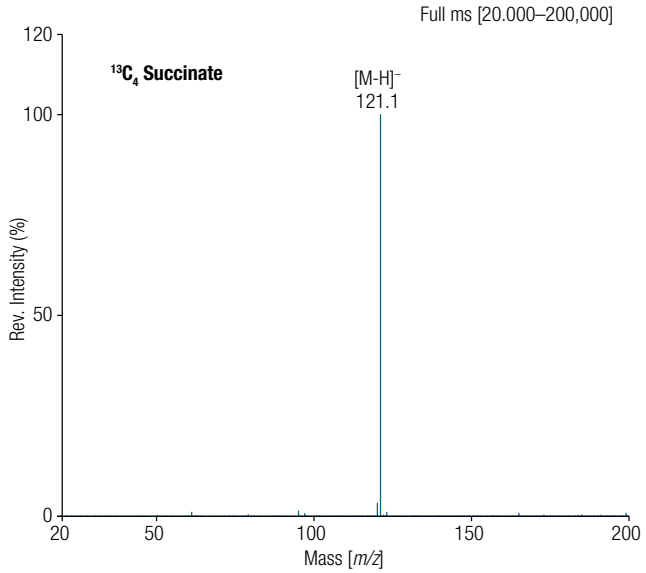

Figure 10. Mass spectrum of $^{13}C_4$ succinate

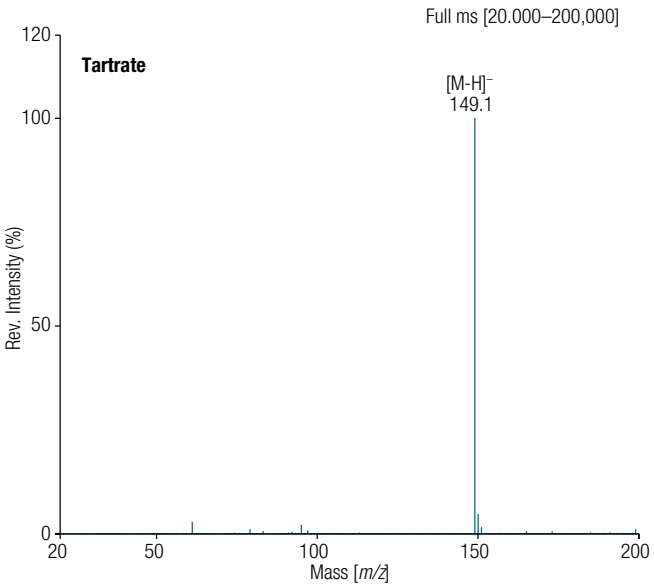

Figure 11. Mass spectrum of tartrate

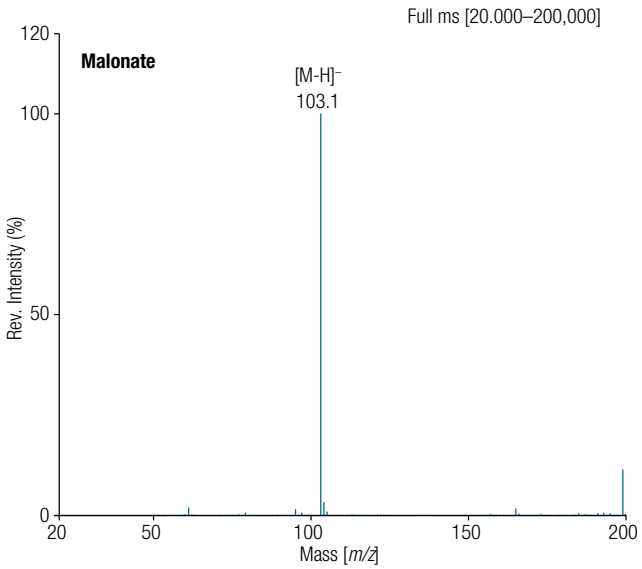

Figure 12. Mass spectrum of malonate

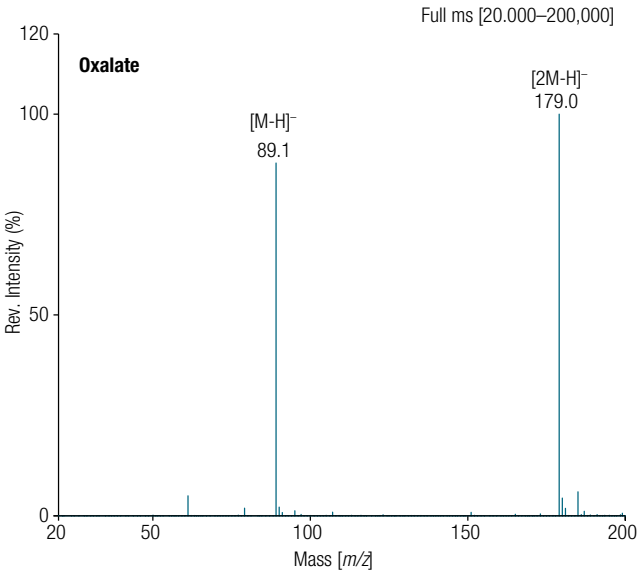

Figure 13. Mass spectrum of oxalate

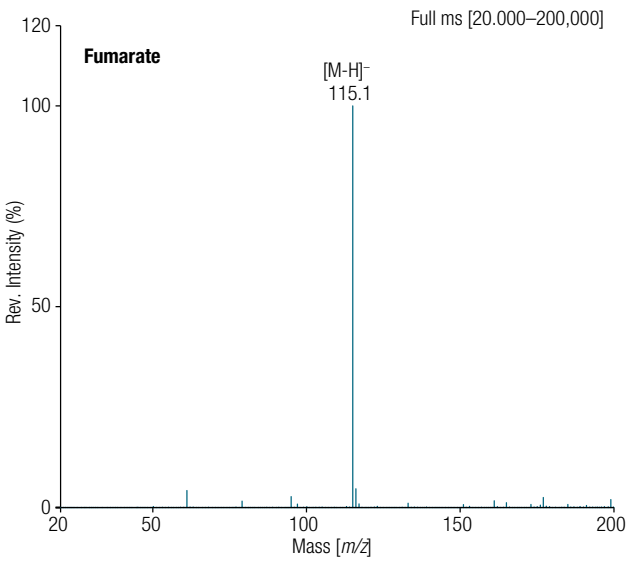

Figure 14. Mass spectrum of fumarate

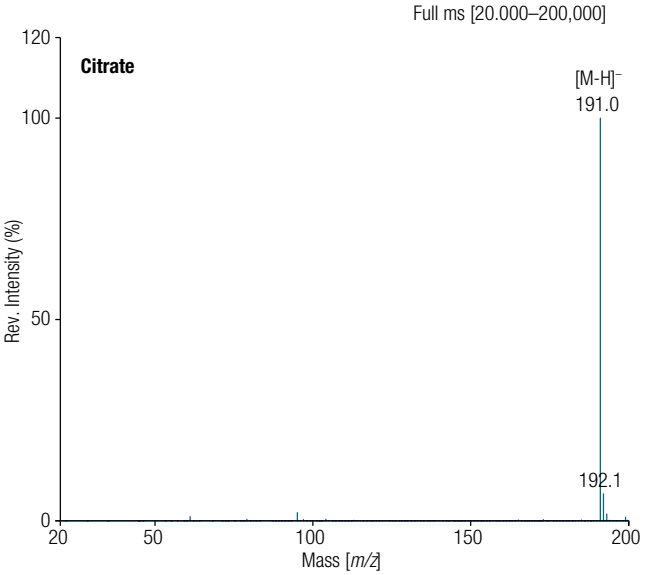

Figure 15. Mass spectrum of citrate

Limit of detection, limit of quantitation, and calibration

The detection limits of suppressed conductivity detection and mass spectrometric detection were compared. Fisseha et al.[8] reported improved signal-to-noise ratios (S/N) and the resolution of co-eluting acids by IC-MS for the qualitative evaluation of organic acids (OA) produced in a smog chamber. Our study evaluated the detection limits of 13 common OAs using IC-CD/MS. We found MS usually outperformed CD, especially for non-resolved OAs because MS gave us an opportunity to explore their sensitivity without chromatographic separation. Figure 16 shows the detection limits of some OAs using the ISQ EC MS are improved compared CD. However, MS did not enhance the sensitivity of formate, oxalate, and citrate compared with CD under the current MS conditions. Note: MS conditions can be optimized for the specific organic acid to improve its sensitivity.

