A Combined Strategy using Data Independent LC/MS and ion Mobility Mass Spectrometry for a Lipidomic Study of the PPAR-α Null Mouse

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INTRODUCTION

The nuclear hormone receptor peroxisome proliferator activated receptor-α (PPAR-α) plays a central role in regulating the switch between the fed and fasted state. Under fasting conditions, PPAR-α deficiency results in hyperglycemia and lipid accumulation in the liver and heart.

In this study, we have investigated the consequences of a failure to express PPAR-α, alongside the effects of ageing, in the PPAR-α null mouse using a combination LC-MS and Ion Mobility based lipidomics to follow metabolic changes in the heart, liver and adipose tissue. The analytical strategy consisted of a two tier levels (screening and verification) comprising of an initial profiling stage using a hybrid quadrupole/Travelling Wave IMS/ oaTOF (Waters Corp) instrument with exact mass. During the analysis, the low and high energy acquisition was obtained from a single injection. The low energy provided intact MS information whilst the high energy provided fragment ion information which was key to catalogue different lipid classes and provided further insight as to the particular key diagnostic fragment ion or neutral loss information[1].

The LC/MS data generated from these experiments was processed utilizing multivariate statistical analysis provided by MarkerLynx XS (Waters Corp) which allowed us to detect significant metabolic changes in the sample set[2]. Structures of key target ions obtained based on the multivariate statistical analysis can be further identified by utilizing the Time Aligned parallel (TAP) fragmentation during an IMS analysis.

In IMS mode, if a single precursor ion was selected in the quadrupole prior to the Tri-Wave region, the fragment ions produced in the trap T-wave (pre-IMS) can be separated based on their charge states and their size as they move through the second T-wave (IMS). These ions separated by their different drift times can then be further fragmented in the transfer T-wave (post-IMS) and fragment ions can be Drift Time aligned with their respective precursor ion. This TAP fragmentation pattern is in effect offering some very selective CID-IMS-CID information for the compound of interest (Figure 2) so that structural elucidation information for small molecule is easily

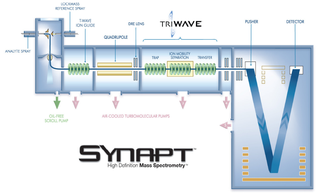

Figure 1. Schematics of the Synapt HDMS instrument configuration.

METHODS

Sample Preparation:

Each of the 150 µl of blood plasma samples was mixed with 600 µl methanol: chloroform (2:1). Samples were sonicated for 15 min. Water and chloroform were added (200 µl of each) and then centrifuged for 20 min. The resulting aqueous and organic layers were separated from the protein pellet. The organic layer was dried overnight in a fume food whilst the aqueous extracts were dried using an evacuated centrifuge

The dried samples from organic layer were reconstituted with a mixture of methanol:chloroform (2:1) prior to injection.

UPLC Conditions:

Instrument:	Waters ACQUITY UPLC® System
Column:	ACQUITY BEH C_8, 1.7 µ m, 2.1 x 100 mm,
Column Temp.:	65°C
Mobile Phase:	A). 10 mM NH_4OAc in H_2O, 0.1% Formic Acid B). 10 mM NH_4OAc in AcN/IPA 5/2, 0.1% Formic Acid
Gradient:	40%A to 0%A linear in 10 minutes, then hold for 12 minutes and equilibrate at 95% A for 4 minutes.
Flow Rate:	0.6 mL/min
Injection Vol.:	10 µ L

MS Conditions:

Mass Spectrometer:	Waters Synapt HDMS™
Mode of Operation:	TOF MS^E or IMS
Ionization:	ESI+
Source Temp.:	120°C
Desolvation Temp.:	450°C
Desolvation Gas:	Nitrogen, 500 L/Hr
Mobility Gas:	Helium, 100 L/Hr

Data Processing:
IMS Data review: DriftScope from MassLynx
Multivariate Statistic Analysis: MarkerLynx XS

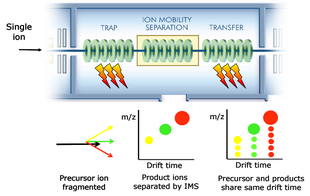

Figure 2. Schematics of the TAP fragmentation in TriWave region. Fragment ions can be generated in Transfer region can be Drift Time aligned with their perspective precursor ions.

