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Application Note

Increased Specificity, Improved Lipidomic Clarity, and Identification Confidence with SONAR

Adam King, Lee A. Gethings, Robert S. Plumb, Ian D. Wilson

Waters Corporation, Imperial College

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

Abstract

This application note demonstrates the improved spectral clarity achieved when using SONAR and thereby enabling increased identification accuracy of co-eluting compounds for complex lipidomic studies.

Benefits

Implementing SONAR improves spectral clarity and thereby increases certainty of lipid identifications.

Introduction

Lipidomic analysis of blood and tissue samples for biomedical research aims to identify and quantify potential

biomarkers or analytes of interest. When investigating these biological samples via LC-MS, high collision energy

fragmentation data is essential for the identification of significant features using database searches. Current data

independent acquisition (DIA) modes switch between low and high collision energy functions in order to

generate fragmentation spectra. Due to the complex nature of biological matrices, liquid chromatography (LC) is

employed to better resolve the components prior to analysis by mass spectrometry (MS). However, due to the

complexity of the lipidome, many lipids co-elute as a result of chemical similarities within lipid classes, and

therefore undergo simultaneous MS/MS fragmentation. This results in high energy spectra comprised

of fragments from multiple precursor ions, making it difficult to attribute which fragments correspond to which

precursor ions, and therefore increasing the difficulty of making accurate identifications. SONAR is a DIA

technique with a low-resolution quadrupole mass filter, which is scanned repetitively, and both precursor and

MS/MS data are acquired at spectral rates approaching 2000 spectra per second (Figure 1). Here we describe

the application of SONAR for the investigation of chimeric humanized mouse liver extracts.
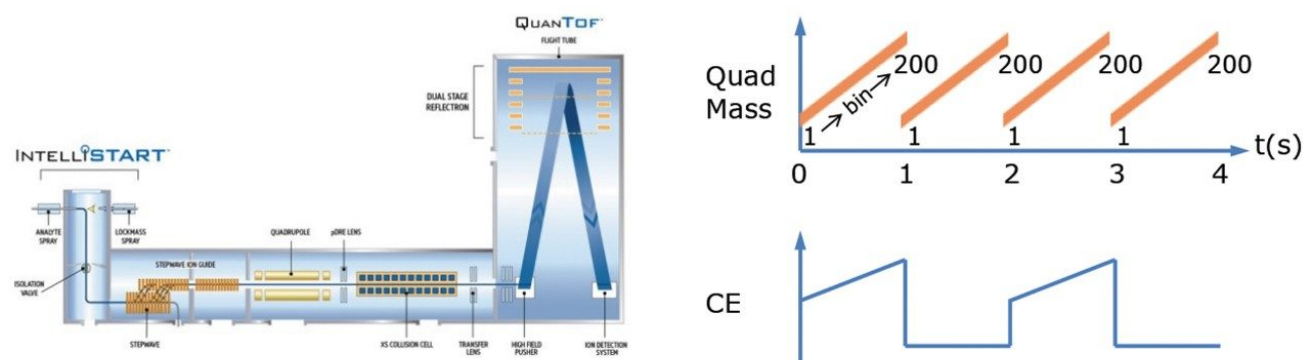

Figure 1. Implementation of SONAR using the Xevo G2-XS QTof. A resolving quadrupole scans over the mass

range of interest at low and elevated collision energy providing accurate mass precursor and product ion data.

Results and Discussion

SONAR has been utilized for the analysis of lipid extracts derived from chimeric humanized mouse livers. Lipids

were extracted from 17 livers using Dichloromethane (DCM):Methanol (3:1) and subsequently evaporated using a

rotary evaporator. Once dry, the extracts were reconstituted in Isopropyl Alcohol (IPA):Methanol:Water prior to analysis. A pooled sample was prepared from each extract and used as a quality control (QC) which was injected every seventh injection. Data were also collected using an alternative DIA method for comparative purposes. Lipids were separated over a 20 minute LC gradient using reversed phase (RP) chromatography (2.1 x 100 mm ACQUITY UPLC CSH C_{18} Column, 1.7 µm). LC-MS data were acquired using a Xevo G2-XS QTof mass spectrometer operating in SONAR mode with a quadrupole window of 10 Da, scanning over a mass range of 350 – 950 *m/z*, while the Tof scanned over 50 – 1200 *m/z* with a scan rate of 0.1 sec.

