Determination of Organic Acids in Herbal Beverages Using IC-MS

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ABSTRACT

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

Methods: The method uses a Thermo Scientific™ Dionex™ IonPac™ AS11-HC-4µm column set, on Thermo Scientific™ Dionex™ Integrion™ HPIC™ system coupled with a recently introduced Thermo Scientific™ ISQ™ EC single quadrupole mass spectrometer (MS).

Results: 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 than conductivity detection alone. A ^{13}C-enriched succinate (^{13}C4, M+4) internal standard (*m/z* 121) is recommended for succinate quantitation to improve accuracy and ruggedness when ion suppression occurs.

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. Among 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 organic acid profile of herbal beverages.

MATERIALS AND METHODS

System Configuration

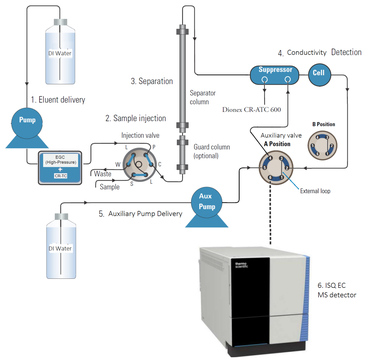

Figure 1. IC-MS Configuration with matrix diversion.

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.

Sample Preparation

For goji samples, 50 g dried goji berries were immersed in 1 L hot water overnight and allowed 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 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.

Test Method

Conditions	
IC System:	Dionex Integrion HPIC system
MS Detector:	Thermo Scientific 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:	Thermo Scientific™ 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 Electrolytically Regenerated Suppressor (2 mm), AutoSuppression, 57 mA, external water mode via Thermo Scientific™ 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 (sec): 25

Table 1. Advanced scan mode parameters.

Scan Name	Mass list (amu)	Time (s)	Width (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

RESULTS

Separation

In this study, an easy IC-CD/MS method was developed to separate 13 common organic acids on the 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 2).

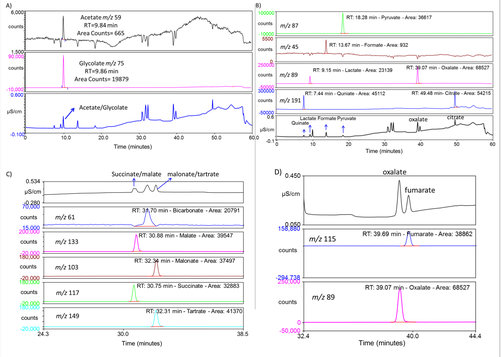

Figure 2. Conductivity and SIM chromatograms of 13 common organic acids (0.5 mg/L each). A) acetate and glycolate B) quinate, lactate, formate, pyruvate, oxalate, and citrate C) succinate, malate, malonate, and tartrate D) oxalate and fumarate.

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 4 selective organic acids including quinate, lactate, pyruvate, acetate. (Figure 3).

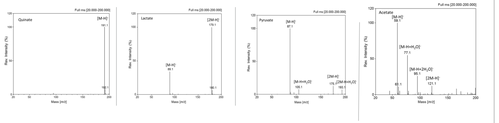

Figure 3. Mass spectra of quinate, lactate, pyruvate, acetate.

Limit of Detection, Limit of Quantitation, and Calibration

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 4 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. While MS conditions were optimized to try improve sensitivity for formate and citrate, such an optimization was not attempted for oxalate.

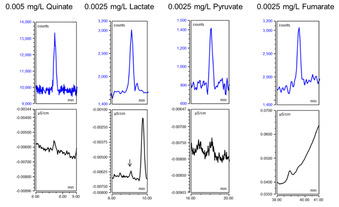

Figure 4. Comparison of signal response between CD and ISQ EC detector in SIM mode for quinate, lactate, pyruvate, and fumarate

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), malate (5 -100 mg/L), malonate (0.05-1 mg/L), tartrate (0.002-0.075 mg/L), and succinate (0.125-1 mg/L). (Figure 5). The calibration curves were constructed for acetate, glycolate, malate, malonate, and tartrate using the external standard method, and for succinate using the internal standard method.

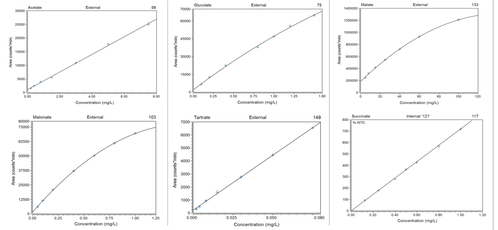

Figure 5. Calibration curves for acetate (0.2–7.5 mg/L), glycolate (0.1–1.5 mg/L), malate (5–100 mg/L), malonate (0.05–1 mg/L), tartrate (0.002–0.075 mg/L), and succinate (0.125–1 mg/L).

Sample Analysis

Sample analysis was done in Full Scan and SIM modes to show that the ISQ EC mass spectrometer 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 6 shows that the ISQ EC mass spectrometer can deliver accurate quantification of co-eluting analytes in aloe, goji, and hawthorn/plum drinks using SIM mode.

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

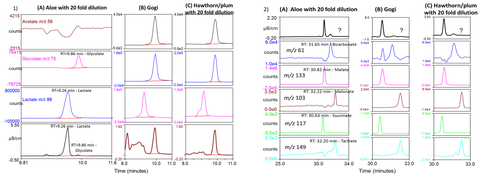

Figure 6. The identification and quantitation of co-eluting OAs by MS in SIM mode. 1) The identification of lactate, acetate, and glycolate, and 2) The identification of malate, malonate, bicarbonate, succinate, and tartrate.

CONCLUSIONS

- This work uses IC with both suppressed conductivity and mass spectrometry detection for organic acid determinations. This two detection approach increases the information available from the sample.
- 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 mass spectrometer provides lower detection limits for most of organic acids, except for formate and citrate, than conductivity detection alone.
- A ^{13}C-enriched succinate (^{13}C4, M+4) internal standard (m/z 121) is recommended for succinate quantitation to improve accuracy and ruggedness when ion suppression occurs.

TRADEMARKS/LICENSING

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