Determination of S/N is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and by establishing the minimum concentration at which the analyte can be reliably detected. A S/N of 3:1 is generally considered acceptable for estimating the limit of detection (LOD), and a S/N of 10:1 for the limit of quantification (LOQ). The LOD and LOQ were then calculated from the average peak height of five injections of each of the standards with known low concentration. Due to co-elution, the CD detector was unable to differentiate succinate and malate, acetate and glycolate, or malonate and tartrate under this study's separation conditions, so there are no LOD and LOQ by CD for those OAs. The results of LOD and LOQ by MS and CD are summarized in Table 3.

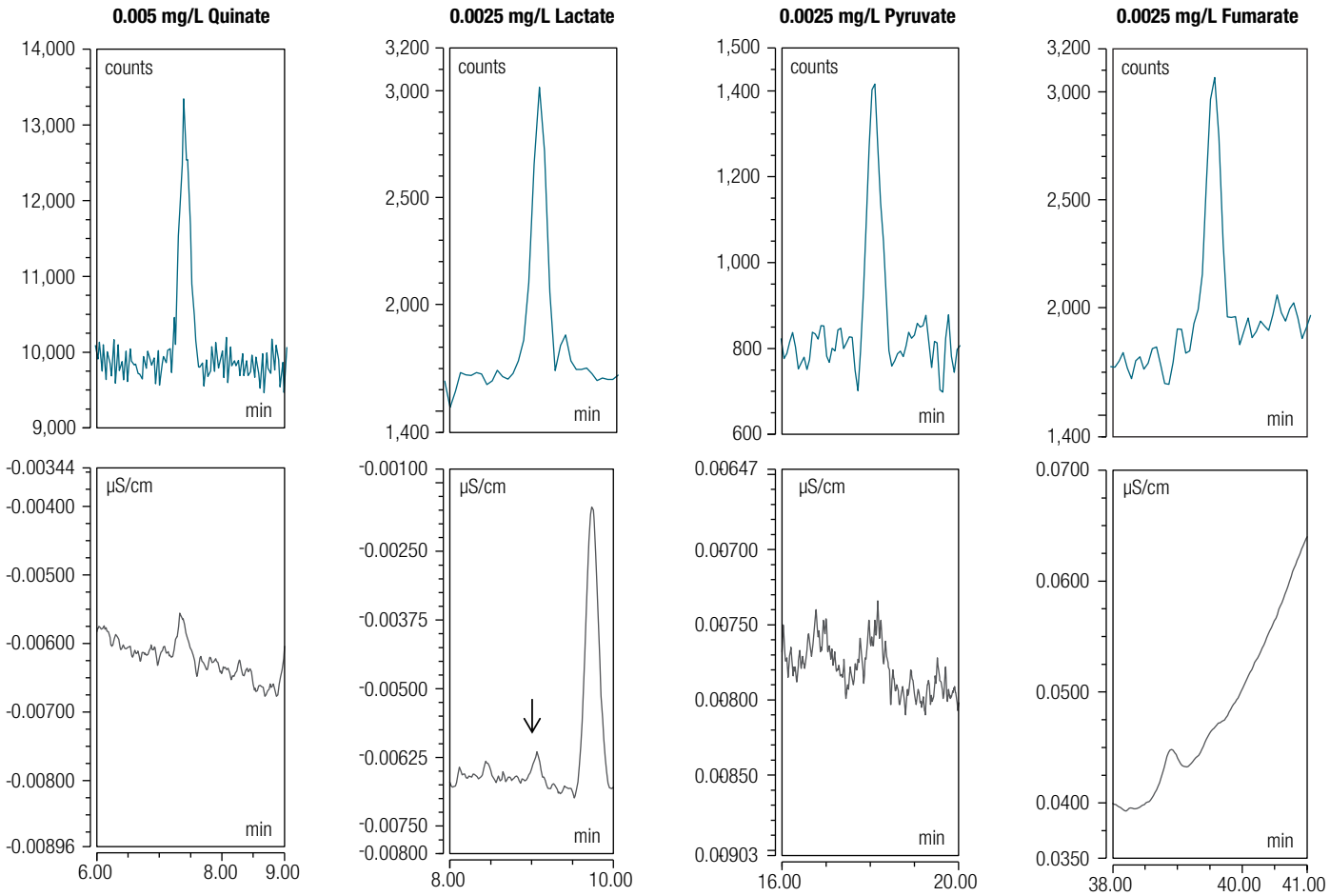

Figure 16. Comparison of signal response between CD and ISQ EC detector in SIM mode for quinate, lactate, pyruvate, and fumarate

Table 3. Method LOD and LOQ by MS and CD detection

Compound	IC-MS		IC-CD	
	LOD[a] (µg/L)	LOQ[b] (µg/L)	LOD[a] (µg/L)	LOQ[b] (µg/L)
Quinate	4.63	15.4	9.96	33.2
Acetate	39.9	133	n.a.	n.a.
Lactate	2.27	7.57	8.45	28.2
Glycolate	1.58	5.26	n.a.	n.a.
Formate	38.3	128	2.89	9.62
Pyruvate	2.11	7.02	12.2	40.8
Malonate	0.664	2.21	n.a.	n.a.
Succinate	2.56	8.53	n.a.	n.a.
Malate	8.75	29.2	n.a.	n.a.
Tartrate	0.595	1.98	n.a.	n.a.
Oxalate	4.65	15.5	3.93	13.1
Fumarate	1.67	5.57	4.24	14.1
Citrate	20.0	66.6	20.0	66.6

[a]LOD=3×S/N
[b]LOQ=10×S/N

Calibration curves with seven concentration levels for MS detection were constructed for each of the non-resolved OAs: acetate (0.2–7.5 mg/L), glycolate (0.1–1.5 mg/L), succinate (0.125–1 mg/L), malate (5–100 mg/L), malonate (0.05–1 mg/L), and tartrate (0.002–0.075 mg/L). For the others that can be fully resolved on a Dionex IonPac AS11-HC-4µm column, the results showed the calibration curves generated by MS are much less linear and have limited working range compared with the conductivity detector. For example, calibration curves for lactate ranging from 0.1 to 20 mg/L were constructed by MS and CD detectors, respectively (Figure 17). Here we only used MS data for the calibration curve construction of non-resolved OAs.

To determine the calibration curves, the MS responses to concentration were determined using triplicate injections of calibration standards. Table 4 shows the quantitation ions, calibration ranges, the coefficients of determination (r^2), and calibration method. A regression model (e.g., linear, quadratic) was chosen to plot peak area versus concentration over the calibration range, as exemplified by the acetate (Figure 18), glycolate (Figure 19), malate (Figure 20), malonate (Figure 21), and tartrate calibration curves (Figure 22). The exception was succinate, which suffered from ion suppression. A ^{13}C-enriched succinate ($^{13}C_4$, M+4) internal standard (*m/z* 121) is recommended for succinate quantitation to improve accuracy and ruggedness.

