EVALUATION OF ION MOBILITY/ TOF MASS SPECTROMETRY WITH MULTIPLE LC METHOD PARAMETERS FOR ENHANCED DETECTION IN METABOLIC PROFILING

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INTRODUCTION

The aim of this study was to evaluate ion mobility spectrometry (IMS) coupled with rapid high resolution LC/MS and determine the impact upon metabolome and lipidome coverage. Metabolic profiling can provide a comprehensive and quantitative view of the metabolite variation in biological systems. The total number of metabolites can vary from 600 for simple yeast cells to over 200,000 metabolites for higher order organisms. Due to this complexity it is very challenging to establish analytical profiling methodologies that can isolate, identify, and quantify analytes in a high throughput environment. IMS offers an extra dimension of separation that when coupled with LC/MS increases the overall peak capacity for metabolic profiling.

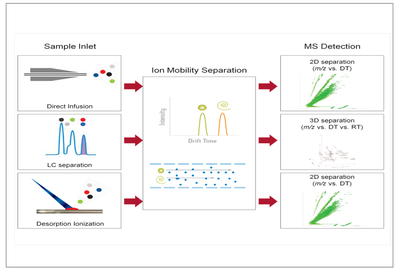


Figure 1. IMS offers an additional separation mode that can be utilized with direct infusion, LC separations , and with desorption ionization techniques.

METHODS

Mid-polar Analytes from Human Urine

Chromatography was carried out on a Waters ACQUITY UPLC
Mobile Phase A: 0.1 % formic acid in water
Mobile Phase B: 0.1 % formic acid in acetonitrile
Flow rate: 500 µL/minute
Columns: ACQUITY UPLC HSS T3 2.1 x 30, 75, and 150 mm
Column Temperature: 35 ° C
Gradient Elution Profile for 150 mm column:

Minutes	% A	% B
0	98	2
1	98	2
3	85	15
9	50	50
15	5	95

Injection Volume: 2 µL
* gradient durations and profiles for the 30 and 75 mm columns were geometrically scaled from the 150 mm length column

Lipid Analytes extracted from Human Plasma

Mobile Phase A: 0.1 % formic acid and 10 mM ammonium formate in 60 / 40 acetonitrile / water
Mobile Phase B: 0.1 % formic acid and 10 mM ammonium formate in 90 / 10 isopropanol / acetonitrile
Flow rate: 500 µL/minute
Columns: ACQUITY UPLC CSH C18 2.1 x 30, 75, and 150 mm
Column Temperature: 65 ° C
Gradient Elution Profile for 150 mm column:

Minutes	% A	% B
0	60	40
2	47	43
2.1	50	50
12	46	54
12.1	30	70
18	1	99

Injection Volume: 10 µL
* gradient durations and profiles for the 30 and 75 mm columns were geometrically scaled from the 150 mm length column

Mass Spectrometry

Mass spectrometry was carried out on a Synapt G2 Si operating in positive ESI mode with MSe and HDMSe acquisition modes. The following parameters were set on the tune page: capillary voltage, 2.0 kV, sampling cone voltage, 40, source offset , 80, source temperature, 120 ° C, desolvation temperature 550 ° C, Desolvation gas flow 1000 L/h and cone gas 20 L/h.

Sample preparation

Human urine was collected and immediately frozen at -80 °C. Aliqouts 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. Lipids from human plasma where extracted via the addition of acetonitrile to plasma (2 : 1) samples where then centrifuged in the same manner as with the human urine samples and the supernatant was then collected for injection onto the LC/MS system.

RESULTS AND DISCUSSION

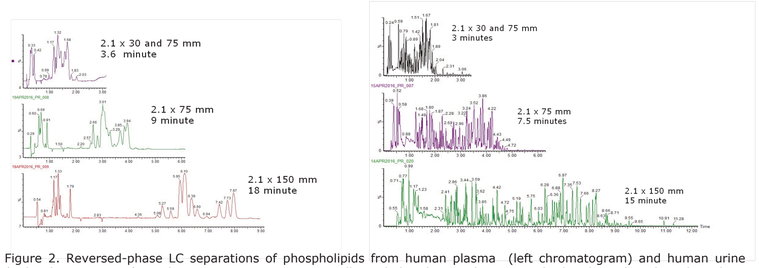


Figure 2. Reversed-phase LC separations of phospholipids from human plasma (left chromatogram) and human urine (right chromatogram). Each separation was geometrically scaled with consideration to both column length and gradient volumes to ensure consistent LC separation conditions were maintained while reducing the time of analysis.

