

# Capillary Ion Chromatography Mass Spectrometry: Recent Advances in Instrumentation and Applications

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## Introduction

Ion chromatography (IC) has been used extensively as a complimentary separation technique to HPLC. Recent applications include coupling to mass spectrometry (MS) for identity confirmation, structural interpretation, and trace level analysis in complex matrices. The introduction of innovative capillary IC further improves the usability of IC-MS for trace-level analysis and sample-size-limited research such as in metabolism/metabolomics. The main challenges of interfacing capillary IC and MS are compatibilities in mobile phase composition and flow rate. This study demonstrates the instrumentation and application solutions for capillary IC-MS utilizing a Thermo Scientific Dionex ICS-5000 Reagent-Free™ IC (RFIC™) system with suppression technology which converts the MS-incompatible mobile phase to deionized (DI) water, and a modified electrospray ionization (ESI) source which optimizes capillary flow sensitivity.

Using capillary IC with an RFIC system in conjunction with the optimized ESI source, this study demonstrates successful applications for simultaneous determination of active pharmaceutical ingredients (API) and impurities, metabolite profiling of organic acids, phosphate compounds in the energy cycle, as well as the profiling of nucleotides in biological samples.

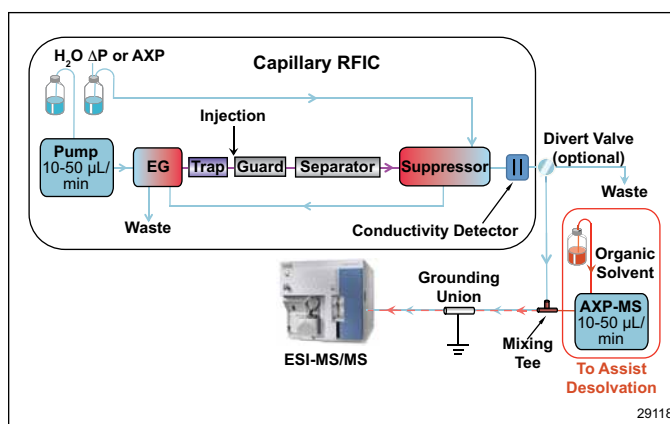
## Instrumentation

A Dionex ICS-5000 RFIC system was used in this study, consisting of an ICS-5000 SP Single Pump, an eluent generator (EG) module with an EGC-KOH (Capillary) Cartridge, and a DC Detector/Chromatography Compartment featuring an IC Cube™ with suppressed conductivity detection. The suppressor was operated in external-water mode with DI water regenerant delivered by an AXP-MS Auxiliary Pump at a flow rate of 50  $\mu\text{L}/\text{min}$ .

As seen in Figure 1, the eluent of the RFIC system conductivity detector was connected to a divert valve which directs the flow to waste or the MS detector flow path. Organic desolvation solvent was delivered by another AXP-MS Auxiliary Pump, combined with the chromatographic eluent via a micromixing tee, and passed through a grounding union before entering the MS detector via the optimized capillary ESI interface.

The detailed chromatographic and MS detection conditions are listed with each chromatogram.

FIGURE 1. Preferred capillary IC-MS system schematics.



## Capillary IC-MS Interface Optimization

The optimization of the capillary IC-MS interface was deemed to be a necessity and of paramount importance for system detection sensitivity. Commercially available interfaces can be divided into two categories based on compatible flow rate ranges: nano-ESI sources usually cover flow rates below 1  $\mu\text{L}/\text{min}$ ; analytical ESI interfaces are designed for a broad flow rate range of 1 to 2000  $\mu\text{L}/\text{min}$ , generally with higher performance in the range of 50 to 1000  $\mu\text{L}/\text{min}$ .

A Thermo Scientific Ion Max source was selected for this study as shown in Figure 2. The parameters related to sensitivity include: the diameter of the ESI spray capillary; the proximity of the spray capillary tip to the MS entrance (controlled by probe depth and probe x-y-z adjustments); the ESI voltage; vaporizer and transfer capillary temperatures; and gas flows including sheath, auxiliary and ion sweep gases.

