High-throughput Targeted Metabolomic Analysis Using Automated Column Switching and Ion Chromatography with HR/AM Mass Spectrometry

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Overview

Purpose: Demonstrate 9-min targeted analysis by IC-HR/AM using automated column switching.

Methods: Polar metabolites were determined using ion chromatography coupled with high resolution accurate mass spectrometry (IC-HR/AM) facilitated by column switching using the dual IC system configuration. On System 1 (Sys 1) the sample was injected, the compounds were separated, and directed to the conductivity detector (CD), desalting suppressor, and MS detector. The alternate column was washed and equilibrated by Sys 2. Valve 2 controls which column is inline: Mode 1: Column 1 is using the separation conditions, Column 2 is in wash/equilibration. Mode 2: switches the column positions.

Results: To further improve the throughput of the previous 20-min run, we have developed a multiplexing method capable of alternating two columns and performing sequential injections of samples. A short gradient of 9-min run time has been achieved using the new platform and good resolution was maintained for most isomeric metabolites.

Introduction

Analysis of small polar metabolites is critical to understanding many metabolic disturbances, such as oral cancer. Recently it has been shown that ion chromatography (IC) when combined with high resolution accurate mass spectroscopy (HR/AM) can provide superior separations and sensitivity for polar ionic species as compared to other separation methods.[1–3] These results have been demonstrated using a capillary IC (45-min run)[1] and replicated using a higher throughput method (20-min run)[2,3] on an analytical IC system. Here we use column switching to facilitate higher throughput.

Methods

Sample Preparation

Oral cancer lysate samples were prepared and provided by Professor Shen Hu and discussed thoroughly in Application Note AN622[3].

Ion Chromatography

Instrument

Thermo Scientific™ Dionex™ ICS-5000⁺ HPIC™ dual IC system with Thermo Scientific Dionex AS-AP Autosampler. Configured for 2 mm column applications using two valves: one 6-port and one 10-port.

Conditions

Column	Thermo Scientific™ Dionex™ IonPac™ AS11HC-4µm guard and separation, 2 mm i.d
Gradient	Sys 1 Separation, 25–99 mM KOH in 9 min Sys 2 Wash/Equilibration, 99 mM KOH for 4 min, 99–25 mM KOH for 5 min
Eluent Source	Thermo Scientific Dionex EGC 500 KOH Cartridge with Thermo Scientific Dionex CR-ATC 500 Anion Trap Column
Flow	0.38 mL/min
Temp	30 °C
Inj. Vol	2 µL of 5 µL loop
Desalter	Thermo Scientific™ Dionex™ AERS™ 500 Anion Electrolytically Regenerated Suppressor in external water mode (Thermo Scientific AXP-MS Pump at 0.8 mL/min)
Makeup Solvent	Methanol at 0.06 mL/min (AXP-MS pump) to low volume mixing tee (Idex, P/N P-890). See Figure 1.

Mass Spectrometry

Instrument

High field Thermo Scientific™ Q Exactive HF™ Quadrupole-Orbitrap mass spectrometer

Thermo SCIENTIFIC

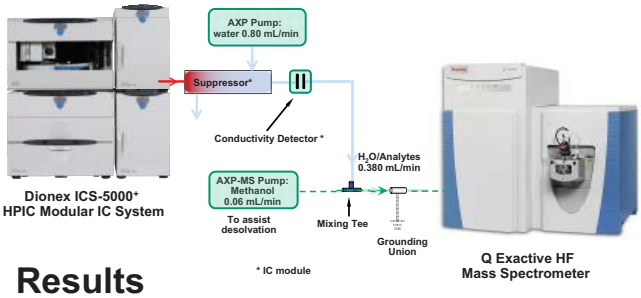
Conditions
ESI negative mode, resolution setting: 15,000–240,000, Full mass scan: m/z 67-1000, resolution: 120 000, automatic gain control (AGC) target: $1x10^6$ ions, maximum ion injection time (IT): 100 ms.

Source ionization parameters: spray voltage: 3.5kV; transfer temp.: 320 °C; S-Lens level: 50; heater temp.: 325 °C; Sheath gas: 36; Aux gas: 5.

