Phospholipid analysis utilising a novel, data independent, mode of acquisition on a QToF instrument in combination with a scanning quadrupole mass filter and an ultra fast data acquisition system

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INTRODUCTION

Quadrupole time of flight (QToF) mass spectrometry is a well established tool for both discovery and quantitative applications. QToF instruments have found great utility in data-dependent modes of operation (DDA) where precursor ion survey acquisitions trigger sequential MS/MS acquisitions in discovery-based experiments. More recently, data independent modes of operation such as MS^E have become increasingly popular owing to the inherent sensitivity and speed of the ToF analyser, which provides simultaneous identification and quantification. Here we describe the SONAR mode of operation in which a resolving quadrupole mass filter is scanned, concomitantly with MS/MS data acquisition, thereby enabling the specificity of MS/MS fragmentation whilst maintaining a powerful, unbiased, data-independent approach for analysis of phospholipids in a complex matrix.

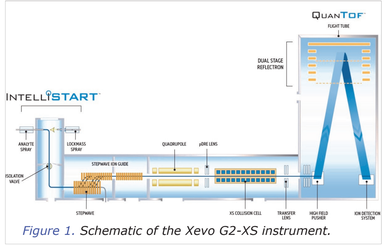

Figure 1. Schematic of the Xevo G2-XS instrument.

METHODS

Experimental

The quadrupole mass filter of a Xevo G2-XS QTof (Figure 1) has been modified, to allow it to operate in a continuously and repetitively scanning mode, over the mass range m/z 200 – 800 with a 0.2 sec cycle time. The instrument can be switched between a post-quadrupole fragmentation mode and a non-fragmentation mode after each quadrupole scan to provide fragment ion profiles, each of which can be assigned to the precursor ion profiles resulting from scanning the quadrupole transmission window (Figure 2). The two- dimensional data format produced by this prototype acquisition mode is similar to IMS (HD) MS^E data and, as such, can be processed using standard $HDMS^E$ software including the Scientific Data Information System (UNIFI) and DriftScope.

LC/MS Conditions

Column: ACQUITY CSH™ C_{18}, 1.7µm, 2.1 x 100 mm

Mobile phase A and B: 10mM ammonium formate in ACN/H_2O (60/40) . 10mM ammonium formate in IPA/ACN (90/10)

Flow rate: 0.4 mL/min

Column temperature: 55°C

Instrument: Xevo G2-XS operated in ES positive ion mode
Acquisition rate: 0.1 scan/sec

2D-MSMS: Method: Low energy 6 eV, high energy 25-50 eV

Time	% A	% B	Curve
-	60	40	-
2.0	57	43	6
2.1	50	50	1
12.0	46	54	6
12.1	30	70	1
18.0	1	99	6
18.1	60	40	6
20.0	60	40	1

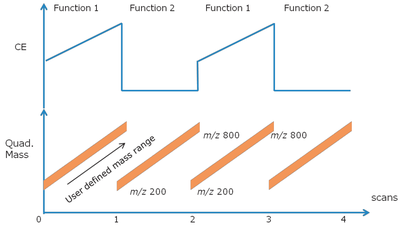

Figure 2. Schematic of the acquisition method. The experiment consists of a 1 cycle at high collision energy (CE) followed by another at low CE. During each period, the quadrupole ramps over a pre-determined mass range.

RESULTS AND DISCUSSION

An extract of phospholipids from rat brain was injected onto a LC system equipped with a C18 analytical reversed phase column. A gradient length of 20 minutes was used. Examples of phospholipids are shown in Figure 3.

Using the complex phospholipid rat brain sample, which contained many closely eluting lipids, with precursor ions that fragment to generate the same (or similar) product ions, are shown to be clearly resolved with clean and unambiguous MS/MS spectra using SONAR.

The main difference between other data independent acquisition methods and SONAR is the behaviour of the quadrupole. Instead of stepping or remaining open and transmitting all ions it slides over the selected mass range during both low and high energy scans. Filtering the of the precursor ions by the quadrupole increases the selectivity of the method (Figure 4).

This set up produces two-dimensional datasets resembling nested ion mobility (IMS)-MS data which can be viewed using DriftScope or UNIFI. This opens up the possibility of precursor and fragment alignment with a tolerance much tighter than the quadrupole window (analogous to RT and drift time alignment in MS^E and $HDMS^E$ experiments). Figure 5 shows how reducing the quadrupole transmission influences the selectivity of the method and results in 'clean' spectra.

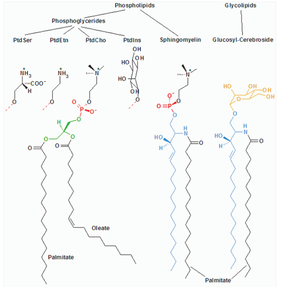

Figure 3. Examples of phospholipids. Here we discuss mainly phosphatidylcholine (PC) however the approach is applicable for lipids analysis in general.

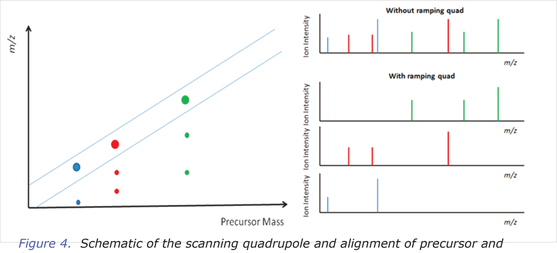

Figure 4. Schematic of the scanning quadrupole and alignment of precursor and fragment ions.