The issue of co-elution is evident with multiple precursors generating mixed fragmentation spectra. The data displayed in Figure 3, however, represents SONAR generated data highlighting the specificity of the technique by resolving co-eluting lipid species. The acquisition mode utilizes a scanning quadrupole, filtering ions over a set *m/z* range, providing significantly cleaner spectra when compared to conventional DIA methods. Representative MS and MS/MS spectra of three co-eluting lipids show the additional specificity afforded by the scanning quadrupole technique when compared with an alternative DIA method, which uses a non-resolving quadrupole. Data from all experiments were subsequently peak picked and database searched using Progenesis QI. For regions of high co-elution, a large number of false positive identifications can result from compound database searches. However, the high selectivity provided by SONAR shows the number of false positives to be significantly reduced while increasing the confidence score of the identifications returned when compared with conventional DIA methods. Figure 4 illustrates this finding, showing an increased number of lipid identifications with higher scoring.
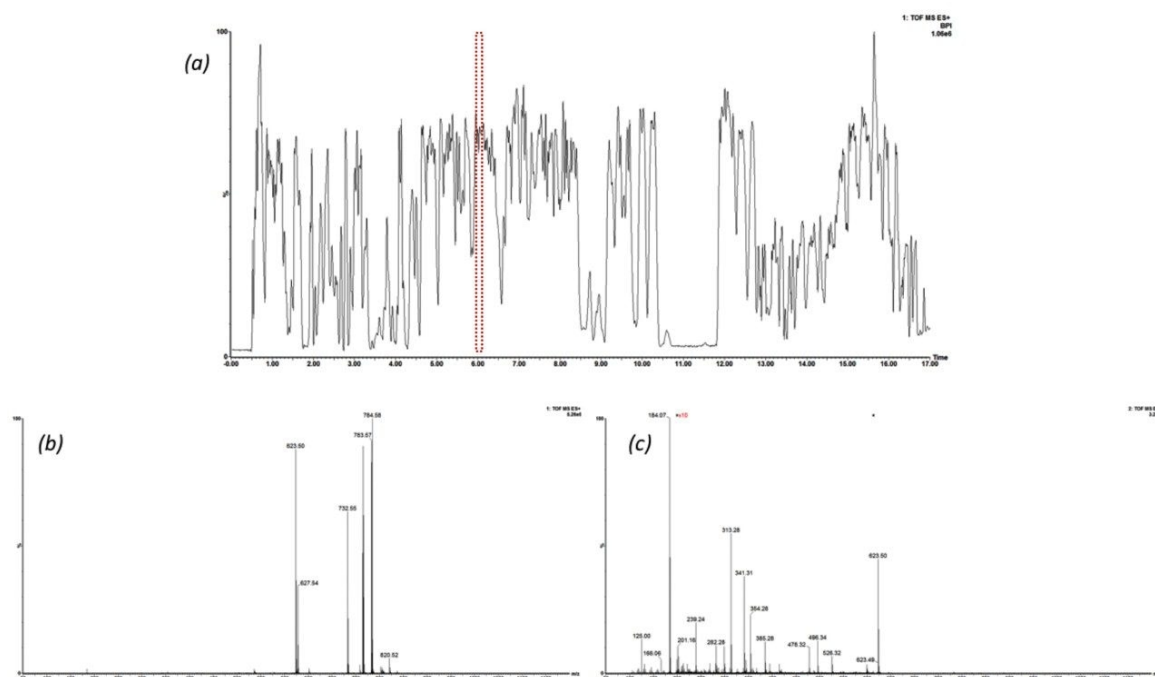
Figure 2. (a) Representative chromatogram of a liver extract generated using an alternative DIA approach (non-resolving quadrupole). Based on a retention window of 5.9 min, the issue of co-elution is highlighted in both the low (b) and elevated (c) energy spectra. The presence of multiple precursors provides a complex high energy spectrum making confident identification and ultimately structural elucidation challenging.