Software and Data Analysis
The programming is managed by new interface software (Thermo Scientific™ Standard Instrument Integration (SII)) for Xcalibur coupling Thermo Scientific™ Xcalibur™ and Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System, version 7.)

Differential analysis of profiling data was performed using Thermo Scientific™ SIEVE 2.2. Targeted analysis of TCA compounds was performed using Thermo Scientific™ Tracefinder™ 3.2.

FIGURE 1. Flow diagram of Dionex ICS-5000⁺ HPIC dual IC system to Q Exactive HF MS.



Results

Configuring the IC for Column Switching

The Dionex ICS-5000⁺ HPIC is a dual system with two pumps, eluent generator cartridges, and traps. System 1 controls the separation conditions and Valve 1; System 2 controls the wash/equilibration conditions. Valve 2 controls which column is inline. In Mode 1 (Fig. 2) and Mode 2 (Fig. 3), Column 1 and Column 2 are performing separation.

FIGURE 2. Column switching configuration. Valve 2, mode 1: Column 1 is inline with separation conditions.

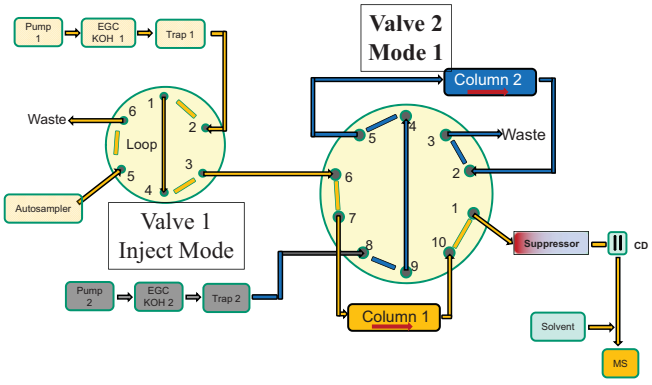


FIGURE 3. Column switching mode 2.

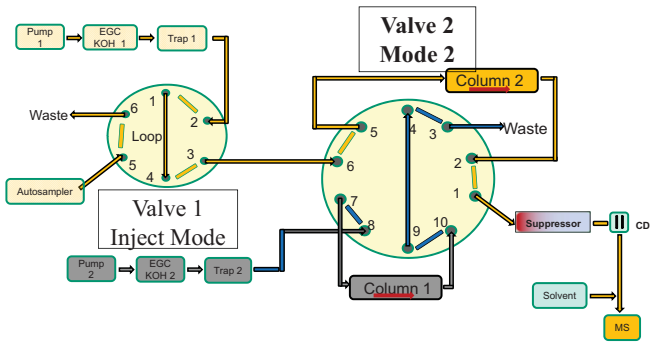


Figure 4 and Table 1 show the conditional programming embedded in the SII-Chromeleon instrument method to initiate column switching. A benign parameter (delay time for leak sensor) was used to define which column was previously used.

FIGURE 4. Multiplexing program using "If, then" Commands.

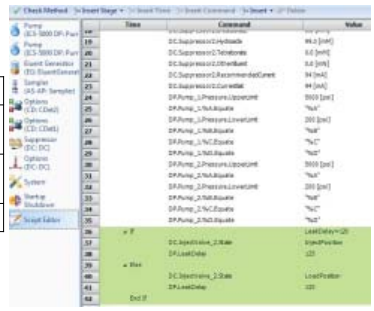


Table 1. "If then" Commands

Conditional	Action
Leak Delay = 120	Switch to Column A, Set leak delay to 125
Leak Delay = 125	Switch to Column B, Set leak delay to 125
Other	Set Leak Delay = 120

To assess the 9-min gradient, the responses of the six stable isotopes (Table 2) were comparable to the previous results using the 20-min gradient[3] (Figure 5). The results were comparable.

