IMPLEMENTATION OF A NOVEL SCANNING QUADRUPOLE DIA ACQUISITION METHOD FOR DESI IMAGING

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INTRODUCTION

Mass spectrometry imaging (MSI) allows for the correlation of spatial and chemical information directly from biological surfaces. Typically untargeted MSI experiments are carried out in full scan MS. After mining of the MSI data, the next step is the identification of potential biomarkers which is usually performed via a limited number of manual MS/MS experiments.

Recently a new Data Independent Acquisition (DIA) method called SONAR utilising a scanning quadrupole mass filter in a Q-Tof geometery has been introduced. In this method, a resolving quadrupole mass filter is scanned repetitively with precursor and MS/MS data acquired at rapid spectral acquisition rates. The method produces a highly specific and unbiased two-dimensional dataset that can be viewed and processed using a variety of informatics tools. The mode of operation has been implemented on a bench top quadrupole - ToF mass spectrometer and has been embedded into a DESI imaging workflow.

Here, we describe two new acquisition methods that collect MS/MS results from a SONAR data independent acquisition (DIA) with either precursor ions recorded using the quadrupole to scan across a specific mass range, or in full MS scan mode, whilst DESI imaging directly from tissue sections.

METHODS

Sample preparation

Experiments have been carried out on mouse brain tissue sections, produced using a cryotome and deposited on a standard microscope slide which was preserved at -80C degrees until analysis by mass spectrometry.
The tissues were directly mounted into the DESI source from the freezer, with no sample preparation or pre-treatment required .

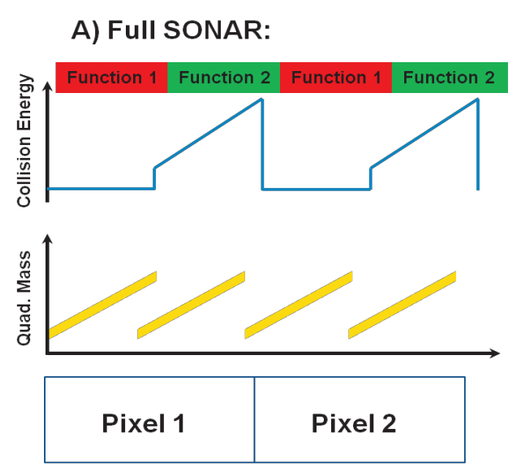
Mass spectrometry

MS imaging experiments were performed in negative ionisation mode using a Prosolia 2D stage (Prosolia, Indianapolis) mounted on a Xevo G2-XS QToF (Waters Corporation), operating in SONAR^{TM} mode.

DESI spray conditions were set at 2 µL/min, 95:5 MeOH:water with nebulising gas pressure of 6 bar.

Full SONAR mode is described in figure 1,A where both precursor and MS/MS data was acquired with the scanning quadrupole, with low collision energy (LE) for the precursor and elevated collision energy (HE) for the fragments.

Hybrid SONAR mode is presented in figure 1,B where the precursor data was acquired in full MS scan (LE), whereas the MS/MS data was acquired with the scanning quadrupole with elevated energy (HE).



Experiment	Quad Scan (Da) MS	Quad Scan (Da) MS/MS	Quad Window (Da)	ToF Scan (Da)	Scan time (s) MS	Scan time (s) MS/MS	LE CE (V)	HE CE (V)
Full SONAR	700 to 900	700 to 900	100 to 1200	5, 10, 20	0.486	0.486	6	25 to 40
Hybrid SONAR	N/A	700 to 900	100 to 1200	5, 10, 20	0.486	0.486	6	25 to 45

Figure 1. Full SONAR^{TM} and Hybrid SONAR^{TM} acquisition method and DIA acquisition parameters used.

Data management

DESI imaging data were processed and visualized using High Definition Imaging 1.4(HDI) software (Waters Corporation) for detailed image analysis. Visualization of the multidimensional data was conducted with DriftScope software (Waters Corporation).

RESULTS

Comparison between Full SONAR and Hybrid DESI acquisition

A series of experiments were carried out to compare the sensitivity and applicability of SONAR vs the Hybrid SONAR in a DESI imaging experiment applied to mouse brain tissue sections, using different quadrupole transmission windows (5, 10 and 20 Da).

Figure 2 displays the MS spectra of around 65-70 pixels for the 10 Da quadruple window. It can be observed that there is a drop in sensitivity in SONAR low CE compared to full scan MS, however, the data is very similar with the SONAR high CE for both experiments, as expected.

