Considerations in IC-ESI-MS Instrumentation and Advances in Applications

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INTRODUCTION

Ion chromatography (IC) has been extensively used as the preferred separation technique for ionic species such as inorganic anions/cations, small amines, organic acids, peptides, proteins, nucleic acids, and carbohydrates. In recent years, the increasing demand for higher sensitivity, selectivity, identity confirmation, and structural information has led to the emergence of mass spectrometry (MS) as a powerful complimentary detector to conductivity, UV, electrochemical detection, and other commonly used IC detection methods.

Here, the authors discuss the general instrument configuration and settings for IC-MS applications. Preferred chromatographic system parameters, such as the analytical column i.d., connection tubing, suppressor, and regenerant settings, are presented. The MS parameters, such as probe temperature, ionization voltage, types and composition of desolvation solvents and flow rates, and the comparison of the direct quantification of extremely low-mass analytes and that of adduct ions are also discussed.

Typical applications using IC-ESI-MS are also presented here, such as the use of MS to analyze coeluted species, common anions, and organic acids; organic acid profiles in beverages and biomasses; ethanolamines as nitrogen mustard degradation products; and the presence of perchlorate in baby formula and milk products.

SYSTEM CONFIGURATIONS FOR IC-MS APPLICATIONS

A typical IC-MS system consists of an IC chromatographic system, a mass spectrometer, and a delivery system for the addition of desolvation solutions (usually an AXP-MS pump and a static mixing tee). Figure 1 shows the configuration of a preferred IC-MS system setup. A standard microbore IC system is recommended for routine MS detection. The microbore system has a suggested flow rate range from 0.1 to 1 mL/min, which is within the preferred flow characteristics of most electrospray ionization (ESI) sources for MS. Higher flow ranges (used for 4 mm formats) can be used but the ESI source is concentration dependent and the excessive flow ends up diverted to waste, with no benefits of sensitivity enhancement. Therefore, the 2 mm columns and consumables (suppressor and trap column) are recommended for MS applications. Reagent-Free™ Ion Chromatography (RFIC™) systems are the preferred systems for the delivery of a highly reproducible mobile phase that is electrolytically generated from an eluent generator. An electrolytic suppressor is used to continuously remove counterions from the eluent, thus converting highly ionic IC eluent to DI water before entering the conductivity and MS detectors. Because the eluent is not recoverable in MS, the suppressor is operated in external mode with DI water as the regenerant delivered by an AXP auxiliary pump. To enhance desolvation (i.e. transfer of the ionic analytes from aqueous solution to gaseous phase), the eluent from the conductivity cell is combined with an organic desolvation solvent, delivered by an AXP-MS pump through a static mixing tee and a grounding union, prior to entering the ESI ionization source on the MS detector.
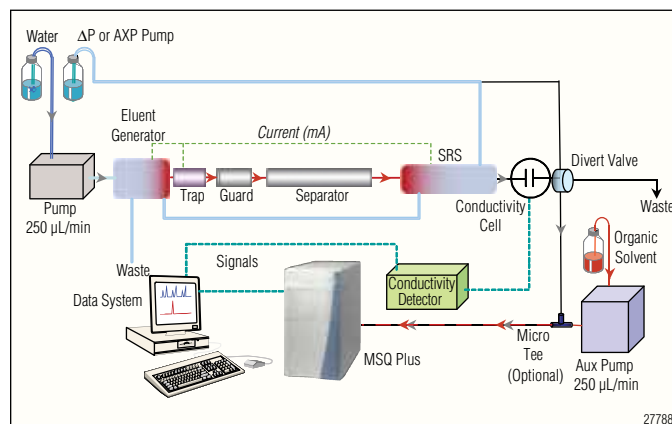

Figure 1. Schematic of an RFIC-MS system.

CONSIDERATIONS FOR IC-MS SYSTEM CONFIGURATION

Besides the general system considerations for any IC application—system backpressure, sufficient eluent degassing, minimizing extracolumn volume—additional considerations are also necessary.

Microbore System or Analytical System?

