Applications of Capillary Ion Chromatography Mass Spectrometry to Metabolomics Research.

Leo Jinyuan Wang¹, William C. Schnute¹, Cees Bruggink²

¹Thermo Fisher Scientific, San Jose, CA, USA, ²Thermo Fisher Scientific, Amsterdam, The Netherlands

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 metabolomic 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, sugars, 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 electrolytically 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 cartridges, 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 regenent 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 mixer before passing 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.

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 demonstrates a metabolic profiling of 19 nucleotides. As seen in Figures 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 analogs, e.g. ADP and ATP. Detection limits were achieved at 1 fmol with 5 µL injection (5fmol 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. 76P 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 8 minutes. And sensitive quantitation can be achieved down to 10 femtomole.

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.