Nota de aplicación

Comparison of the SYNAPT XS™ and SELECT SERIES™ Cyclic™ IMS for the Analysis of Human Urine

Adam King, Lee A. Gethings, Robert S. Plumb

Waters Corporation

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

Untargeted analysis in metabolomic studies involve the analysis of complex biological matrices which contain thousands of compounds. Ultimately, analysis of these matrices relies on the capacity of the analytical instrument to detect as many features as possible to determine potential biomarkers. Advances in liquid chromatography (LC) separation and hyphenated mass spectrometers (MS) incorporating orthogonal separation techniques such as ion mobility (IM), have assisted in increasing the number of detected features.

Benefits

Improved IMS separation, feature detection, and spectral quality

Introduction

The hyphenation of separation techniques increases the resolution between compounds, enhancing spectral quality, in turn, improving compound annotation and identification. Previous studies[1] have demonstrated the

benefits of implementing IM into the LC-MS workflow for metabolomic studies, improving peak capacity and providing the ability to achieve high throughput without sacrificing performance (i.e., feature detection and identification confidence).[2] Further advancements in ion mobility capability of performance with the SELECT SERIES Cyclic IMS mass spectrometer, has allowed the possibility of increasing this analyte resolution further with multipass IMS capabilities. In this mode the ions are subjected to several passes of the ion mobility device, significantly increasing the separation path length. However, even when performing a single pass IM separation, the mobility path on the SELECT SERIES Cyclic IMS instrument is almost double the length of the SYNAPT XS ion mobility separation cell, thereby significantly improving the ion mobility resolution which can be achieved. In this application brief, we compare and highlight the benefits that the SELECT SERIES Cyclic IMS provides to the analysis of metabolomic samples of human urine.

Experimental

All chromatographic separations were performed using an ACQUITY™ Premier UPLC™ System configured with an ACQUITY Premier BEH™ Amide Column. Mobile phase and gradient conditions are outlined in Table 1. Mass spectrometer source conditions and Time-of-flight (Tof) acquisition settings were maintained for both instruments (SYNAPT XS and SELECT SERIES Cyclic IMS) and are summarized in Table 2. Ion mobility separation conditions were maintained at the default settings respectively for each instrument.

A single human urine sample (20 µL) was prepared by adding 30 µL of LC-MS grade water and 350 µL of LC-MS grade acetonitrile. The sample was then shaken for ten minutes prior to centrifugation at 13,000 rpm. The supernatant was then transferred to a Waters™ total recovery vial for analysis.

Table 1. UPLC Method Parameters

System:	ACQUITY Premier
Mobile phase A:	5:95 acetonitrile:water 0.1% formic acid, 10 mM ammonium formate
Mobile phase B:	95:5 acetonitrile:water 0.1% formic acid, 10 mM

	ammonium formate
Seal wash:	10% Isopropanol in water
Weak wash:	80:20 (v/v) Water/acetonitrile
Strong wash:	Isopropanol
Lockspray:	Leucine enkephalin 200 pg/µL (50:50 water:acetonitrile)
Column:	Waters ACQUITY Premier UPLC BEH Amide, 1.7 µm, 2.1 x 100 mm
Column temperature:	40 °C
Injection volume:	2 µL
Autosampler temperature:	8 °C

Gradient Table

Gradient	Time (mins)	Flow (mL/min)	%A	%B	Curve
1	Initial	0.700	0	100	Initial
2	0.1	0.700	0	100	6
3	5.0	0.700	20	80	6
4	6.0	0.700	50	50	6
5	6.5	0.700	50	50	6
6	7.0	0.700	0	100	6
7	10	0.700	0	100	6

Table 2. MS Parameters for SYNAPT XS and SELECT SERIES Cyclic IMS

Capillary voltage:	2.0 kv
ESI polarity:	Positive
Sampling cone:	30 V
Source offset:	80 V
Source temperature:	120 °C
Desolvation temperature:	600 °C
Cone gas flow:	50 L/hr
Desolvation flow:	800 L/hr
Quad profile:	Auto
Lockspray flow:	10 µL/min
Analyzer mode:	Resolution
Tof mass range:	50–1200 Da
Scan time:	0.3 seconds
Data format:	Continuum
Acquisition mode:	HDMS^{E} ™
Collision energy:	Transfer cell ramp: 20–40 eV

Results and Discussion

Feature Detection

All MS data collected from both the SYNAPT XS and the SELECT SERIES Cyclic IMS were imported separately into Progenesis™ QI where the repeat injections of extracted urine (n=3) were aligned and subject to peak picking using the same algorithm. Upon importing the raw data into Progenesis QI, the software determined the number of low energy ions detected from each imported injection. Figure 1 shows that the SELECT SERIES Cyclic IMS allowed the detection of just over 3.5 times more low energy ions on average when compared to the same sample analysed using the SYNAPT XS. The peak picking performed by Progenesis QI produced a list of features (*m/z* and retention time pairs) for all peaks that were detected across all imported sample analyses. The bar chart below (Figure 1), additionally, shows that the SELECT SERIES Cyclic IMS enabled Progenesis QI to detect approximately 50% more features than were detected from the datafiles generated using the SYNAPT XS.