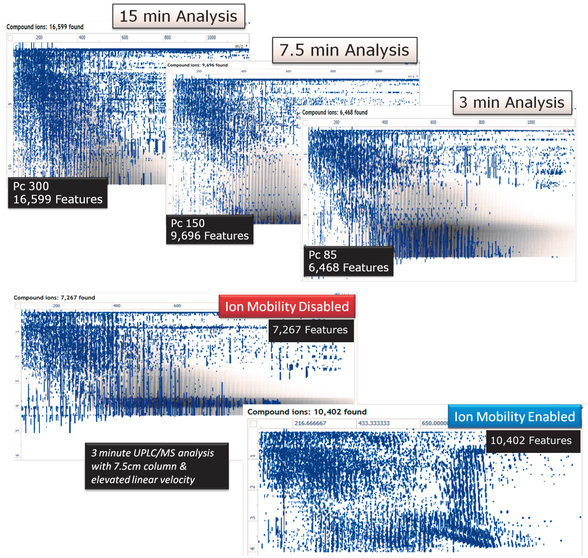
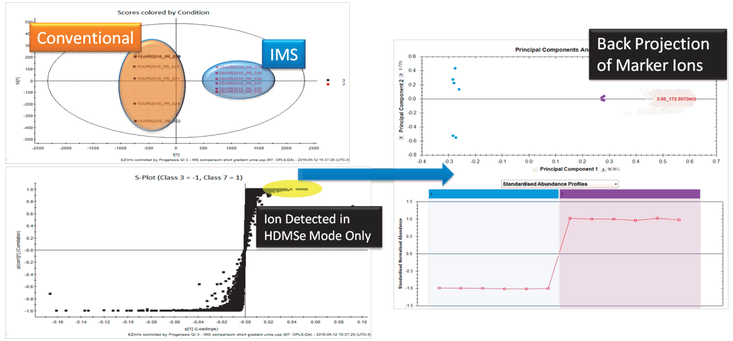


Figure 3. (Top) Comparison of features detected from a conventional LC/MS separation of human urine under 15, 7.5 and 3 minute gradients. This data shows the overall loss in chromatographic peak capacity, from 300 to 85 and thus compound resolution as the gradient time is reduced. However, when ion mobility is utilized as part of separation method (bottom) the overall detected features can increase. In this example, features detected increased to 10402 features from 7267 for a 3 minute separation of human urine when IMS was utilized as part of the separation method. Similar results were obtained for the separation of phospholipids from human plasma albeit the features detected were not as high due to the abundance of the analytes in the sample . Data processed with Progenesis QI software.



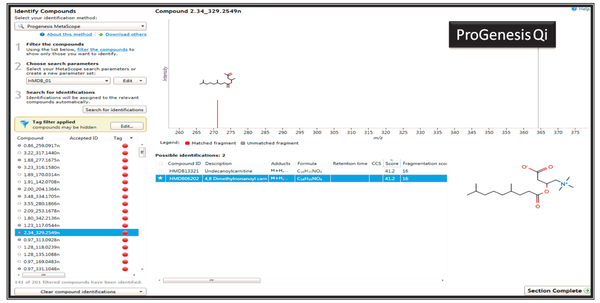


Figure 4. Scores plot from principle component analysis (PCA) of conventional LC/MS separation versus LC/MS separation with IMS. S plot highlights ions of particular interest detected (yellow highlighted area) with IMS but not with the conventional LC/MS separation. This result is further shown in the abundance plot as an individual feature is shown as detected for six consecutive injections of human urine with IMS enables but not detected for six repetitive injections of the same sample. The possible identification of this feature or analyte is further proposed by Progenesis QI.

CONCLUSION

- Chromatographic peak capacity and thus resolution of analytes can be compromised when reducing the overall gradient duration of an analysis.
- The use of addition separation techniques such as ion mobility mass spectrometry can increase the detection of analytes found in complex biofluid samples.
- The combination of compressed LC gradients with ion mobility may enable the reduction in overall analysis time in metabolic profiling studies and thus expedite the analysis of large cohorts.

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