Waters™

Note d'application

Evaluation of Ion Mobility/Tof Mass Spectrometry with Multiple LC Method Parameters for Enhanced Detection in Metabolic Profiling

Paul D. Rainville, Ian D. Wilson, Robert S. Plumb

Waters Corporation, Division of Computational and Systems Medicine, Department of Surgery and Cancer, Imperial College, London UK

Abstract

This application note investigates the metabotyping of human urine by integrating IMS with gradient reversed-phase UPLC as a means of enhancing “peak recovery”.

The incorporation of ion mobility as a separation modality between LC separations and MS detection significantly increases the number of features detected in metabolic phenotyping.

The reason(s) for the observed increase in feature detection warrants further investigation but is most likely due to a combination of the separation of co-eluting compounds, noise reduction, resolution of isobaric components and separation of fragment ions. Therefore the incorporation of IMS with shorter LC gradient times may provide a means for the rapid profiling of large sample cohorts on metabonomics studies.

Benefits

- Rapid omics profiling
- Greater feature coverage
- Faster analysis

Introduction

The use of metabolic phenotyping (metabonomics/metabolomics) to discover biomarkers of organismal response to environmental and physiological change is now widespread. In biomedical applications, metabolic phenotyping, or metabotyping,[1,2] is being deployed as a method for finding novel, mechanistic, biomarkers of disease with obvious potential for improving diagnosis, patient stratification, and both predicting and monitoring patient response to therapy.

In liquid chromatography/mass spectrometry (LC-MS)-based phenotyping, the need for rapid and efficient high-throughput analysis often requires compromises to be made between speed and metabolome coverage. As the separation time is reduced to increase throughput, ion suppression (due to peaks co-elution) increases, reducing the number of features detected.[3,4]

One potential means of maximizing metabolite detection without increasing analysis time is to employ ion mobility spectrometry (IMS) prior to MS detection in a hyphenated UPLC-IM-MS system. The ion mobility

separation is performed post ionization in the vacuum region of the mass spectrometer and has a rapid time scale, typically in the 10s of milliseconds range. This makes such a configuration ideal for coupling between UPLC-based separations, with peaks eluting over a few seconds, and ToF mass spectrometry which operates on a microsecond time scale. The use of the collision cross-section (CCS) within the mass spectrometer allows analytes of interest to be separated and detected even in the presence of a co-eluting isobaric species. This orthogonal separation therefore provides an increase in peak capacity.

Here we describe the results of the investigation of the effect of integrating IMS with gradient reversed-phase UPLC as a means of enhancing "peak recovery" for the metabotyping of human urine.

Experimental

LC conditions

LC system:	ACQUITY UPLC I-Class
Detection:	MS
Column:	ACQUITY UPLC HSS T3 2.1 x 150 mm, 2.1 x 75 mm, or 2.1 x 30 mm
Column temp.:	40 °C
Sample temp.:	10 °C
Injection volume :	2 µL
Flow rate:	600 µL/min
Mobile phase A:	Aqueous formic acid (0.1% v/v)
Mobile phase B:	Acetonitrile, formic acid (0.1% v/v)

MS conditions

MS system:	Synapt G2-S*i* operating in MS^E or $HDMS^E$ mode
Ionization mode:	ESI positive ion
Acquisition range:	100–1200 *m/z*
Capillary voltage:	2.5 kV
Collision energy:	5–40 eV
Cone voltage:	30 V

Informatics

MassLynx Software with Progenesis QI Software

Method conditions

Column length and gradient duration:

150 mm column:	Initial hold at 2% B for 1 minute followed by a linear gradient of 2–15% B over 3 minutes; raise to 50% B at 9.0 minutes; raise to 95% at 15 minutes; hold for 1 minute and return to initial conditions.
75 mm column:	Initial hold at 2% B for 0.5 minute followed by a linear gradient of 2–15% B over 1.5 minutes; raise to 50% B at 4.5 minutes; raise to 95% at 7.5 minutes; hold for 1.0 minute and return to initial conditions.
30 mm column:	Initial hold at 2% B for 0.3 minute followed by a linear gradient of 2–15% B over 0.6 minutes;

	raise to 50% B at 2.2 minutes; raise to 95% at 3 minutes; hold for 1.0 minute and return to initial conditions.

In addition, a further analysis was run at accelerated linear velocity of 800 µL/min on the 75 mm column length under the following gradient conditions:

75 mm column:	Initial hold at 2% B for 0.3 minutes followed by a linear gradient of 2–15% B over 0.6 minutes; raise to 50% B at 2.2 minutes; raise to 95% at 3 minutes; hold for 1.0 minute and return to initial conditions.

Sample preparation

Human urine was collected and immediately frozen at -80 °C. Aliquots were then prepared by diluting 1:4 with water followed by centrifugation at 15,000 relative centrifugal force (RCF) for 5 minutes. The supernatant was then collected for injection onto the LC-MS system.

Results and Discussion

Effects of column length and gradient duration on feature detection in UPLC-MS

In this study, the effect of LC column length and gradient duration on feature detection in metabonomics study was first determined using human urine from 6 volunteers. Chromatographic analysis of the urine using a 15 cm length column enabled the detection of over 16,000 features (Table 1) in positive ion electrospray ionization (ESI) mode (Figure 1). The base peak ion (BPI) chromatogram of the urine analysis shows that the component peaks were well distributed across the first 10 min of the analysis.