Figure 1. Comparison of the average number of peak-picked features and detected ions between the SYNAPT XS

and SELECT SERIES Cyclic IMS.

Mobility Separation

All data were acquired with ion mobility enabled using the HDMS^E acquisition mode to provide the best separation of precursor ions and to also produce fragment ion information. The addition of this mobility separation can increase the overall peak capacity of the analysis, where previously co-eluting LC features can be resolved from one another in the mobility cell. Figure 2 highlights this improvement where two drift time separated features of the same *m/z* and retention time, once separated, can produce different spectra that otherwise would have been difficult to resolve and accurately assign fragment ions to precursors.

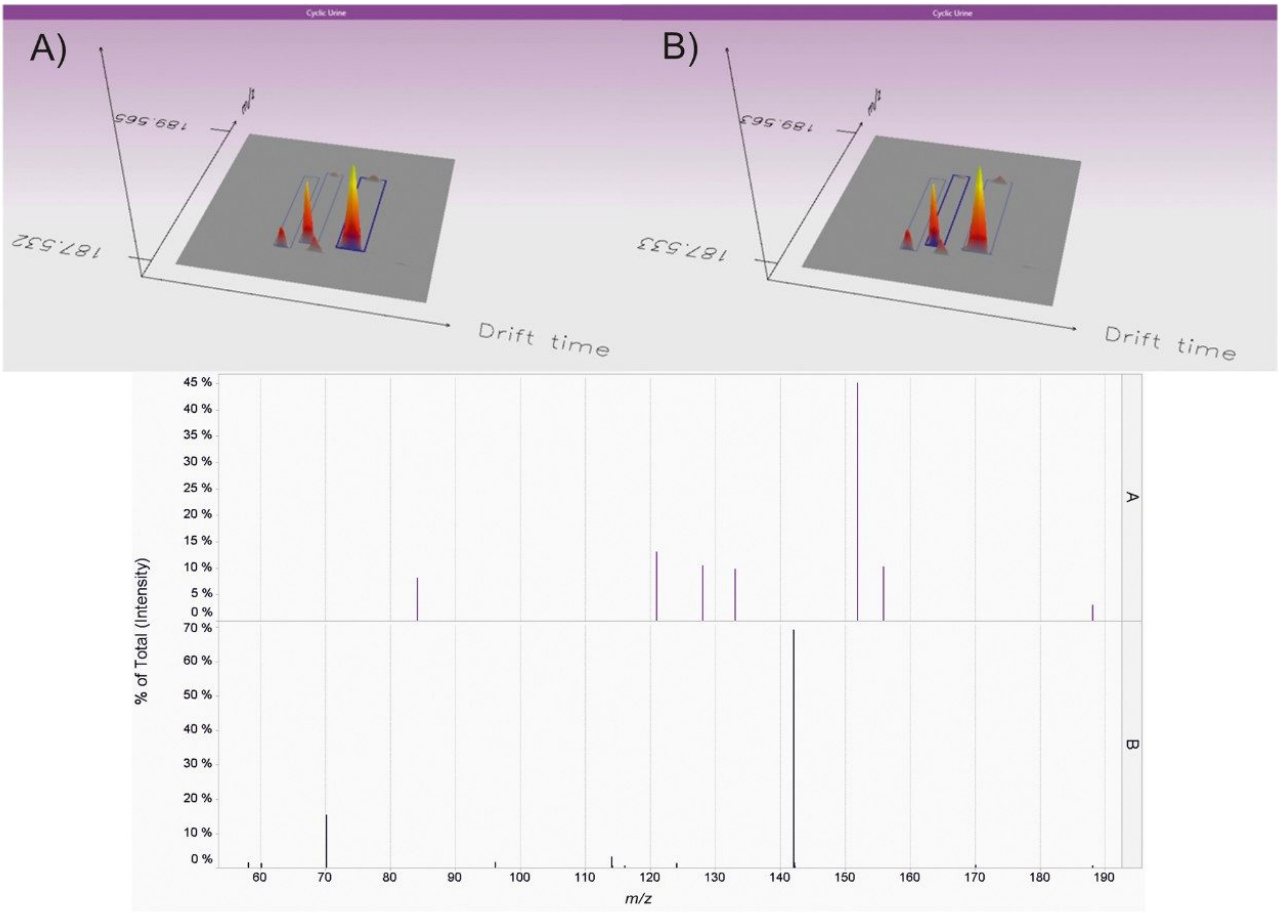

Figure 2. Three-dimensional drift time plot highlighting two features separated by a single pass with the SELECT SERIES Cyclic ion mobility with each feature's corresponding high energy fragmentation spectra. Selected features in the three-dimensional plots are outlined in the dark blue box.