A microbore system is preferred for IC-MS applications due to its more compatible flow range to MS detection, improved sensitivity from relatively higher analyte concentration, less eluent consumption, and less generated waste. Comparable microbore-compatible consumables are highly recommended for IC-MS applications, such as 2 mm guard and analytical columns, suppressor, trap columns (CR-TC), and red connection tubing (0.005 in. i.d.) instead of black tubing (0.010 in. i.d.) for the connections prior to the suppressor.

Backpressure on Suppressor and Conductivity Cell

An additional backpressure of 30 to 40 psi is recommended for the proper functioning of suppressor and cell to prevent eluent in the cell from out-gassing due to abrupt volume changes. However, excessive backpressure may cause adverse effects such as peak tailing, broadening, and decrease in resolution due to the expanded flow path, and may cause irreversible damage to the suppressor hardware (the current SRS® suppressors are rated at 150 psi, and recommended to run at backpressures < 100 psi for IC-MS applications). Figure 2 shows the comparison of two chromatograms obtained with proper and excessive backpressure conditions. The chromatogram on the left demonstrates well-maintained chromatographic resolution, and sharp and symmetric peaks, while the right chromatogram demonstrates peak tailing and decreased resolution.
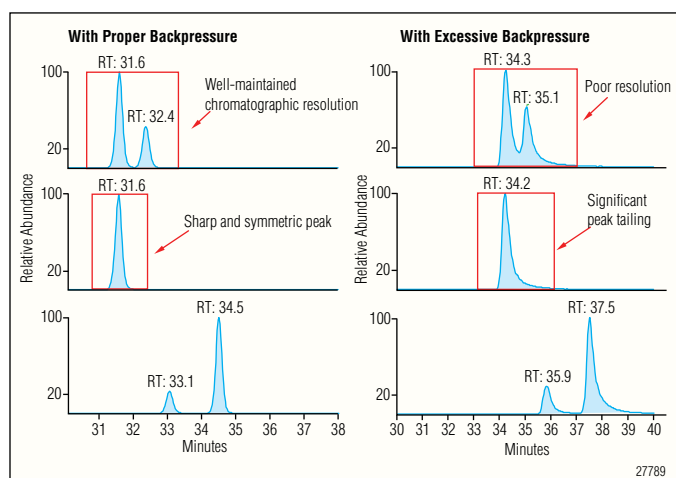

Figure 2. Effect of suppressor/cell backpressure on chromatographic performance.

Desolvation Solvent

Suppressed IC eluent is essentially 100% aqueous and requires a higher nebulizer gas volume and temperature for desolvation. Therefore, an organic solvent is usually used in IC-MS applications to improve the desolvation/ionization process. Different solvents may be used for this purpose, such as the frequently used HPLC solvents—acetonitrile, methanol, and isopropanol (IPA). These three solvents have been evaluated for various IC-MS desolvation applications and provided the following findings: 1) 100% organic solvent demonstrated the best signal improvements; 2) acetonitrile demonstrated the best improvements for anions and organic acids; and 3) isopropanol demonstrated the best signal improvements for cation applications.

As shown in Figure 3, lithium (Li) with isopropanol as the desolvation solvent demonstrated the cleanest adduct MS spectrum with the best intensity for the main adduct. The use of organic desolvation solvents improves the desolvation/ionization process and the detection sensitivity, and also forms adducts that facilitate the detection for some hard-to-analyze species, such as extremely low-mass analytes. Lithium ions are a singly charged species with a mass to charge (m/z) of 7, which is below the lowest calibrated mass on any MS detectors. With the addition of isopropanol, $[Li+nIPA]^+$ clusters were observed, i.e., $[Li+2IPA]^+$, $[Li+3IPA]^+$, $[Li+5IPA]^+$ with the observed m/z at 127, 187, and 307 respectively, which are within the calibrated mass range.
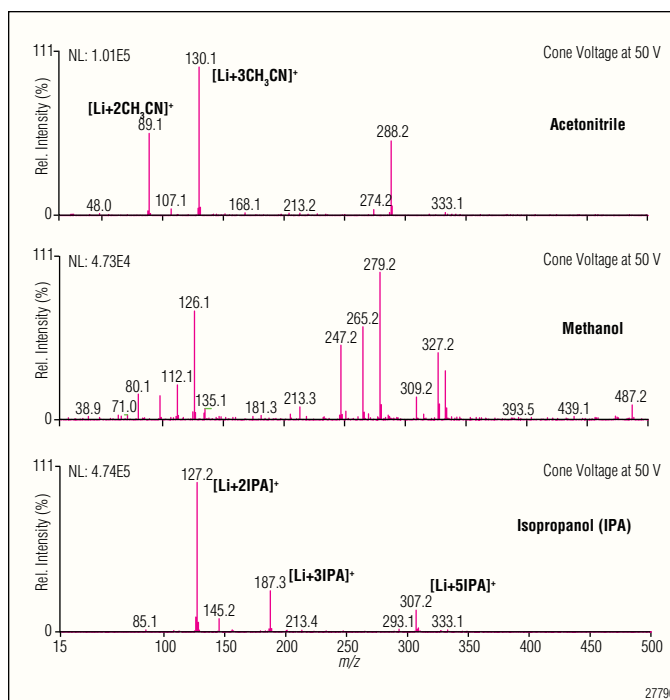