**FIGURE 2. The Thermo Scientific Ion Max source interface and optimization parameters.**

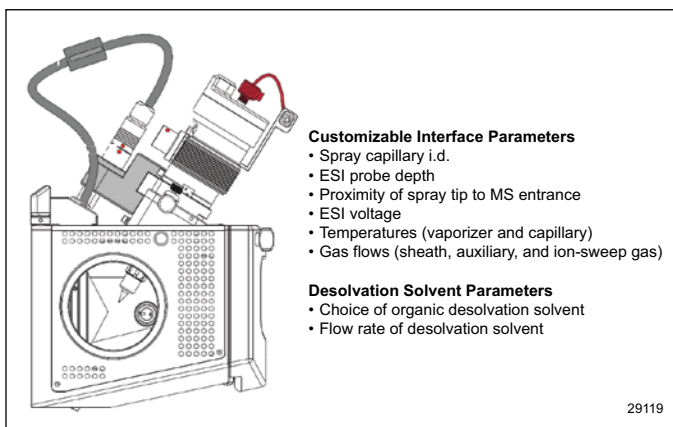
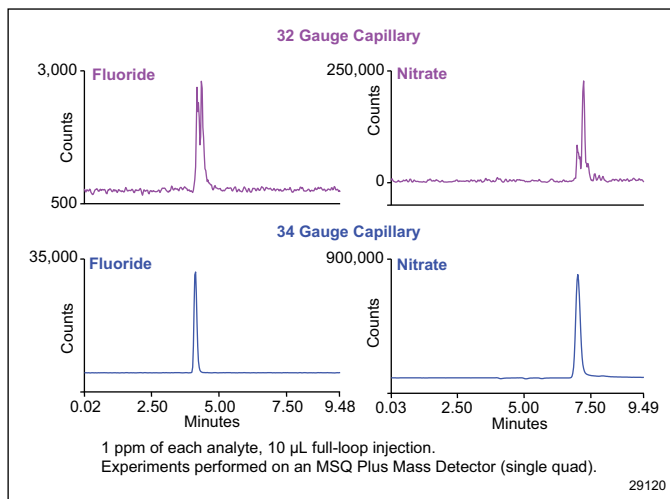


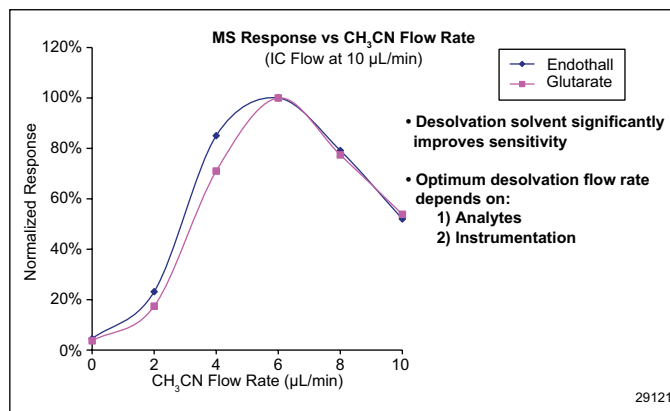
Figure 3 demonstrates the significant effect on MS sensitivity from the spray capillary internal diameter. The top chromatograms were obtained using a 32 gauge (0.235 mm) capillary, and the bottom chromatograms were obtained on a 34 gauge capillary (0.184 mm). With the same injection volume, significantly higher sensitivity can be achieved via the smaller internal diameter ESI capillary.

**FIGURE 3. Spray capillary i.d. affects sensitivity.**



The desolvation solvent parameters also need to be optimized for each application. The choice of organic solvent and the flow rate of its introduction were observed to be of particular importance in improving MS detection sensitivity. As seen in Figure 4, more than a ten-fold increase in MS response was achieved at the experimentally determined optimal flow rate of desolvation solvent (acetonitrile in this experiment).

