IMPROVED DETECTION AND IDENTIFICATION OF LIPIDS USING THE XEVO™ G3 QTOF MASS SPECTROMETER

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INTRODUCTION

To elucidate lipid biosynthesis pathways and find potential lipid biomarkers for diseases or authentication of foods for example, requires accurate identification of lipids. Unintended analyte fragmentation typically occurs due to dissociation of unstable molecular bonds caused by the energy imparted during ion transfer from the ion source to the mass analyser. This unintended fragmentation can lead to a reduction, or even complete loss of intact analyte ion signal and can potentially result in undetected or misidentification of key analytes. Previously, we have shown the benefits of using the new ion optics and StepWave™ XS ion guide to improve the detection of labile drug compounds [1] Here we show how a new adjustable voltage StepWave XS ion guide (Figure 1) can be used to improve the transmission of labile components, with a particular focus on lipids to improve the identification of key biologically relevant lipids using a high-resolution QTof.

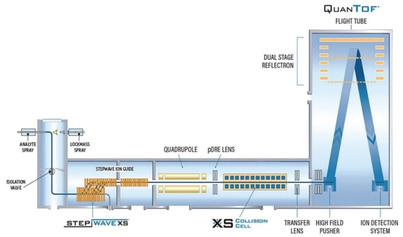

Figure 1. The Xevo™ G3 schematic depicting the StepWave™ XS design.

EXPERIMENTAL

- **Test samples consisted of a 100 times dilution of AVANTI EquiSPLASH™ in IPA and plasma extracts using a simple protein precipitation method with 100x EquiSPLASH in IPA [2].**
- **12 injections of Standard mix and plasma extracts in both positive and negative ESI mode.**
- **High throughput Reversed-Phase Lipid profiling method for large samples sets gradient and MS conditions (12 min run time).** Mobile Phase B (900:90:10 IPA: Acetonitrile:1M Ammonium Formate, 0.1% Formic Acid) starts from 50% to 99% over 12 minutes.
- **ACQUITY™ Premier UPLC™ I-Class system and ACQUITY Premier UPLC™ CSH™ C18 (2.1 x 100 mm, 1.7 µm) column.**
- **Mass Spectrometers used: Previous generation StepWave instrument and Xevo G3 QToF instrument with adjustable voltage StepWave XS ion guide.**
- **Data processing by UNIFI™ software informatics.** waters_connect

Variable	Description
MS Function	ToF MSe
Analyser Mode	Resolution
Dynamic Range	Extended
Mass Range	50 – 1200 Da
Scan Time	0.1 Secs
Data Format	Continuum
Collision Energy	20eV-45eV
StepWave DC	
Default	**Body Gradient = 10V**
	RF Settings enabled (150)
AGC	Automatic Detector check optional
Lockspray function Settings	Acquire lockspray & apply correction,
	Scan time = 0.1 Secs, Interval =30 Secs, Scans to average = 4
	Mass window = +/-0.5.

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RESULTS AND DISCUSSION

Overall, when using the Xevo G3 QTof instrument, there was a significant improvement of the precursor intensity for phospholipids (Cer, PC, PE, PG, PI, PS) was observed compared to previous generation instruments (**Figure 2**). The bar graphs in **Figure 3** show there is an up to 30 times increase in precursor ion response in the Xevo G3 QToF instrument when using default settings compared to the previous generation instrument. The bar graph show responses of standards measured within a plasma matrix. The CV of mean responses were >5% showing good reproducibility using the Xevo G3 QTof instrument. Low energy spectra in **Figures 4** and **5** show better precursor ion transmission (higher intensity) using Xevo G3 QToF. Although in-source fragmentation (ISF) of PG in positive mode is still observed, the Xevo G3 QToF reduces this significantly (**Figure 4**).

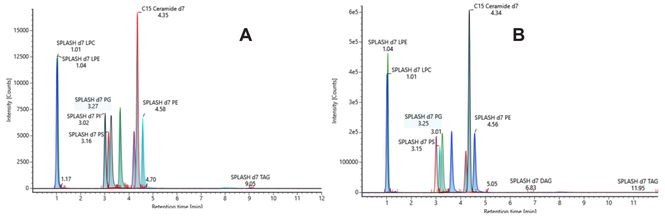

Figure 2. Example chromatograms showing the response of 100x dilution EquiSPLASH in IPA using the same LC system, column and mobile phase coupled to a previous generation StepWave instrument (A) and the Xevo™ G3 QToF instrument with adjustable voltage StepWave XS ion guide (B).

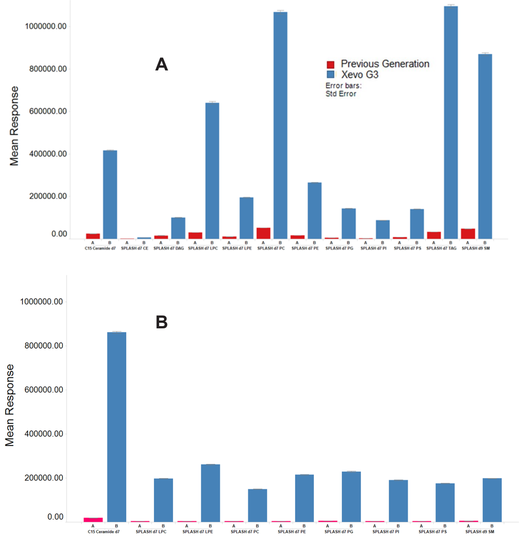

Figure 3. Average response of EquiSPLASH lipids spiked in plasma (n=12) in positive mode (A) and negative mode (B). The error bar s used represent standard error (SE).

