Applications of Capillary Ion Chromatography Mass Spectrometry to Metabolomics Research.

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Overview

Purpose:

To review considerations of capillary ion chromatography (Cap IC) mass spectrometry (MS) instrument configurations and to illustrate preferred setup. To demonstrate applications using Cap IC-MS for metabolite(omics) research.

Methods:

A capillary reagent free IC (RFIC) system was used to perform ion exchange chromatographic separation on a packed or monolithic capillary column, with electrolytically generated hydroxide gradient mobile phase. A capillary suppressor was used to convert the non-volatile mobile phase to water thus compatible with MS detection. Targeted metabolite quantitation was achieved using selective and sensitive SRM MS/MS mode acquisition with isotope labeled internal standard (when applicable) for quantitation accuracy.

Results:

Preferred Cap IC-MS configuration is illustrated. This setup was applied to

- Metabolic profiling of 19 nucleotides
- Quantitation of isobaric sugar phosphates (trehalose 6-phosphate and
- sucrose 6-phosphate)
- Targeted organic acid metabolites in oxalate, glycolytic and citric acid metabolism cycles;

Introduction

Ion chromatography (IC) has been used extensively as a complimentary separation technique to HPLC, and recent applications include coupling to mass spectrometry (MS) for identity confirmation, structural interpretation, and trace level analysis in complex matrices. With its unique selectivity, IC has been successfully applied to the identification and quantification of targeted and untargeted charged metabolites, such as organic acids, sugar phosphates, and nucleotides in biological samples. Capillary IC- MS (Cap IC-MS) furthers the capability of IC with respect to metabolite identification and quantitation by improving the system sensitivity and stability as well as reducing the amount of sample required.

In this study, we demonstrate the application to the targeted analysis of organic acid metabolites, quantitation of isobaric sugar phosphates and the metabolic profiling of 19 nucleotides. Experiments were performed on a reagent free IC (RFIC) system using hydroxide gradient as mobile phase, which was then post-column suppressed to water by electrolyticaly removing potassium ions and neutralizing the eluent which was then MS compatible. Separations were achieved on capillary format packed (0.4 mm ID) or monolith (0.25 mm ID) ion exchange columns, and the MS/MS detector was operated in selected reaction monitoring (SRM) mode for selective and sensitive quantitation.

Using this configuration, targeted metabolites can be quantified at fmol levels with a small number of µL sample consumption, and linearity can be maintained over two orders of magnitude.

Methods

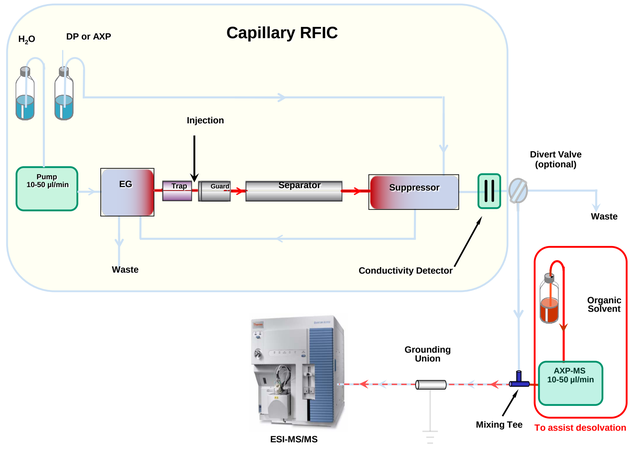
Instrumentation

A Thermo Scientific Dionex ICS-5000 RFIC chromatography system was used in this study consisting of a capillary pump, an eluent generator (EG) with capillary KOH cartridge, and a detection compartment (DC) featuring a capillary IC module with suppressed conductivity detection. The suppressor was operated in external-water mode with DI water regenerant delivered by an AXP-MS pump at a flow rate of 30 µL/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 pump and combined with the chromatographic eluent via a micro mixing 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 Cap IC-MS System Schematics



Optimization of Cap IC-MS Interface

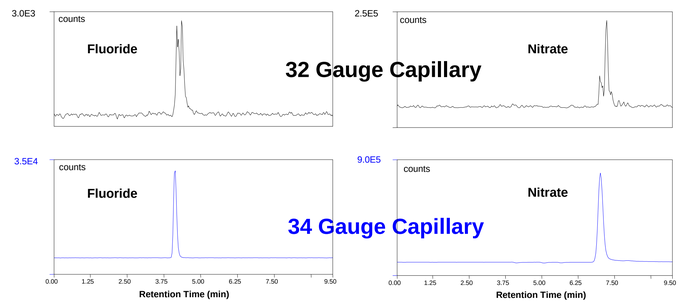
The optimization of the Cap IC-MS interface was deemed to be a necessity and of paramount importance for system detection sensitivity.