Figure 3. MS spectra of Li with different desolvation solvents.

Other Considerations

Other considerations are important for IC-MS configurations including 1) the grounding union between the conductivity cell and the high-voltage potential ESI probe to prevent possible current back-streaming if the connection tubing becomes filled with a conductive solution; 2) the connection tubing between IC and MS detectors must also be minimized in i.d. and length to reduce the extracolumn volume but with the total additional backpressure not exceeding 100 psi; and 3) the need for calibration of the MS detector while maintaining a high-transmission efficiency at low-mass ranges. The MSQ Plus™ Mass Spectrometer was used for most of the IC-MS applications presented here due to its native mass calibration and extremely high-mass transmission efficiency down to 17 m/z, thus making it suitable for high-sensitivity detection of low-mass analytes.

General Parameter Settings on the MSQ Plus Detector

Previously, the authors presented an example of systematic optimization of MSQ Plus parameters for anions and organic acids.[1] As suggested, general conditions can be used where sensitivity is not crucial or can be used as a starting point to further optimize system performance: when operating IC at 0.25 mL/min, the probe temperature is set between 400 ~ 450 °C; nebulizer gas at 85 psi; and CH_3CN is used as the desolvation solvent with a flow rate at 0.20 ~ 0.30 mL/min.

ADVANCES IN IC-MS APPLICATIONS

Many applications have been reported using IC-MS and MS/MS methods for unknown screening, identity confirmation, structure elucidation, and selective quantification. Here, the authors present applications from simple unknown identification with coeluting peaks to specific quantification of target analytes in a complex matrix. Figure 4 shows the identification of an unknown under the fluoride peak, observed erroneously as peak tailing in the conductivity trace. By using selective MS detection, the peak tailing is explained by the presence of co-eluted acetate. Figures 5 and 6 demonstrate IC-MS analyses for two widely used IC applications: anions and organic acids, and cations and amines, respectively. Inorganic species, small organic acids, and small amines can be detected with excellent sensitivity and selectivity, even for low-mass species such as potassium and chloride (39 and 35 m/z respectively).
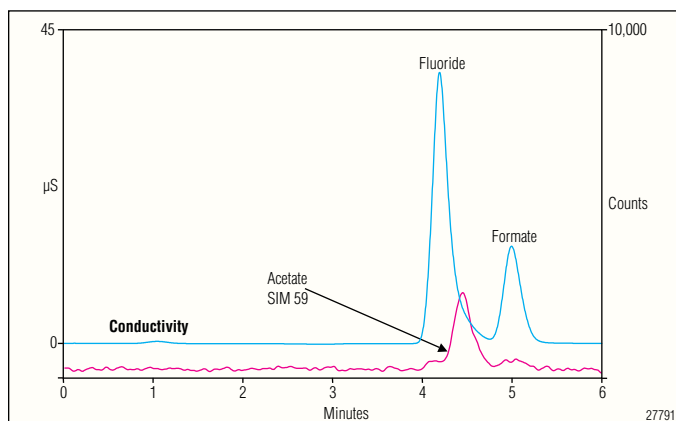