UPLC/TOF MS^E RESULTS

Figure 3 shows the schematics of the mechanism of the TOF MS^E experiment. The low energy data contains the information about intact compounds, and the high energy data contains the information about the fragment ions.

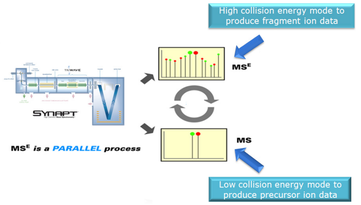

Figure 3. The schematic of the oaTOF MS^E experiment.

Figure 4 shows two chromatograms obtained from the LC/ *oa*TOF MS^E analysis. 4A shows the total ion chromatogram (TIC) of the plasma extract obtained from the low energy scan. 4B shows the selected ion chromatogram of m/z 184 obtained from the high energy scan. The result shown in 4B indicated that the Glycerophoshocholines were eluted in the range of 4.5 minutes to 7.5 minutes. The m/z 184 ion is the diagnostic product ion for Glycerophosphocholine (PC) as it is the $[M+H]^+$ ion of the polar head of phosphocholine. Also shown in Figure 4a that the triacylglycerols (TAG) eluted after the phosphocholines.

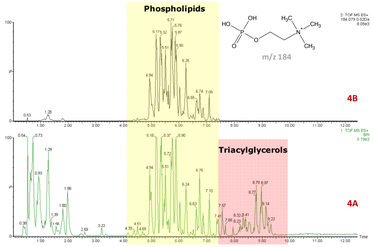

Figure 4. Results from UPLC/oaTOF MS^E experiment. 4a). Total ion chromatogram (TIC) of the plasma extract; 4B). Selected Ion Chromatogram of the same plasma extract (m/z 184).

UPLC/IMS/TOF RESULTS

If the same sample is analyzed in IMS/*oa*TOF mode, the results will also contain IMS information that can be reviewed using DriftScope from MassLynx. Figure 5 shows the screen shot of the DriftScope obtained from the same plasma extract analyzed by UPLC/IMS/TOF method.

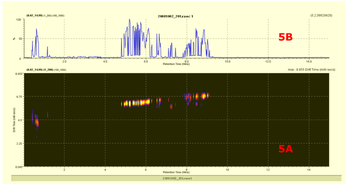

Figure 5. DriftScope display of the plasma extract. Result was obtained from the UPLC/IMS/oaTOF analysis..

Figure 5B shows the TIC for the plasma extract, and Figure 5A shows the DriftScope that corresponding to what's shown on the TIC. IMS is a 4D dataset with LC run time, m/z value, IMS drift time, and signal intensity. There are three different possible ways of displaying the LC/IMS/TOF results from the DriftScope. Showing here, the x-axis displays the LC run time, and the y-axis the drift time, the signal intensity is shown by the intensity of the colors. The 3D plot from the DriftScope can be selectively cut so that only the area of interest is investigated. Shown in Figure 6B, the TAG components were isolated and displayed in time vs m/z format, this result can be exported into MassLynx as a 2D format shown in Figure 6A. Shown here a clear correlation between ascending drift time as the number of acyl carbons increases.

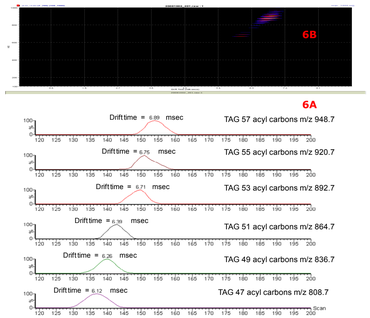

Figure 6. The TAG region from the plasma extract UPLC/IMS/ oaTOF result.