**FIGURE 4. Desolvation solvent improves sensitivity.**



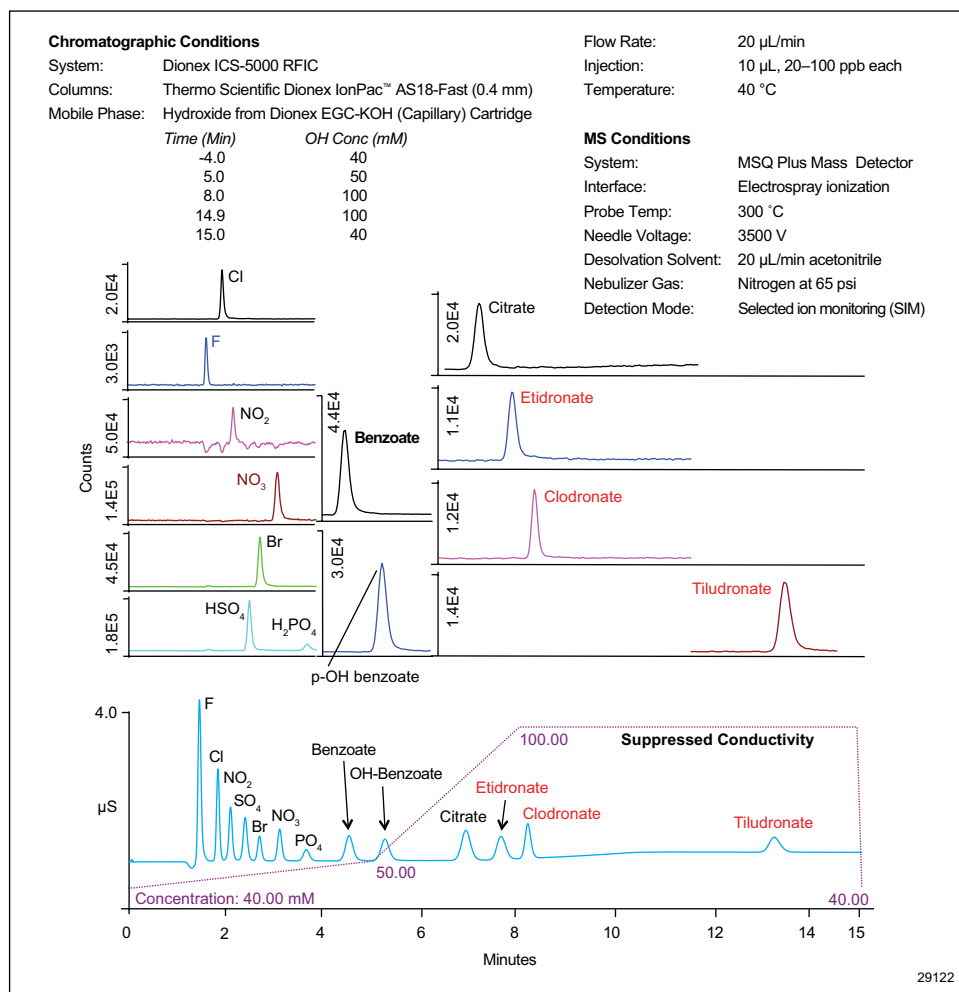
## Applications

### Bisphosphonates, Excipients and Impurities

Bisphosphonates are a group of compounds that are used as active pharmaceutical ingredients (APIs) to treat bone disorders. This application demonstrates simultaneous determination of bisphosphonate APIs, common excipients such as benzoate, hydroxybenzoate,

citrate, and anions such as phosphate which may be observed as impurities. Figure 5 shows the complete separation of seven common anions, three excipients, and three bisphosphonate APIs by capillary IC (as seen in the suppressed conductivity chromatogram), and the selective and sensitive detection using the Thermo Scientific MSQ Plus Mass Detector.