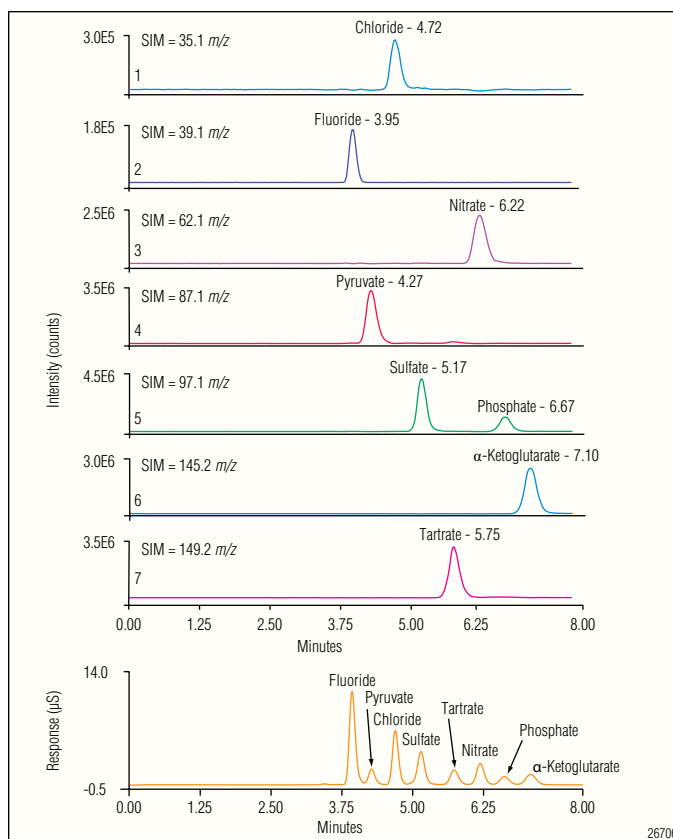Figure 4. Acetate found under fluoride peak using IC-MS.

Figure 5. Conductivity and SIM chromatograms of five common and three selected organic acids.
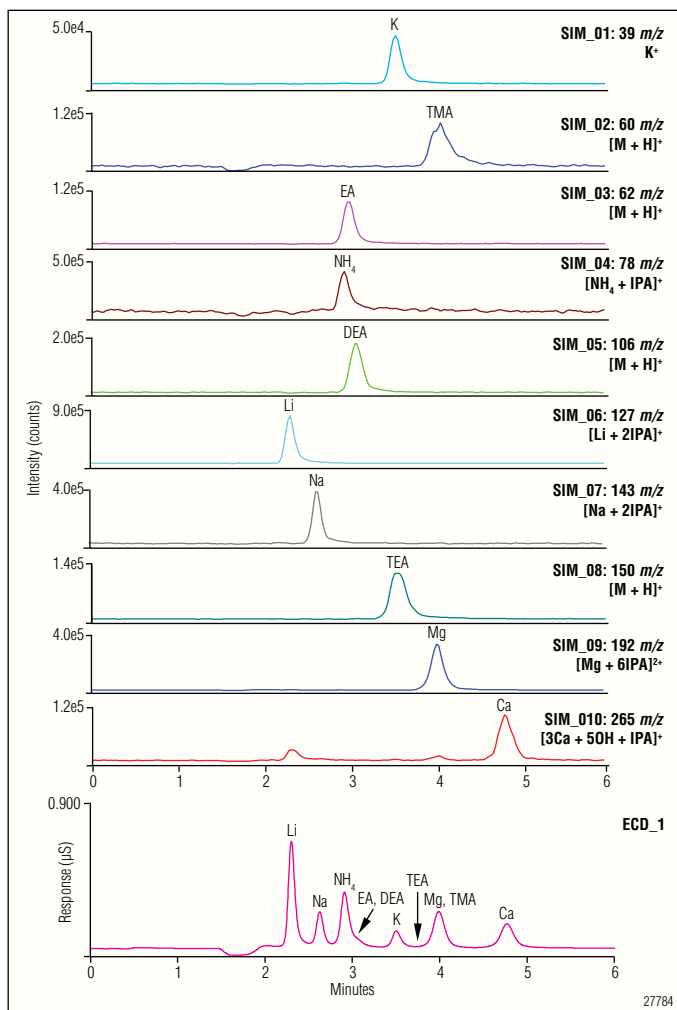
Figure 6. SIM and conductivity chromatograms of six cations and four amines.