TAP FRAGMENTATION (I)

As displayed in Figure 2, during TAP fragmentation, the fragment ions obtained in the Trap region are drift time aligned with their respective precursor ions after they traveled though the drift tube. TAP can effectively simplify structural elucidations and offers confirmation for compound identifications. Here is an example of how a specific phosphocholine was positive identified.

The workflow for this project was to use UPLC/*oa*TOF MS^E with exact mass coupled with a multivariate statistical tool to identify the markers that showed significant concentration changes between control group and stimulus group. TAP fragmentation pattern of specific marker of interest was obtained to help positively identify the marker. In this example, a glycerophosphocholine (PC) with m/z 732.5538 was identified as a significant marker. This ion has the diagnostic m/z 184 fragment indicating it belongs to the PC class. In addition, TAP fragmentation pattern offering fatty acid side chain info was obtained as shown in Figure 8. 8A shows the Drift Scope of the fragment ions. This result can be exported into MassLynx and reviewed in 2D format. Figure 8B shows the 2D Driftogram of the m/z 496/494 ion.

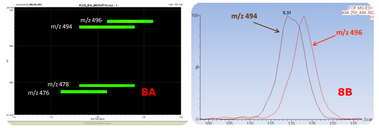

Figure 8. Fragmentation pattern obtained for PC 732.5538. 8A shows the DriftScope. 8B shows the 2D Driftogram after exporting the DriftScope result into MassLYnx.

As displayed in Figure 8, the major fragment ions after losing one of the fatty acid chain for this PC are m/z 498 and m/z 494, and after further loss of water, m/z 496 generates m/z 478, and m/z 494 generates m/z 476. This information indicated that the two fatty acid side chains of this PC is 2 amu apart, i.e.: they differ by a single double bond. Searching the Lipid maps database in PC class with m/z 732.5538 resulted a list of possible candidates as shown in 9. However, only the two heighted in yellow here are the possible hit since they contain the correct fatty acid fragment ions. At this point, to further specify the identify of the PC indentified would require the actual injection of the pure standards for both PCs.

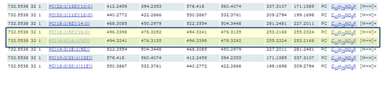

Figure 9. Glycerophosphocholine with m/z 732.5538 obtained from lipidmaps database search.

TAP FRAGMENTATION (II)

Figure 10 shows the proposed fragment pathway of the PC identified. The parent structure was obtained based on the match identified from the lipidmaps database. Each compound entry in the database is associated with the proposed structure. This agrees with our observation as the two fatty acid side chains were both C16. One has a double bond, and the other one is fully saturated. Loss of the C16:0 generates m/z 494 fragment, then m/z 476 after loss of water. (Figure 10a). Loss of C16:1 generates m/z 496 fragment, then m/z 478 after loss of water.

As a result, the PC (16:1(Z9)/16:0) was positively identified.

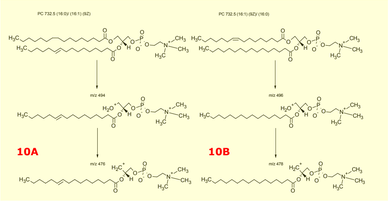

Figure 10. Fragmentation pathway for PC(16:0/16:1(9Z) or PC (16:1(9Z)/16:0).

CONCLUSION

- **UPLC/oaTOF MS^E allows intact ion and fragment ion information to be obtained within a single injection. By selectively review of the diagnostic fragment ions, the lipid class can be easily separated and observed.**
- **Multivariate Statistical Analysis can be used to identify markers with significant concentration changes between control and study group.**
- **UPLC/IMS/oaTOF analysis offers additional dimension of data set which can be easily reviewed by the DriftScope.**
- **IMS TAP fragmentation is an effective tool for structural identifications. Combining with TOF exact mass information, positive compound identification is simplified.**

References

1. Bateman, K. et al, Rapid Communications of Mass Spec., 2007, 21: 1485-1496.
2. Yu, K et al, Waters Application Note 720002541.
3. Yu, K et al, ASMS Sanibel Conference 2008 Poster.

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