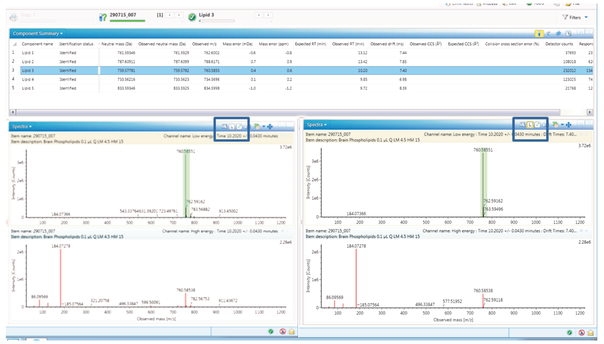

Figure 6. Alignment of data in both retention time and quadrupole m/z dimensions (left) and retention time dimension only (right). Employing the additional dimension of quadrupole m/z, to deconvolve data, aids in unambiguous lipid identification and assignment.

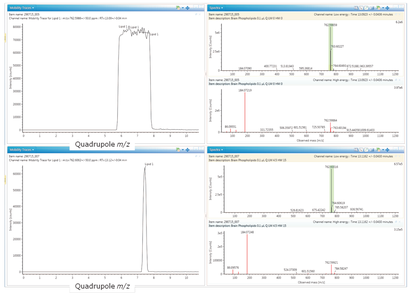

Figure 5. Plots show the effect of using a narrower quadrupole window in order to achieve a 'clean' high energy MS^E spectrum.

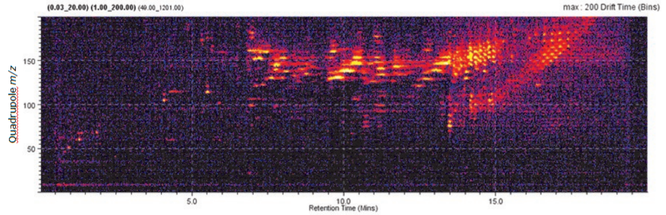

Figure 7. SONAR high energy data function with a 5 Da unit quadrupole window over the 20 minute chromatographic run time. The horizontal axis corresponds to retention time (min) and the vertical axis corresponds to quadrupole m/z. The cluster of ions around 10 minutes represents the phospholipids and triglycerides at around 15 minutes.

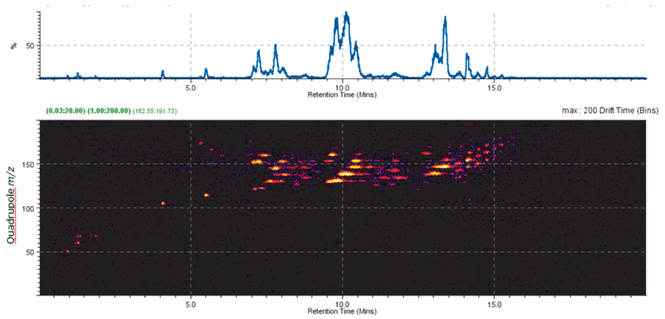

Figure 8. XIC of m/z 184 in the high energy function showing based line resolved PC lipids that would otherwise have overlapped resulting in ambiguous MS^E spectra. Vertically aligned features correspond to co eluting precursor ions that give rise to the fragment ion indicative to the PC lipid class.

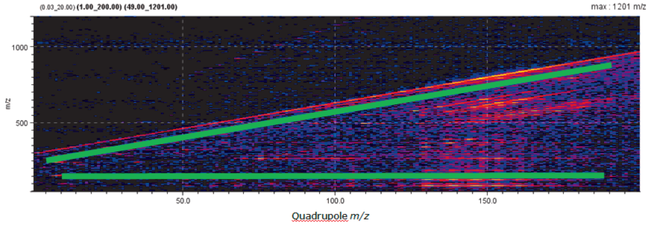

Figure 9. DriftScope plot illustrates some of the types of ions observed in SONAR experiments, the x axis represents the quadrupole m/z and the y axis the ToF m/z. The horizontal green line represents the location of fragment ion at m/z 184, indicative of the PC lipid class. The diagonal green line represents the neutral loss of 141 Da, indicative of the PE lipid class.

Data alignment, within UNIFI, using both the quadrupole *m/z* **and** retention time dimension is shown in Figure 6, by toggling on and off the alignment button. The low intensity fragment ions in the region ~ *m/z* 500 in Figures 5 and 6 provide valuable information on the length and saturation of the sn1 and sn2 fatty acid chains of the phospholipids. It is, therefore, important to have clean and unambiguous fragmentation data for assignment and identification.

DriftScope was used in order to highlight the complexity of the sample set and how use of a narrow quadrupole window resolves the high density of lipids within the complex sample (Figure 7 and 8). Shown in Figure 8 is the XIC for *m/z* 184 in the high energy function, a fragment indicative of the PC lipid class, and the heat map of retention time *vs* the quadrupole *m/z*. Here, it is possible to differentiate precursor ions that fragment to give the ion at *m/z* 184 and that have been shown to co-elute.

Although preliminary data, Figure 9 highlights the additional possibility of monitoring for neutral losses (as well as common fragment ions) within the SONAR data set, applicable for the PE lipid class, for example.

CONCLUSIONS

Here we have shown the benefits of SONAR for the application of lipid analysis including:

1. Cleaner spectra compared to other DIA methods
2. High spectra/second scan rate compatible with HR MS
3. Increased confidence in identification through selectivity
4. Cataloguing of a complex sample within one experiment
5. Unbiased data acquisition within a targeted mass range

SONAR has been shown to be a valuable tool in the analysis of complex lipid sample sets by providing confidence in fragment ion assignment and thus the identification of lipids.

REFERENCES

1. http://www.waters.com/webassets/cms/librarydocs/2015asms_richardson_2dmsms.pdf

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