For the IonMax electrospray (ESI) source used in this study, the parameters related to sensitivity include: the diameter of the ESI spray capillary, the proximity of the capillary spray 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 and Figure 3 demonstrate spray capillary ID and flow rate of the desolvation solvent significantly affect sensitivity.

As shown in Figure 2, the top chromatograms were obtained using a 32 gauge (100 µm) capillary and the bottom chromatograms were obtained on a 34 gauge capillary (75 µm). With the same injection volume, significantly higher sensitivity can be achieved via the smaller internal diameter ESI capillary.

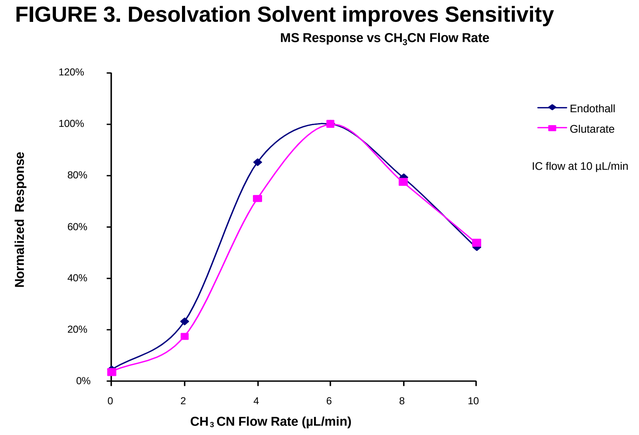
FIGURE 2. Spray Capillary ID Affects Sensitivity



1 ppm of each analyte,10 µL full-loop injection. Experiments performed on a single quad mass spectrometer.

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 3, more than a 10-fold increase in MS response was achieved at the experimentally determined optimal flow rate of desolvation solvent (acetonitrile in this experiment).

FIGURE 3. Desolvation Solvent improves Sensitivity



Applications

Metabolic Profiling of 19 Nucleotides (Mono-, Di- and Tri-phosphates)

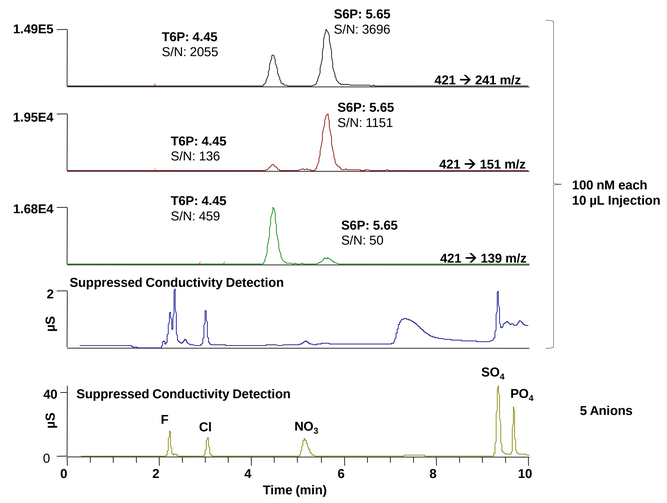
Nucleotides are essential compounds active in many cell functions. In recent years, there have been extensive studies of using nucleoside analogs as prodrugs in anti-cancer, anti-viral and immunosuppressive therapy, and monitoring of their activated nucleotide metabolites is of paramount importance to understand the pharmacology. This application demonstrate s metabolic profiling of 19 nucleotides.
As seen in Figure 4, target nucleotides were chromatographically resolved within 35 minutes at a flow rate of 15 µL/min. Two SRM transitions were used for quantitation and confirmation for each analyte. The chromatographic separation was essential to eliminate the SRM interferences from structurally related analytes, e.g. ADP and ATP. Detection limits were achieved at 1 nM with 5 µL injection (5 fmol on column) for all analytes

Quantitation of Isobaric Sugar Phosphate Metabolites

Trehalose 6-phosphate (T6P) is an intermediate in trehalose production pathway and is recognized as an important signaling molecule that regulates starch synthesis. T6P and sucrose 6-phosphate (S6P) are isobaric compounds with similar structure thus require chromatographic separation for accurate quantitation.

As seen in Figure 5, fast separation of target sugar phosphates was achieved on a MAX-100 capillary monolith column within 6 minutes. And sensitive quantitation can be achieved down to 10 femtomole.