**FIGURE 5. Bisphosphonate APIs, excipients and impurities.**

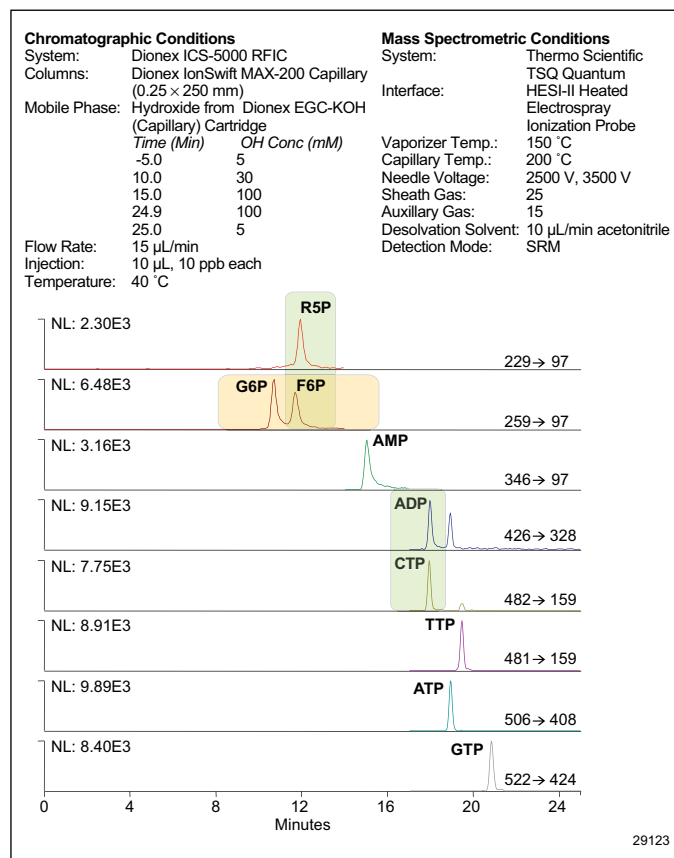


## Sugar and Energy Phosphates

Sugar phosphates such as fructose 6-phosphate (F6P), ribose 5-phosphate (R5P), and glucose 6-phosphate (G6P) are important metabolites, are highly polar, and thus may not be well retained and separated by reversed-phase chromatography. Triphosphate nucleotides are important energy compounds involved in energy cycles and have also been monitored as biomarkers in various research, such as that for cancer therapy. This application demonstrates the simultaneous quantitation of sugar and energy phosphates using capillary IC-MS/MS with separation achieved on a monolith capillary ion exchange column. As shown in Figure 6, the Thermo Scientific Dionex IonSwift MAX-200 Capillary Column provided all the necessary chromatographic resolution, as seen by the multiple peaks completely resolved in the individual selected reaction monitoring (SRM) channels. Additionally, MS/MS detection can easily differentiate closely eluting peak pairs such as R5P/F6P and ADP/CTP.

To establish the complete profile of nucleotide metabolites, another application was developed for the simultaneous quantitation of 19 nucleotides using isotope-labeled analogue as internal standards (as seen in Figure 7), including mono-, di- and tri-phosphates of adenosine, cytosine, guanosine, uridine, and deoxytriphosphate-nucleotides.

FIGURE 6. Sugar and energy phosphates.