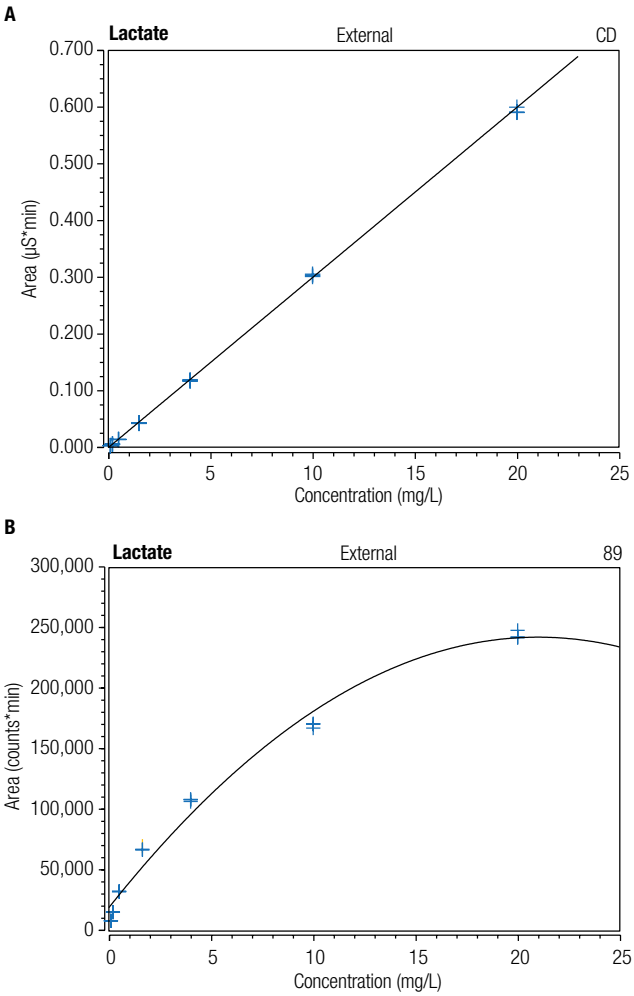

Figure 17. Lactate calibration curves ranging from 0.1 to 20 mg/L were constructed with A) CD detector, and B) MS detector.

Table 4. Method calibration for six non-resolved OAs

Analyte	Range (mg/L)	Quantitation Ion	Calibration Method	Cal. Type	Coefficent of Determination (r^2)
Acetate	0.2–7.5	*m/z* 59	External standard	Lin, WithOffset	0.9985
Glycolate	0.1–1.5	*m/z* 75	External standard	Quad, WithOffset	0.9985
Malate	5–100	*m/z* 133	External standard	Quad, WithOffset	0.9992
Malonate	0.05–1	*m/z* 103	External standard	Quad, WithOffset	0.9998
Tartrate	0.002–0.075	*m/z* 149	External standard	Lin, WithOffset	0.9991
Succinate	0.125–1	*m/z* 117	Internal standard (*m/z* 121)	Lin	0.9994

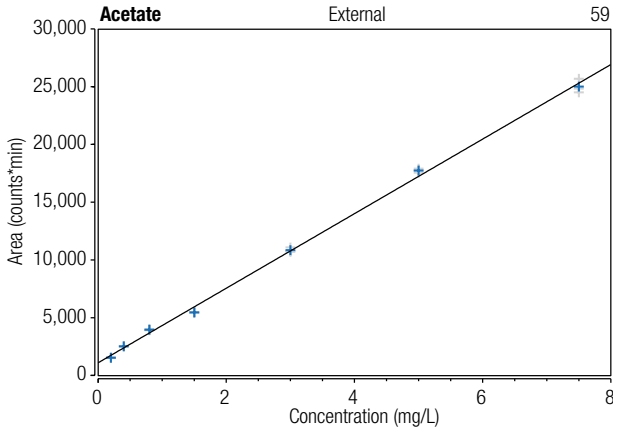

Figure 18. Acetate calibration curve ranging from 0.2 to 7.5 mg/L was constructed with the MS detector.

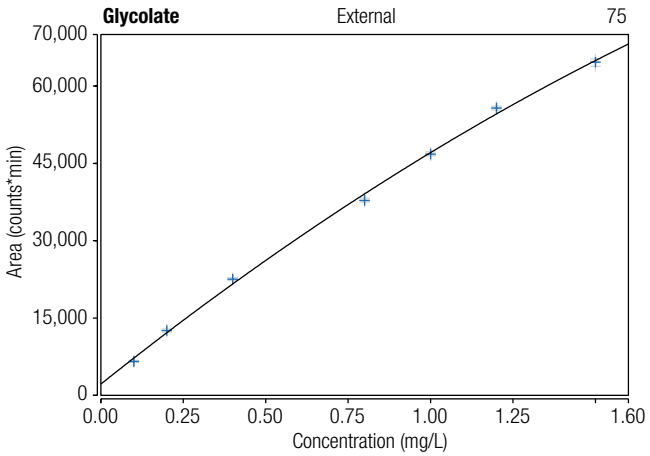

Figure 19. Glycolate calibration curve ranging from 0.1 to 1.5 mg/L was constructed with the MS detector.

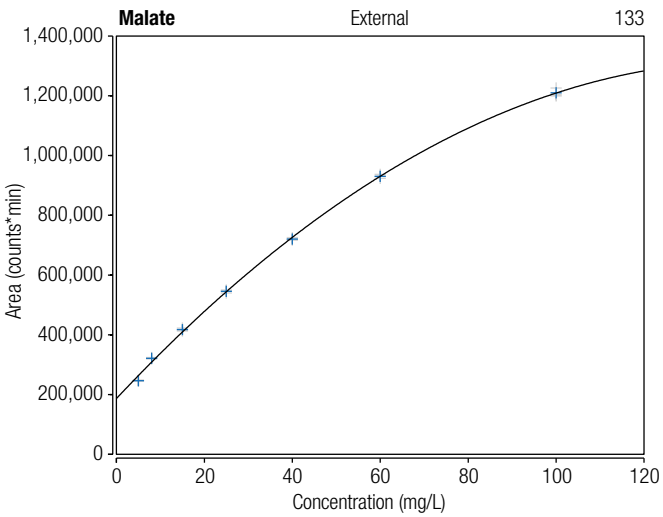

Figure 20. Malate calibration curve ranging from 5 to 100 mg/L was constructed with the MS detector.

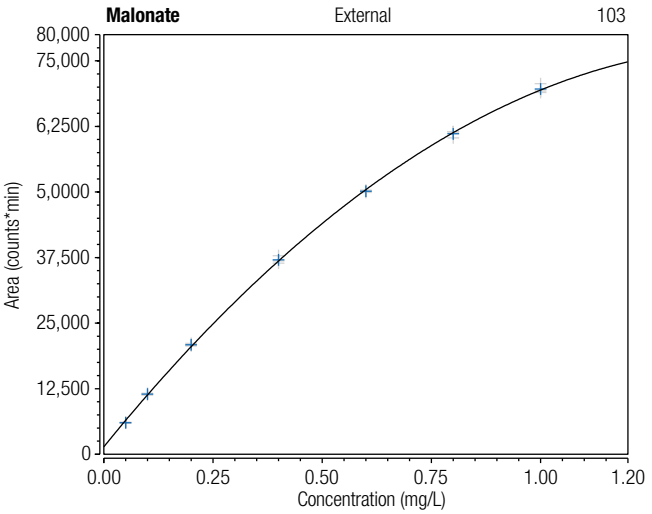

Figure 21. Malonate calibration curve ranging from 0.05 to 1 mg/L was constructed with the MS detector.