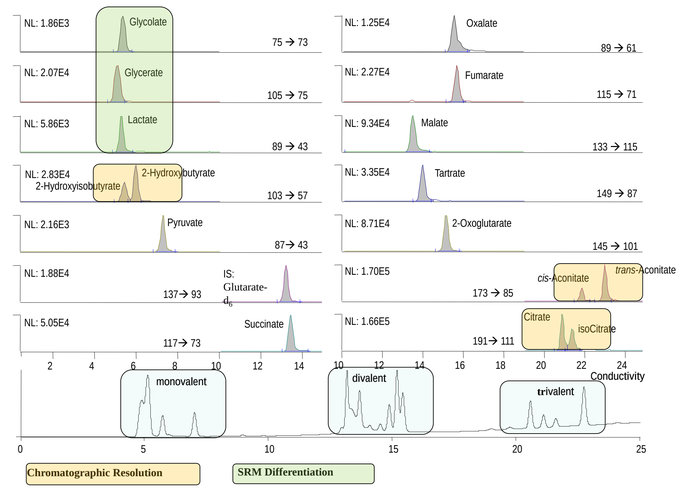
FIGURE 5. Quantitation of Isobaric Sugar Phosphates



Quantitation of Targeted Organic Acid Metabolites in Oxalate, Glycolytic and Citric Acid Metabolism Cycles

Organic acids are compounds of interests in many areas; many of them are metabolic intermediates and metabolites and 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.

FIGURE 6. Profiling Organic Acid Metabolites



As seen in Figure 6, 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. 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.

Conclusion

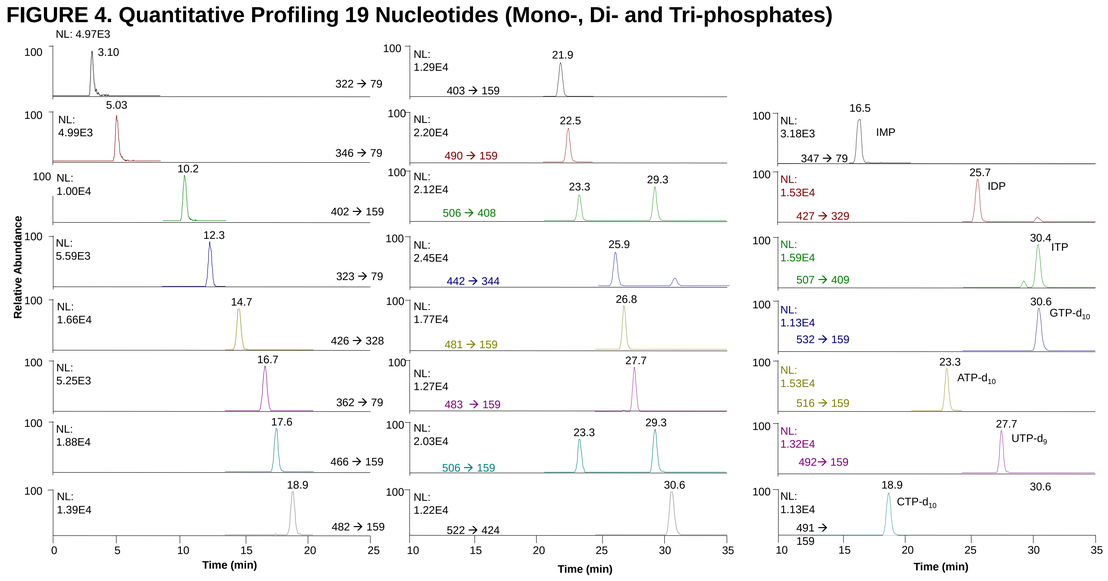
This study illustrated the preferred Cap IC-MS configuration. Using this setup, successful applications are demonstrated including

- Metabolic profiling 19 nucleotides (mono-, di- and tri-phosphates);
- Quantitation of isobaric sugar phosphate metabolites
- Quantitation of targeted organic acid metabolites

Cap IC-MS offers unique chromatographic selectivity for polar metabolites and combines selective and sensitive SRM detection, ensuring low nM quantitation limit.

Cap IC-MS can be used as complimentary technique to reversed phase LC-MS to solve analytical challenges.

FIGURE 4. Quantitative Profiling 19 Nucleotides (Mono-, Di- and Tri-phosphates)



Chromatographic Conditions

System:	Dionex ICS5000 Capillary Ion chromatography
Column:	MAX-100 Monolith Capillary Column (0.2 × 250 mm)
Column Temp.:	40 °C
Flow Rate	15 µL/min
Mobile Phase:	Hydroxide gradient
Eluent Source:	Eluent Generator with KOH Capillary cartridge
Detection:	1st: Suppressed Conductivity External Mode with DI water as regenerant at 50 µL/min delivered by AXP-MS pump 2nd: Quantum TSQ Access MAX

Mass Spectrometric Conditions

System:	Quantum TSQ Access MAX
Interface:	Heated Electrospray Ionization with HESI II probe
Spray Voltage:	3500 V
Vaporizer Temp.:	150 °C
Sheath Gas:	25 Arbitrary Units
Auxiliary Gas:	15 Arbitrary Units
Capillary Temp.:	200 °C

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