Table 2. Stable Isotope Standards

#	Metabolite Name	Formula	Obs. *m/z*	Ion
1	Sodium pyruvate (13C3, 99%)	[13]C3H4O3	90.0188	[M-H]-
2	Succinic acid (13C4, 99%))	[13]C4H6O4	121.0328	[M-H]-
3	Malic acid (13C4, 99%)	[13]C4H6O5	137.0277	[M-H]-
4	alpha-ketoglutaric acid (13C5, 99%)	[13]C5H6O5	150.0310	[M-H]-
5	Fumaric acid (13C4, 99%)	[13]C4H4O4	119.0172	[M-H]-
6	Citric acid (2,2,4,4-D4, 98%)	C6H4[2]H4O7	195.0449	[M-H]-

FIGURE 5. Response of stable isotope standards at 20-min and 9-min gradient conditions.

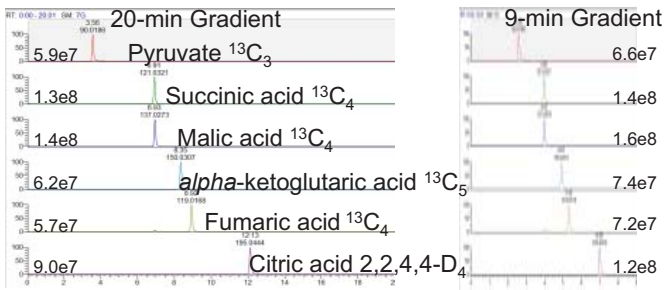


Figure 6 the chromatograms of the stable isotopic standards on both columns alternating in column switching mode. The average run time is 11 min with the 9.1 min gradient.

FIGURE 6. Stable isotopic standards running in column switching mode.

Column 1
•New
•5-cm guard column

Column 2
- 1 year old
- No guard column

Figure 7 compares the difficult separations of mono- and di-phosphate sugars using both gradients. There is resolution loss of 2–3 isomers with the shorter gradient. Figure 8 compares the resolution of fructose 6-phosphate (F6P) from glucose 6-phosphate (G6P), citrate from isocitrate, and *cis*- from *trans*- aconitate.

FIGURE 7. Resolution of mono- and di-phosphate sugars in oral cancer cell lysate samples.

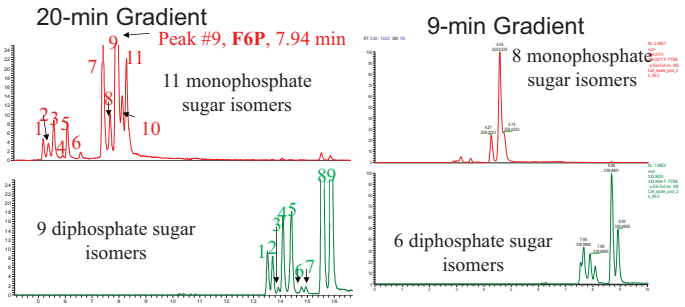
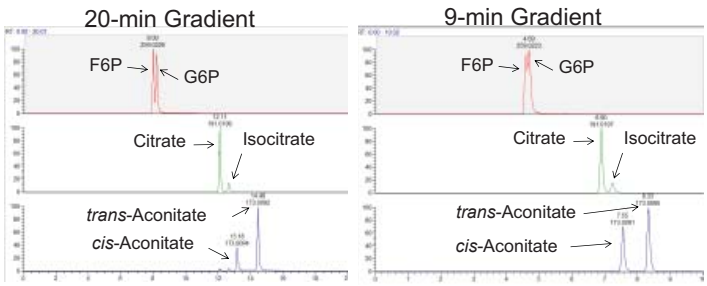


FIGURE 8. The resolution of isobaric compounds using the 9-min and 20-min gradients.



Conclusion

- IC coupled with HR/AM of Q Exactive Orbitrap instruments provides a superior method to resolve small polar metabolites.
- For higher throughput runs,
  - The separation method was reduced from 20 (plus 3 min equilibration time) to 9 min
  - The dual capabilities of the Dionex ICS-5000⁺ HPIC system enabled column switching by directing one column for separation and the second column for conditioning aided by SII software conditional programming.

References

1. Wang, J., Christison, T.; et al. *Anal. Chem.*, **2014**, *86 (10)*, 5116–5124.
2. Hu, Shen; Wang, J., et al. *Anal. Chem.*, **2015** DOI:10.1021/acs.analchem.5b01350
3. Wang, J.; Christison, et al. AN622. Thermo Fisher Scientific 2015.

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