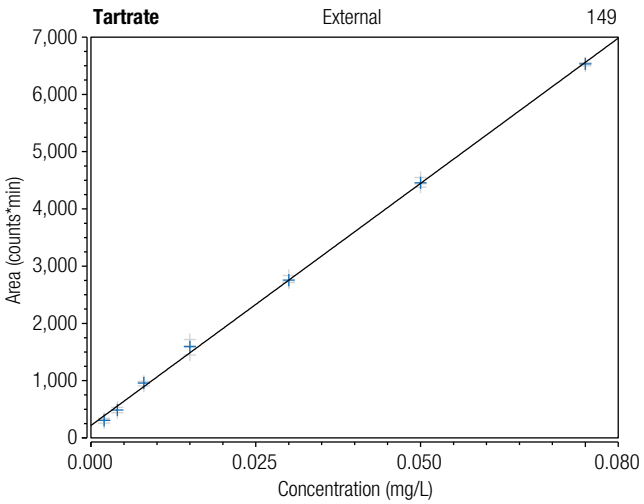

Figure 22. Tartrate calibration curve ranging from 0.002 to 0.075 mg/L was constructed with the MS detector.

The calibration curves were constructed for succinate using the external standard method; Figure 23B displays succinate intensity over a series of concentrations when malate is at a comparingly high concentration (> 5 mg/L). The succinate plot has a linear section in the low concentration, followed by “saturation” with increasing concentration, and a small decrease in intensity at the highest concentration. It indicates that a high concentration of co-eluting malate greatly affects the calibration curve for succinate using the external standard method. Internal standard calibration is one of the most widely used techniques to compensate for ion suppression. An internal standard allows the response of a given analyte to be normalized, thus compensating for matrix effects and possible variations in injection, chromatography, and sample preparation.

If a stable isotope-labeled analog is used as the internal standard, which has identical chemical and structural properties to those of the analyte, the analyte and internal standard will behave identically not only during chromatography but also during sample preparation. Isotopic analogs are therefore the best choice of internal standard to reduce signal variability and improve precision. Using ^{13}C-enriched succinate as the internal standard resulted in reliable quantification and high precision for succinate calibration (Figure 23A).

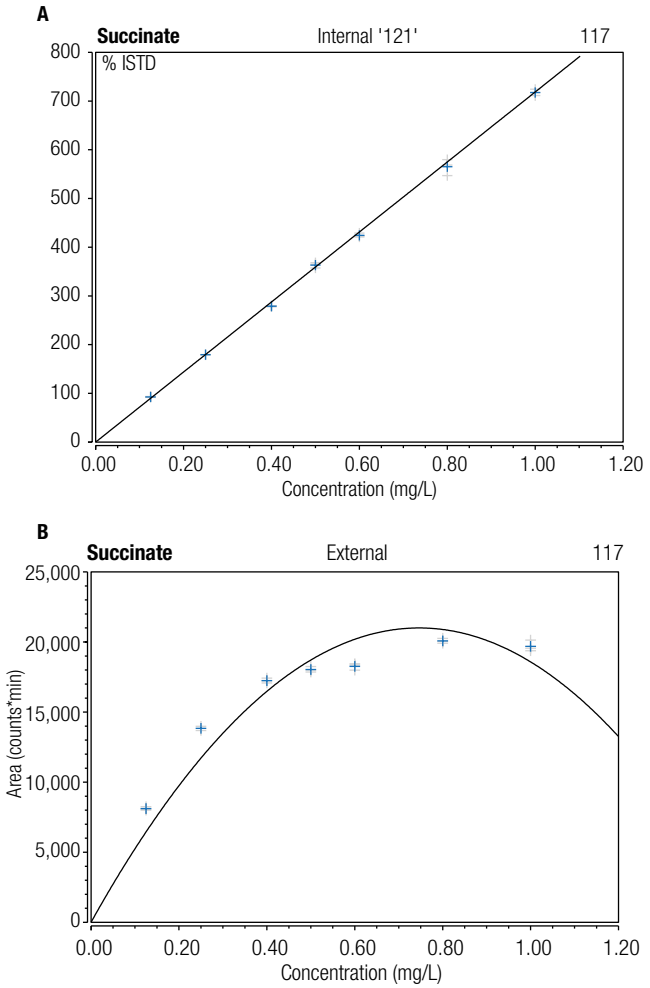

Figure 23. Succinate calibration curve ranging from 0.125 to 1 mg/L using the internal standard (A) and external methods (B)

Ion suppression

The matrix effect phenomenon, as the result of co-eluting sample components, can affect the detection capability, precision, and accuracy for the analytes of interest.[9] Ion suppression appears as one particular manifestation of matrix effects, which is associated with influencing the extent of analyte ionization. This change often is observed as a loss in response, and thus the term ionization suppression. In our study, ion suppression has been found to occur between succinate and malate, especially at high concentrations. Experimentally determined ion intensities at different concentrations of succinate with malate in solution are shown in Figure 24. In these experiments, the concentration of succinate was kept constant (e.g. 0.125 ppm) and the concentration of malate was increased from 0 to 100 ppm. The plots obtained in Figure 24 illustrate how succinate's intensity is suppressed by the presence of a second analyte malate, which coelutes with succinate. When the succinate concentration is 50 ppm, the loss of intensity is from 4.7% to 62.1% as the of malate concentration increases from 0 ppm to 100 ppm; this is the smallest decrease observed. When succinate concentration is 0.125 ppm, the intensity decrease is highest at 88% when the malate concentration is 100 ppm. Similarly, succinate also suppressed the ion intensity of malate (Figure 25). When malate concentration is above 10 ppm, and succinate concentration is below 10 ppm, the ion suppression is not severe in that the ion intensity of malate decreases between 0.5 and 6.1%. The highest loss of malate intensity is observed with 1 ppm malate and 50 ppm succinate, a 56% loss.

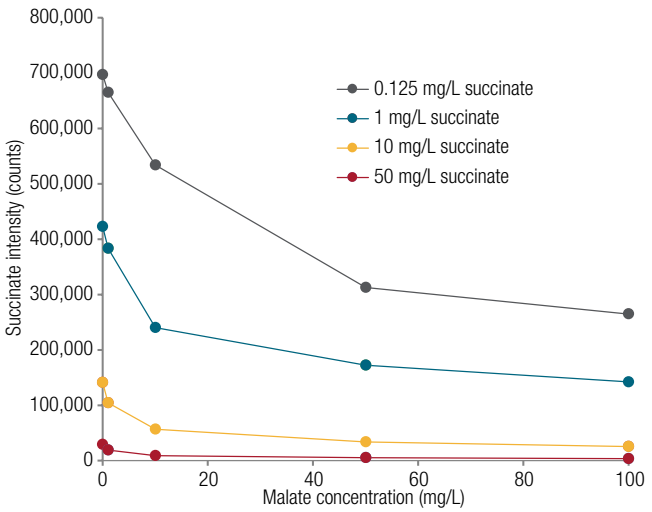

Figure 24. Change of ion intensity of succinate at constant concentration when malate is increased at concentrations ranging from 0 to 100 mg/L

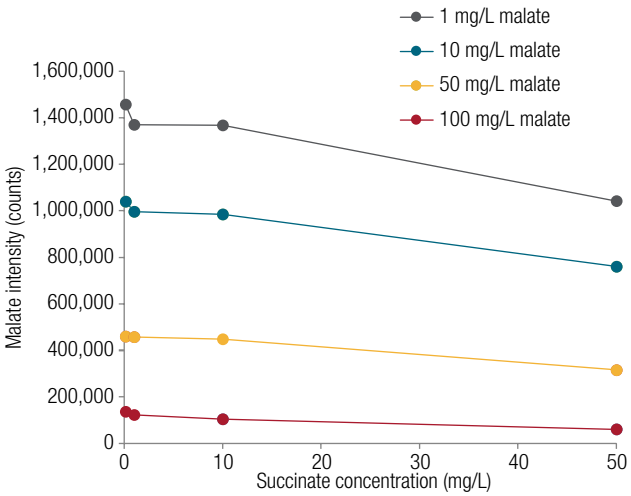

Figure 25. Change of ion intensity of malate at constant concentration when succinate is increased at concentrations ranging from 0.125 to 50 mg/L

Sample analysis

Sample analysis was done in Full Scan and SIM modes to show that the ISQ EC MS can confirm the masses of chromatographic peaks, eliminate false negatives and positives, and deliver accurate quantification of non-resolved analytes using their mass-to-charge ratios.