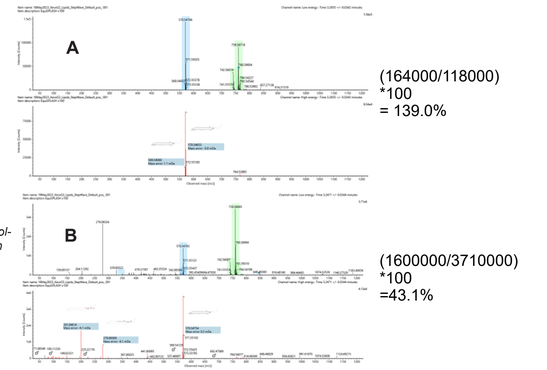

Figure 4. High and low energy spectra from UNIFI of the PG standard in IPA (positive mode). The spectra from the previous generation is (A) and for the Xevo G3 QToF instrument, B) are shown here. In the low energy trace the precursor peak is highlighted in green. Theoretical fragments are also used to monitor in source fragmentation and these are highlighted in blue. The % in source fragmentation is estimated for the two models.

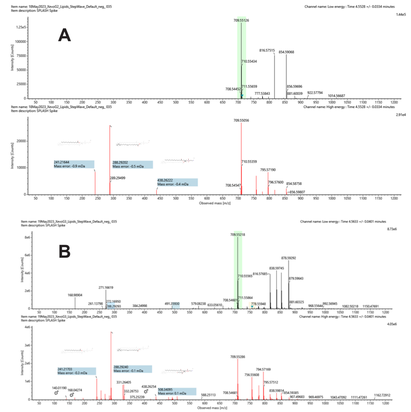

Figure 5. High and low energy spectra from UNIFI of the PE standard in plasma (negative mode). The spectra from the previous generation is (A) and for the Xevo G3 QTOF instrument, B).

Figure 5 shows that spectra of lipids generated on the Xevo G3 QToF instrument are more fragment rich allowing for easier to elucidate and I more confident identification. In **Figure 6** and example of an endogenous lipid is used to show that more theoretical fragments can be matched using the Xevo G3 QToF instrument (12/14) compared to previous generation instruments (6/14) to improve identification.

m/z	Ion Description	Structure	Previous Generation	Xevo G3
804.5760	Precursor ion (M+HCOO)-			
744.5549	Loss of CH3 and formate from precursor ion			
673.4814	Loss of choline and formate from precursor ion			
506.3252	Loss of sn1 acyl chain as ketene (RCH=C=O), CH3 and formate from			
488.3147	Neutral loss of sn1 RCOOH group, loss of CH3 and formate from precursor ion			
480.3096	Loss of sn2 acyl chain as ketene (RCH=C=O), CH3 and formate from			
462.2990	Neutral loss of sn2 RCOOH group, loss of CH3 and formate from precursor ion			
281.2486	sn2 RCOO- ion			
255.2330	sn1 RCOO- ion			
224.0693	Glycerophosphocholine with loss of CH3 and H2O			
168.0431	Phosphocholine with loss of CH3			
152.9958	Glycerol-3-phosphate ion with loss of H2O			
96.9696	H2PO4- ion (from phosphate)			
78.9591	PO3- ion (from phosphate)			

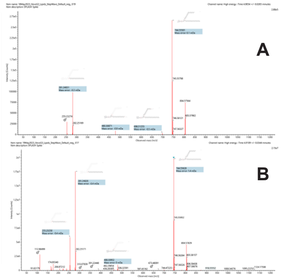

Figure 6. the theoretical fragments of endogenous PC(16:0/18:1) in negative mode are according to LIPIDMAPS were used to illustrate the advantages of the Xevo G3 QTof instrument to improve structural elucidation. The matched fragments are assigned a green colour if identified by UNIFI, An amber colour if theoretical fragment can be found by visual inspection and of spectra and red if not detected. (A) represents a high energy spectra using the previous generation instrument and (B) shows the spectra of Xevo G3 QTof instrument.

CONCLUSION

- **Improved precursor ion transmission compared to previous generation StepWave**
- **Reduction of unintended fragmentation effects leads to improved detection of the analyte**
- **Improved structural elucidation and higher confidence in identification of endogenous lipids**
- **Fast data processing and visualization using UNIFI informatics for maximum flexibility.**

References

[1] Reid, L. and Pickles, D. (2022) Improved Transmission of Labile Species on the Xevo™ G3 QTof Mass Spectrometer with the StepWave™ XS, Waters Corp Application Note

[2] Munjoma, N.; Isaac, G.; Muazzam, A.; Cexus, O.; Azhar, F.; Pandha, H.; Whetton, A.D.; Townsend, P.A.; Wilson, I.D.; Gethings, L.A.; Plumb, R.S. (2022) High Throughput LC-MS Platform for Large Scale Screening of Bioactive Polar Lipids in Human Plasma and Serum. J Proteome Res. 2022 Nov 4;21(11):2596-2608. doi: 10.1021/acs.jproteome.2c00297. Epub 2022 Oct 20. PMID: 36264332; PMCID: PMC9639203.

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