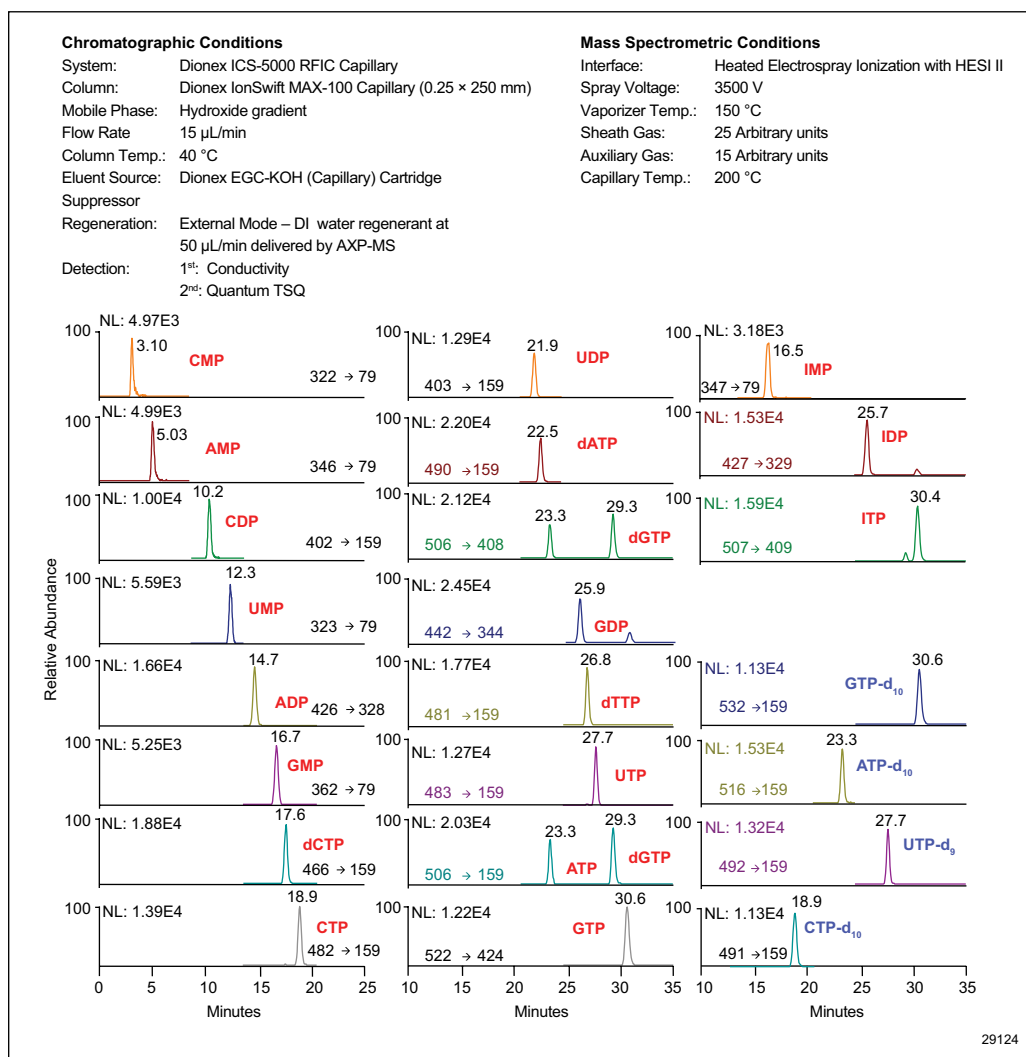


## Organic Acid Metabolites

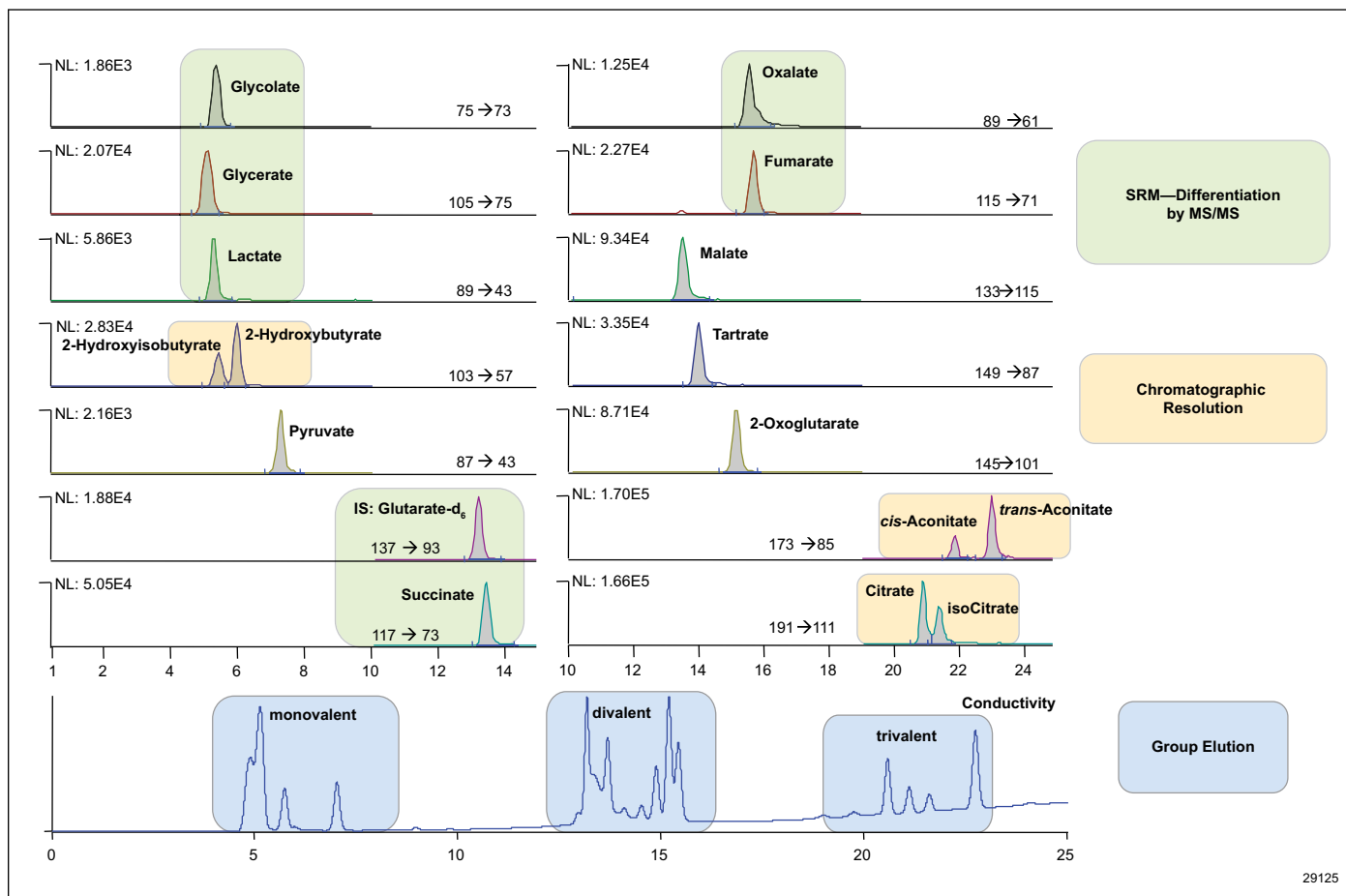
Organic acids are compounds of interests in many areas; many of them are metabolic intermediates and metabolites that have been monitored as biomarkers in clinical studies for diagnostic purposes. This application demonstrates the quantitative determination of selected organic acids involved in three metabolic cycles, including: oxalate metabolism (glyceric, glycolic, oxalic acids); glycolytic metabolism (lactic, pyruvic, 2-hydroxybutyric acid and its structurally similar analogue 2-hydroxyisobutyric acid); and citric acid

metabolism (succinic, fumaric, malic, 2-oxoglutaric, aconitic (cis- and trans-), citric and isocitric acids). As seen in Figure 7, all target analytes were substantially retained and eluted in groups based on their valences. Complete resolution by chromatography was not time efficient, thus a second dimension of selectivity was introduced by using MS/MS detection. As shown in Figure 8, closely eluted compounds, e.g., glycolate, glycerate, and lactate, were differentiated by MS/MS detection. In addition, all analytes sharing the same MS/MS transitions were chromatographically separated, thus ensuring quantitation accuracy.

**FIGURE 7. Profiling metabolic nucleotides by capillary IC-MS/MS.**



**FIGURE 8. SRM chromatograms of 16 organic acid metabolites and related compounds.**



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## Conclusion

This study describes a set of preferential instrumentation for capillary ion chromatography coupled with single and tandem mass spectrometry, and demonstrates the significant advantages of optimizing interface parameters to improve detection sensitivity. Analytical methods developed using this configuration showed successful application to pharmaceutical analysis, as well as for metabolite quantitation and profiling.

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