Figure 26 shows the analysis of aloe beverage with 20-fold dilution using Full Scan mode to identify OAs by comparing their retention times and mass spectra with those of reference substances. Figure 27 shows that the ISQ EC MS can deliver accurate quantification of co-eluting analytes in aloe, goji, and hawthorn/plum drinks using SIM mode. The MS data help to confirm the identities of OAs (Figures 27–29). There are differences in the organic acid compositions and contents of the herbal drink samples. Acetate was not detected by MS in the aloe drink, and acetate and glycolate were resolved by MS in SIM mode in gogi and hawthorn/plum drinks. The MS detector provided greater sensitivity for lactate than CD. Co-eluting OAs including malate/succinate and malonate/tartrate were well resolved by MS in SIM mode. The sensitivity of tartrate and malonate were increased with the MS detector. Quinate and fumarate were not detected by CD and MS in the aloe drink but were detected in the gogi and hawthorn/plum drinks.

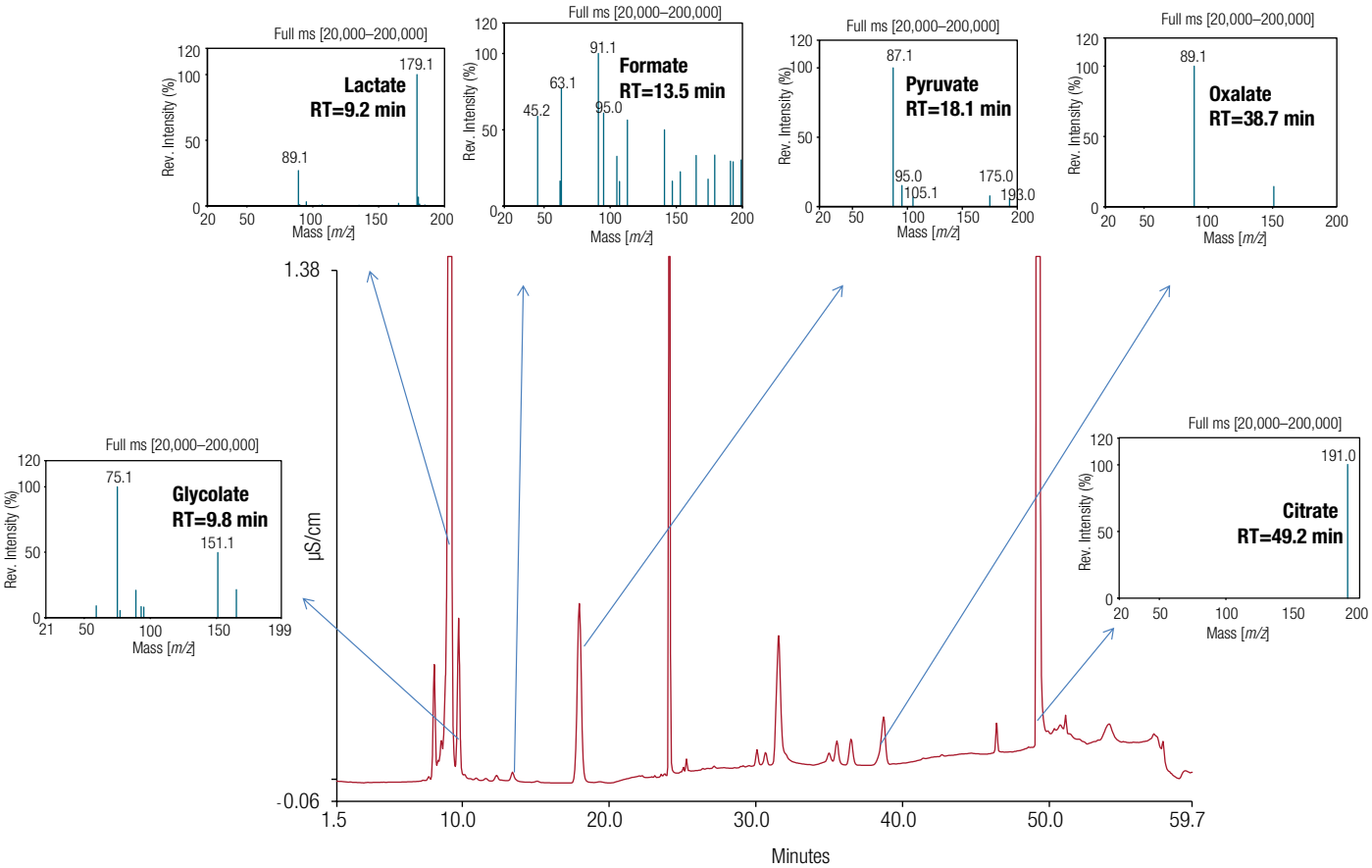

Figure 26. The identification of OAs using Full Scan mode by comparing their retention times (RT) and mass spectra with those of reference substances in aloe with 20-fold dilution

Peaks	(A) mg/L	(B) mg/L	(C) mg/L
Acetate	< LOQ	6.75	4.95
Glycolate	1.30	1.14	0.41