Ion Chromatography Conditions

System:	ICS-3000 RFIC system
Column:	AS16 & AG16 Hydroxide
	Selective anion-exchange columns
	ASRS® 300 self-regenerated suppressor
	(external water mode)
Column Temp:	30 °C
Flow Rate:	300 µL/min
Inj. Volume:	100 µL
Mobile Phase:	Isocratic 45 mM hydroxide
	from EG-II KOH catridge
Solvent:	150 µL/min acetonitrile
	delivered by AXP-MS pump
Detection:	1st detector: Suppressed Conductivity
	2nd detector: TSQ Access Mass Spectrometer

Mass Spectrometric Conditions

System:	TSQ Quantum Access™
	Triple Quadrupole Mass Spectrometer
Interface:	Electrospray Ionization (ESI) Source
Spray Voltage:	4000 V
Sheath Gas:	40 arbitrary units
Auxiliary Gas:	5 arbitrary units
Iom Sweep Gas:	15 arbitrary units
Capillary Temp:	300 °C
Collision Gas:	Argon at 1.8 m Torr
Operating Mode:	Selected Reaction Monitoring (SRM)

Figure 7. SRM chromatograms for A) a standard prepared at 20 ppt; B) an infant formula sample quantified at 2.44 ppb; and C) a milk sample quantified at 4.65 ppb.

IC-MS methodology also has the additional benefit of simplifying sample preparation for ultratrace-level quantification in a complex matrix. As shown in Figure 7, perchlorate can be specifically detected at low ppt levels in infant formula and milk product with an isotope-labeled analogue as internal standard. IC-MS can improve the detection and quantification of inorganic species, and also ionic organic analytes. As seen in Figure 8, nitrogen mustard degradation products, such as ethanolamines, were separated from major cations and quantified by MS in Selected Ion Monitoring (SIM) mode. The IC-MS method also demonstrated excellent results on anionic organics such as organic acids (Figure 9).
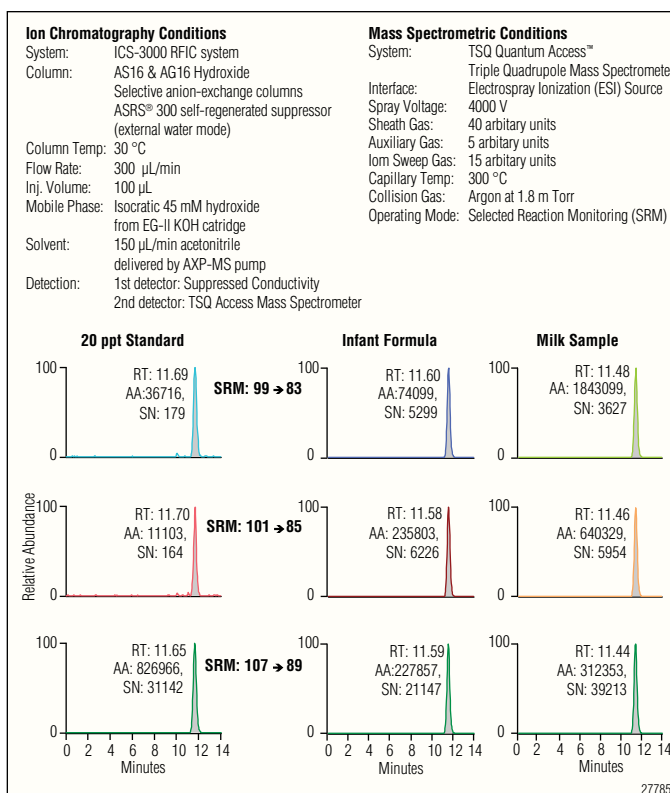
Chromatographic Conditions

System: ICS-2000 RFIC
Columns: IonPac CS15 & CG15 (2 mm)
 CR-CTC (2 mm)
Temperature: 40 °C
Mobile Phase: MSA from EGC II MSA cartridge