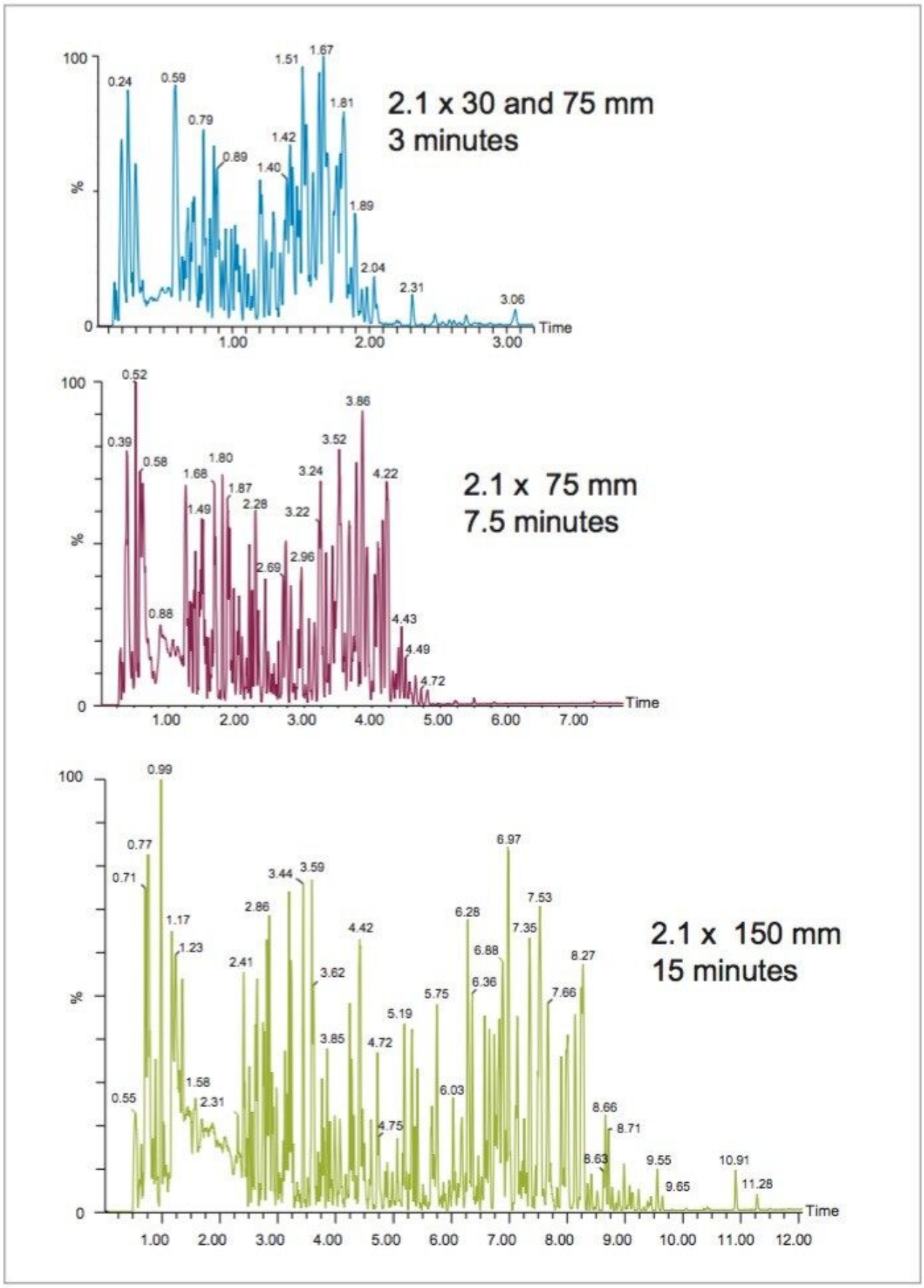

Figure 1. Analysis of human urine using gradient durations of 15, 7.5, or 3 minutes and column lengths of 150,

The relationship between the number of features detected and the chromatographic conditions, with respect

The number of features detected in the urine sample varied depending on the length of the column and corr

2. Gavaghan C.L.; Wilson, I.D.; Nicholson, J.K. *FEBS Lett*., 2002, 530, 191–196.

3. Wilson, I.D.; Nicholson, J.K.; Castro-Perez, J.; Granger, J.H.; Johnson, K.A.; Smith, B.W.; Plumb, R.S. *J Proteome Res*. 2005 4, 591–598.

   J Proteome Res. 2005 4, 591–598.

4. Gray, N.; Adesina-Georgiadis, K.; Chekmeneva, E.; Plumb, R.S.; Wilson, I.D.; Nicholson, J.K. *Anal. Chem*. 2016, 88, 5742–5751.

5. Plumb, R.S.; Granger, J.H.; Stump, C.L.; Johnson, K.; Smith, B.W.; Gaulitz, S.; Wilson, I.D.; Castro-Perez, *J. Analyst*, 2005, 130, 844–849.

6. Paglia, G.; Williams, J.P.; Menikarachchi, L.; Thompson, J.W.; Tyldesley-Worster, R.; Halldorsson, S.; Rolfsson, O.; Moseley, A.; Grant, D.; Langridge, J.; Palsson, B.O.; Astarita, G. *Anal. Chem*. 2014, 86, 3985–399.

Featured Products

ACQUITY UPLC I-Class PLUS System <https://www.waters.com/134613317>

SYNAPT G2-Si High Definition Mass Spectrometry <https://www.waters.com/134740622>

Progenesis QI <https://www.waters.com/134790652>

720005933, March 2017

©2019 Waters Corporation. All Rights Reserved.