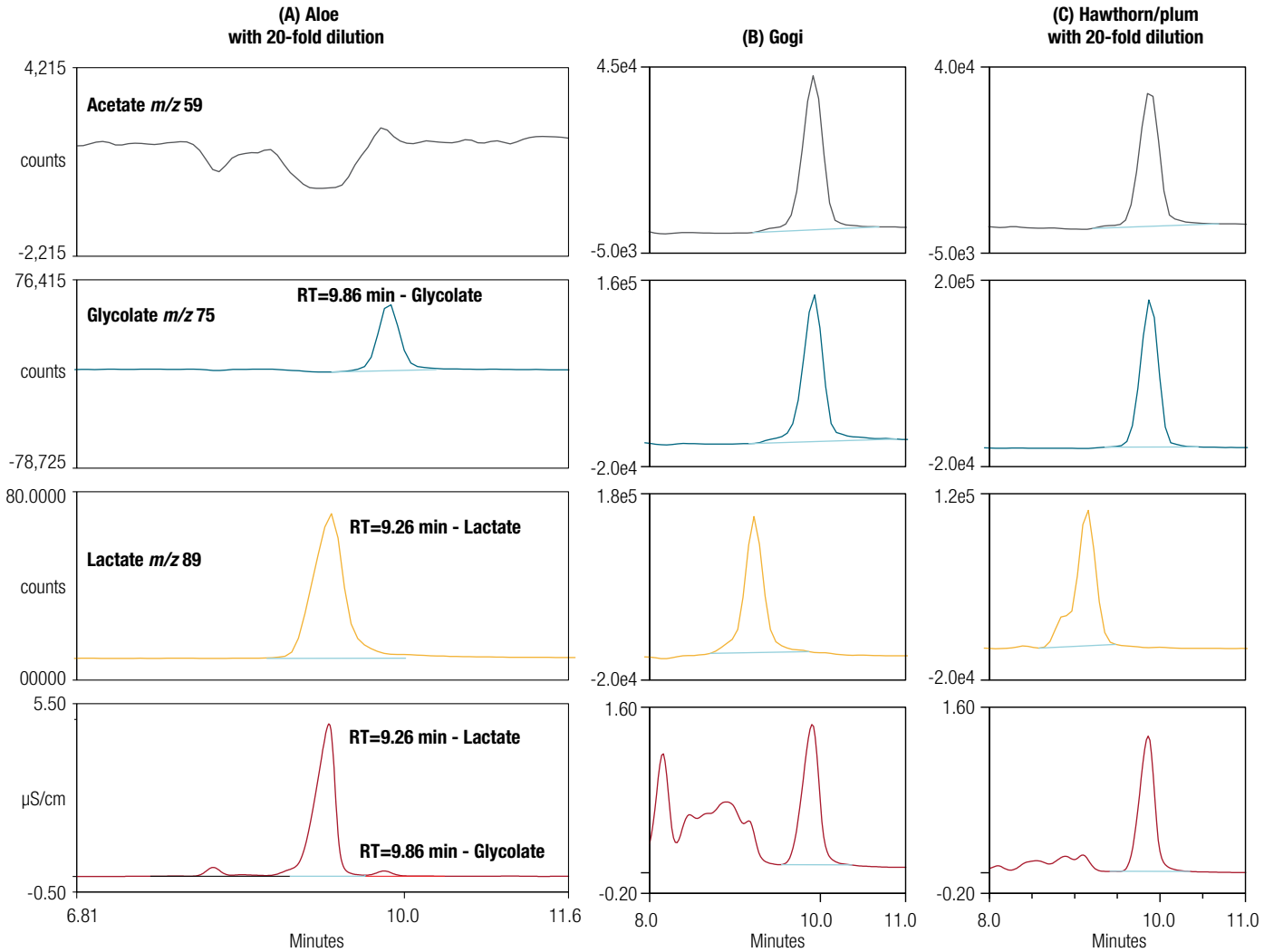

Figure 27-1. The identification and quantitation of co-eluting OAs lactate, acetate, and glycolate by MS in SIM mode

Peaks	(A) mg/L	(B) mg/L	(C) mg/L
Malate	7.42	42.89	11.05
Succinate	< LOQ	0.72	0.34
Malonate	< LOQ	0.63	0.16
Tartrate	0.020	0.069	0.053

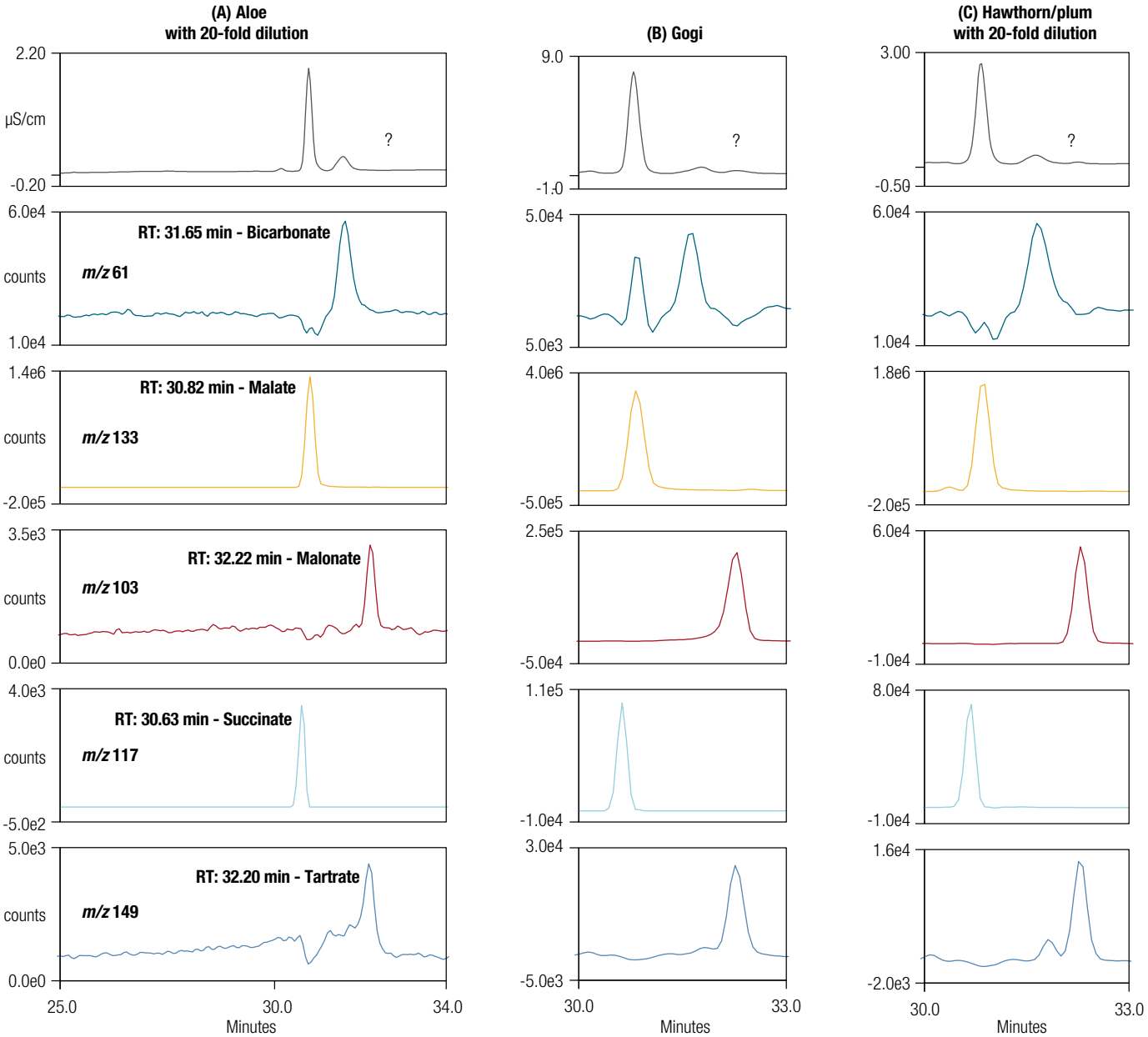

Figure 27-2. The identification and quantitation of co-eluting OAs malate, malonate, bicarbonate, succinate, and tartrate by MS in SIM mode

The levels of all co-eluting OAs listed in Figure 27 were determined from their respective calibration curves by MS detection in SIM mode, except for succinate quantitation, which was determined by the internal standard method using ^{13}C-enriched succinate.