Time /min	MSA concentration
−4.0	2 mM
8.0	2 mM
18.0	30 mM
22.0	30 mM

Flow Rate: 0.40 mL/min
Injection: 20 µL
Detection: Conductivity, MSQ Plus

Mass Spectrometric Conditions

Interface: ESI positive
Desolvation Solvent: 0.2 mL isopropanol
Scan Mode: SIM
Needle Voltage: 3000 V
Probe Temperature: 450 °C
Nebulizer Gas: Nitrogen at 80 psi

Analytes:
DEA: Diethanolamine
MDEA: N-methyldiethanolamine
EDEA: N-ethyldiethanolamine
TEA: Triethanolamine

DEA – 11.7 SIM_01: 106 m/z
DEA-d_8 – 11.8 SIM_02: 114 m/z
MDEA – 12.5 SIM_03: 120 m/z
EDEA – 12.9 SIM_04: 134 m/z
TEA – 12.5 SIM_05: 150 m/z

Ethanolamines and Cations ECD_1

Figure 8. Ethanolamines by IC-MS.

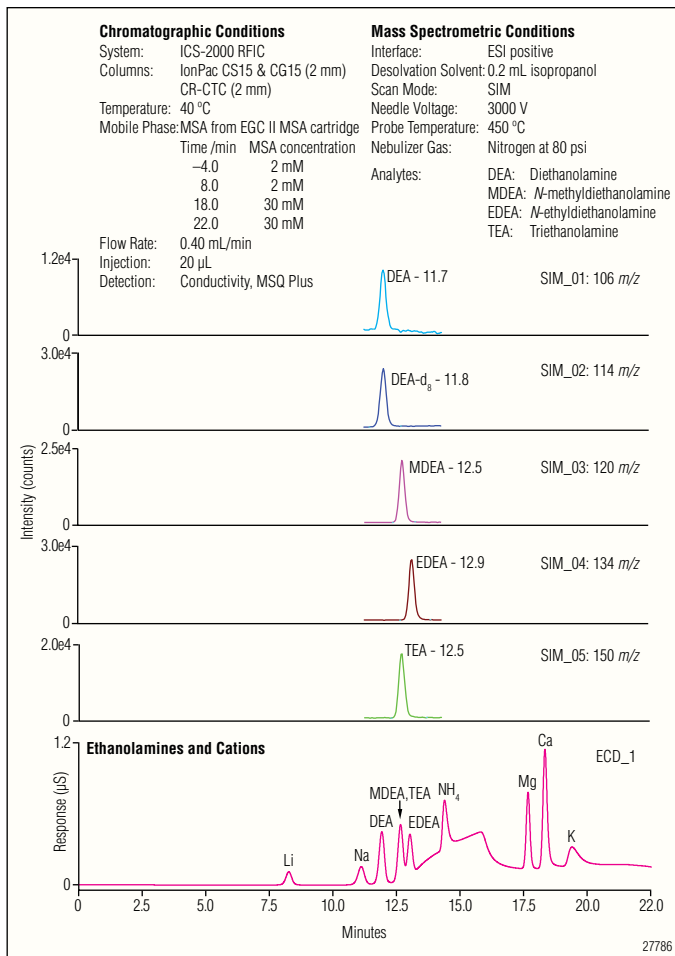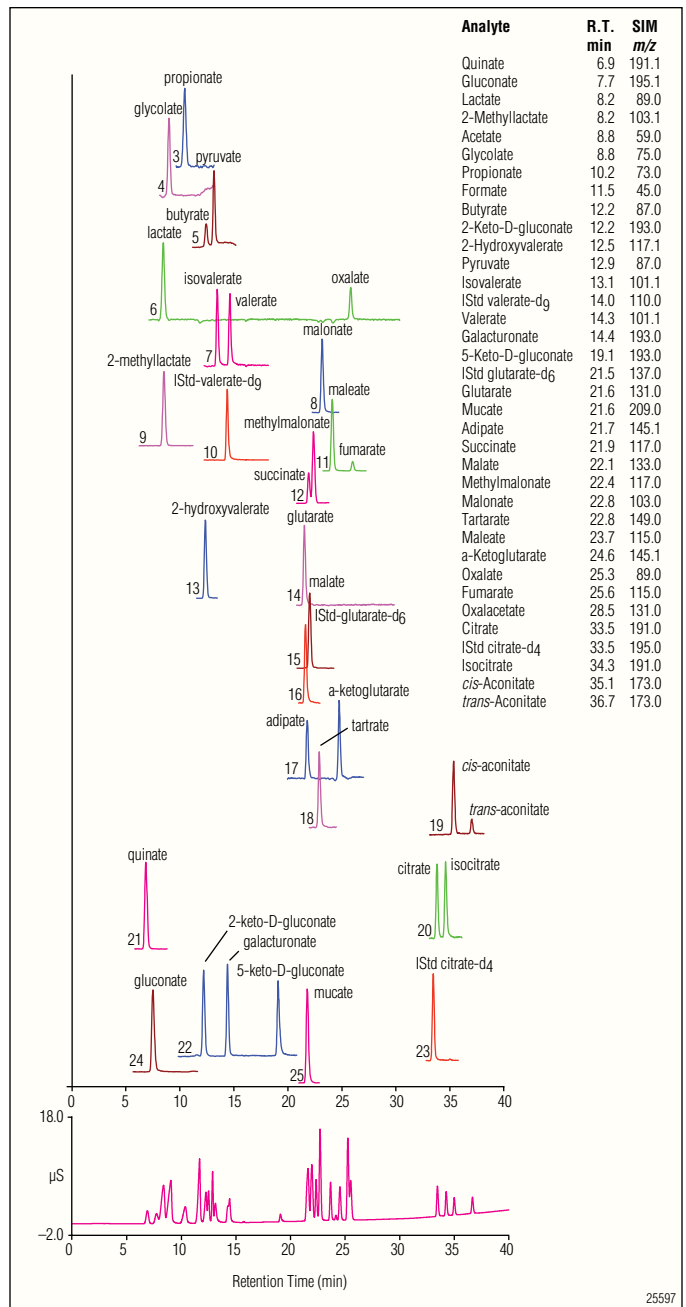| Analyte	R.T. min	SIM m/z
Quinate	6.9	191.1
Gluconate	7.7	195.1
Lactate	8.2	89.0
2-Methyllactate	8.2	103.1
Acetate	8.8	59.0
Glycolate	8.8	75.0
Propionate	10.2	73.0
Formate	11.5	45.0
Butyrate	12.2	87.0
2-Keto-D-gluconate	12.2	193.0
2-Hydroxyvalerate	12.5	117.1
Pyruvate	12.9	87.0
Isovalerate	13.1	101.1
IStd valerate-d_9	14.0	110.0
Valerate	14.3	101.1
Galacturonate	14.4	193.0
5-Keto-D-gluconate	19.1	193.0
IStd glutarate-d_6	21.5	137.0
Glutarate	21.6	131.0
Mucate	21.6	209.0