Database Anotation

To investigate the benefit that the increased number and drift time separated features had, these were searched against the HMDB database using their precursor mass and theoretical fragmentation, both set to a tolerance of +/- 10 ppm. For the urine data obtained from the SYNAPT XS, 1450 features returned a possible annotation from the database query out of 2363 detected features. Comparatively, the results from the SELECT SERIES Cyclic IMS analysis produced possible annotations for 944 more features than that annotated on the SYNAPT XS when using the same database and search criteria (Figure 3).

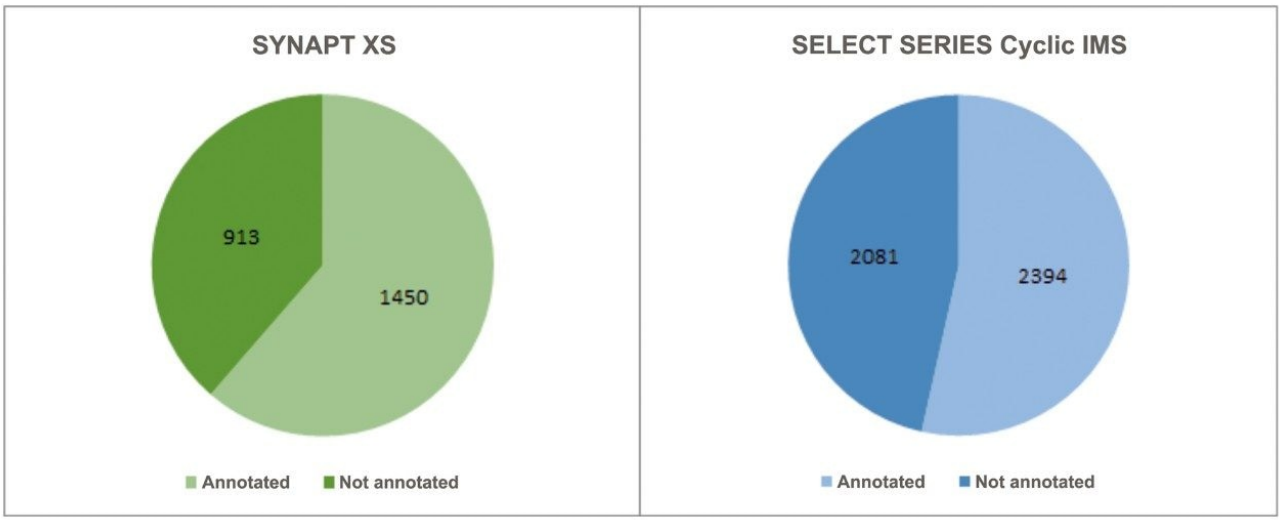

Figure 3. Pie charts outlining the number of features with tentative database annotations and those features without for both the SYNAPT XS and SELECT SERIES Cyclic IMS.

Conclusion

With the SELECT SERIES Cyclic IMS possessing a longer ion mobility cell, this provided improved ion separation due to the enhanced mobility resolution and thereby increased peak capacity. This contributed to the number of ions and peak picked features almost doubling when compared to the SYNAPT XS. This increase in feature detection and drift time separation improved the spectral quality of the feature fragment ions and ultimately increased the total number of database annotations possible. Thus, increasing the confidence and accuracy of eventual biomarker identification.

References

1. Rainville, P.D., *et al.*, Ion Mobility Spectrometry Combined With Ultra Performance Liquid Chromatography/Mass Spectrometry for Metabolic Phenotyping of Urine: Effects of Column Length, Gradient

Duration and Ion Mobility Spectrometry on Metabolite Detection. *Anal Chim Acta*, 2017. 982: p. 1–8.

2. King, A.M., *et al*., Rapid Profiling Method for the Analysis of Lipids in Human Plasma Using Ion Mobility-enabled Reversed- phase Ultra-high Performance Liquid Chromatography/Mass Spectrometry. *J Chromatogr A*, 2020. 1611: p. 460597.

Featured Products

ACQUITY Premier System <https://www.waters.com/waters/nav.htm?cid=135077739>

SELECT SERIES Cyclic IMS <https://www.waters.com/waters/nav.htm?cid=135021297>

SYNAPT XS High Resolution Mass Spectrometer <https://www.waters.com/waters/nav.htm?cid=135020928>

waters_connect <https://www.waters.com/waters/nav.htm?cid=135040165>

720007537, February 2022