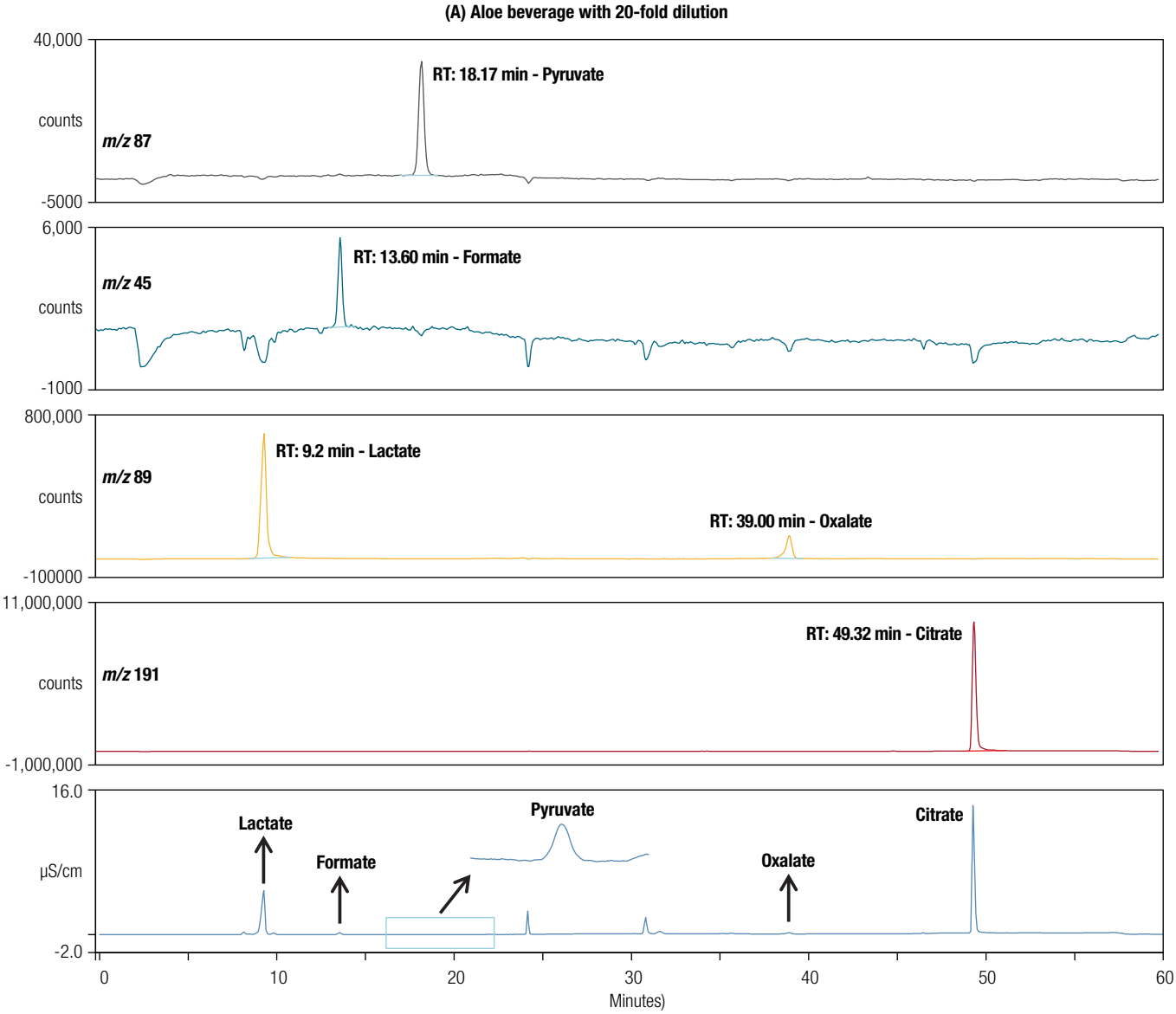

Figure 28A. The identification of quinate, pyruvate, formate, lactate, oxalate, and citrate was confirmed by MS in aloe beverage with 20-fold dilution. Quinate was not detected by CD and MS.

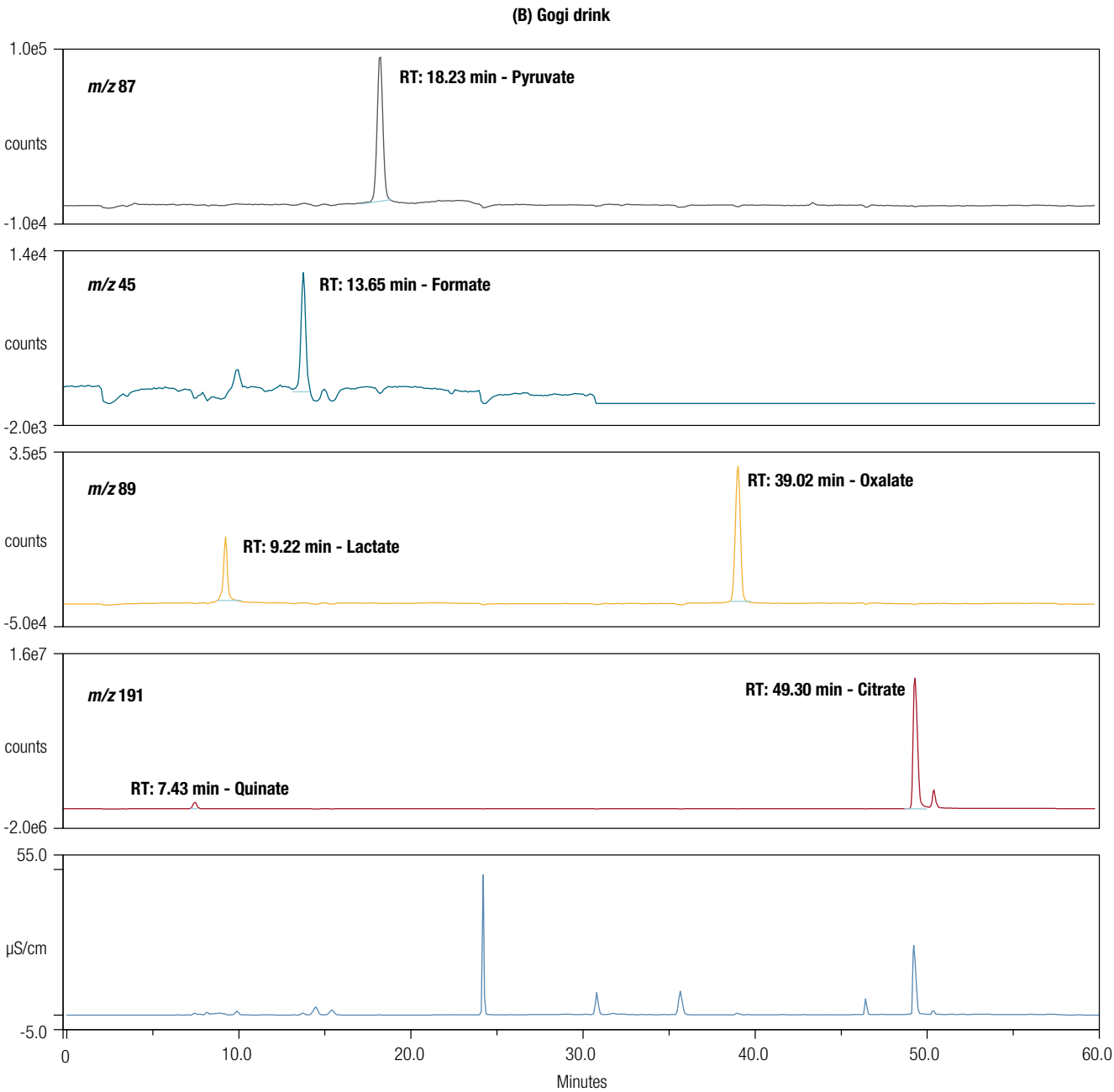

Figure 28B. The identification of quinate, pyruvate, formate, lactate, oxalate, and citrate was confirmed by MS in Gogi drinks.