Figure 3. SONAR acquired data showing increased specificity over the alternative DIA approach. SONAR data can be represented as a two dimensional ion map (quadrupole m/z vs. retention time) with the scanning quadrupole showing increased specificity for co-eluting lipids at a retention time of 5.9 min. Fragmentation data from the elevated energy spectra (a,b,c) indicate the presence of three individual phospholipid species.
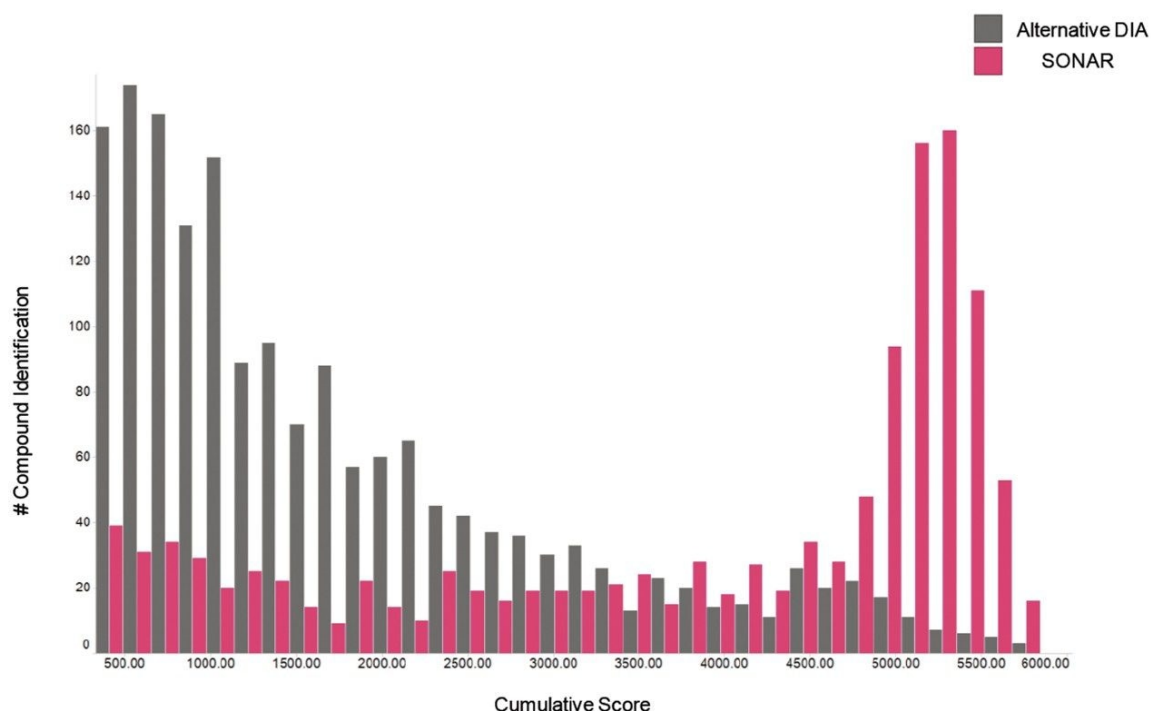
Figure 4. Bar graph illustrating increased compound identification scores with SONAR generated data. The cumulative score (x-axis) is the product of confidence score and fragmentation score from Progenesis QI searches. The increased specificity of SONAR shows higher scoring identifications due to increased specificity and higher quality fragmentation data.

Conclusion

This study demonstrates the benefits of SONAR for omics studies, providing enhanced specificity, clearer spectra, and ultimately fewer false positives when conducting compound database searches. A comparison of SONAR with an alternative DIA methodology shows how increased specificity in the workflow can reduce false

positives that result from simultaneous fragmentation from multiple species within the collision cell.

References

1. Lipid Separation Using UPLC with Charged Surface Hybrid Technology, G. Isaac, S. McDonald, and G. Astarita, 720004107EN, 2011.

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SONAR <https://www.waters.com/134908444>

Xevo G2-XS QTof Quadrupole Time-of-Flight Mass Spectrometry <https://www.waters.com/134798222>

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