Adipate	21.7	145.1
Succinate	21.9	117.0
Malate	22.1	133.0
Methylmalonate	22.4	117.0
Malonate	22.8	103.0
Tartarate	22.8	149.0
Maleate	23.7	115.0
a-Ketoglutarate	24.6	145.1
Oxalate	25.3	89.0
Fumarate	25.6	115.0
Oxalacetate	28.5	131.0
Citrate	33.5	191.0
IStd citrate-d_4	33.5	195.0
Isocitrate	34.3	191.0
cis-Aconitate	35.1	173.0
$trans$-Aconitate	36.7	173.0

Figure 9. IC-MS for small organic acids.

CONCLUSION

Here, general concerns of IC-MS instrumentation are discussed and possible preferred configurations are presented. Typical applications using IC-MS methodology are also presented that demonstrate the benefits of identifying unknowns, sensitive and selective detection, simplified sample preparation, and accurate quantification by using isotope-labeled analogues as internal standards.

For more applications using IC-MS methods, refer to the Dionex MS Application Guide and application literature available at www.dionex.com.

REFERENCE

1. Wang J.; Schnute W.C. Optimizing Mass Spectrometric Detection for Ion Chromatographic Analysis. I. Common Anions and Selected Organic Acids. *Rapid Commun. Mass. Spectrom.* **2009,** *23* (21), 3439–47.

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