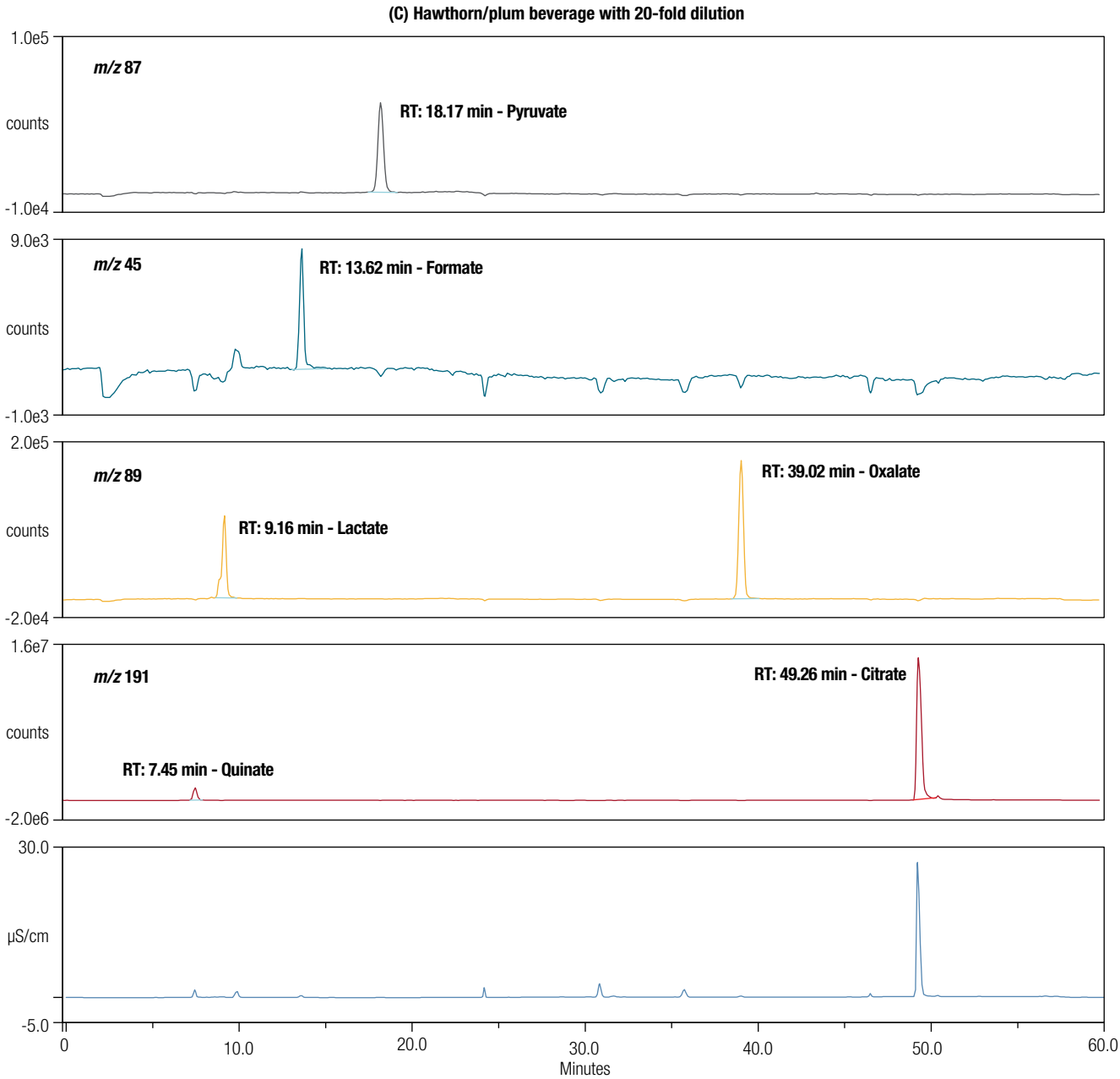

Figure 28C. The identification of quinate, pyruvate, formate, lactate, oxalate, and citrate was confirmed by MS in Hawthorn/plum beverage with 20-fold dilution.

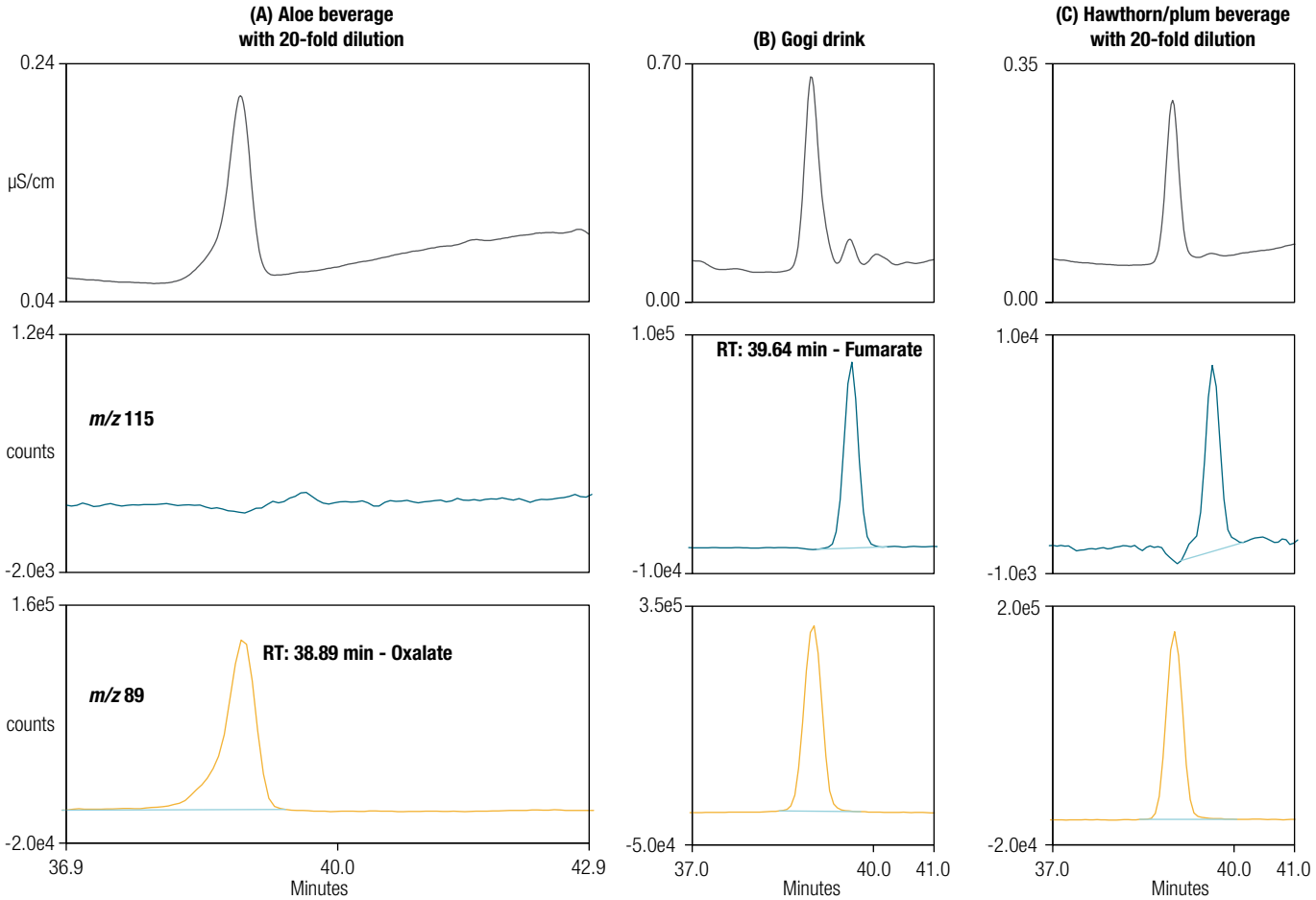

Figure 29. The identification of oxalate and fumarate was confirmed by MS in (A) Aloe beverage with 20-fold dilution. Fumarate was not detectable by CD and MS. (B) Gogi drink, (C) Hawthorn/plum beverage with 20-fold dilution

Conclusion

This work uses a Dionex IonPac AS11-HC-4µm column on an Integrion IC system that electrolytically generated the eluent for separation coupled to an ISQ EC single quadrupole mass spectrometer. Co-eluting OAs including acetate/glycolate, malate/succinate, and malonate/tartrate were accurately quantified with MS detection. The results showed that this IC-MS method allows the accurate direct determination of OAs in aloe, hawthorn/plum, and goji drinks, especially showing selectivity in detection for co-eluting OAs and confirmation of identity. The ISQ EC MS provides lower detection limits for most of organic acids except for formate and citrate than conductivity detection alone. A ^{13}C-enriched succinate ($^{13}C_4$, M+4) internal standard (*m/z* 121) is recommended to be used for succinate quantitation to improve accuracy and ruggedness when ion suppression occurs.

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