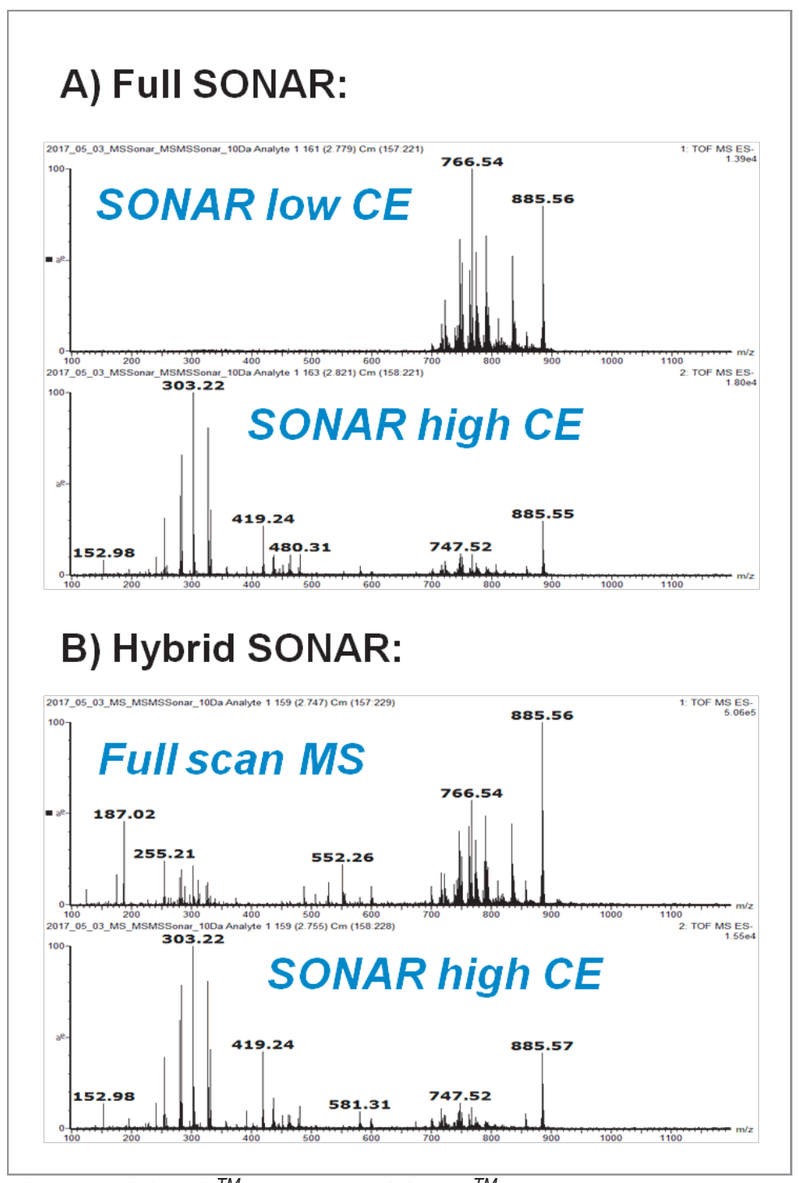


Figure 2. SONAR^{TM} and Hybrid SONAR^{TM} acquisition method and DIA acquisition parameters used.

Hybrid SONAR DESI Imaging acquisition for higher specificity and lipid identification

An hybrid SONAR DESI imaging experiment was acquired with a 5 Da scanning quadrupole window directly from a coronal mouse brain section.

To demonstrate the specificity of SONAR^{TM} in a DESI imaging experiment, figure 3 displays the example of lipids 788.54 and 790.54, which have significantly different MS ion images. By mining the fragmentation information in the SONAR^{TM} HE function and using Lipid maps (http://www.lipidmaps.org/).

Lipid at m/z 790.54 was identified to be PE (18:0/22:6) H^- with all the main intense fragments matching the predicted fragments (highlighted in red). However for precursor m/z 788.54, some of the main intense fragments match the PE (18:1/22:6) H^- predicted fragments but fragment m/z 701. 51 corresponds to the loss of serine, indicating that there is also a lipid PS (18:1/18:0) present in the mouse brain.

As a result of the acquisition of the SONAR^{TM} HE function in imaging mode, ion images of the fragments can also be displayed for each of the specific lipids. It can be see that the two PE lipids have similar distribution , whereas PS (18:1/18:0) is less abundant in the medulla of the mouse brain versus the cerebellum.

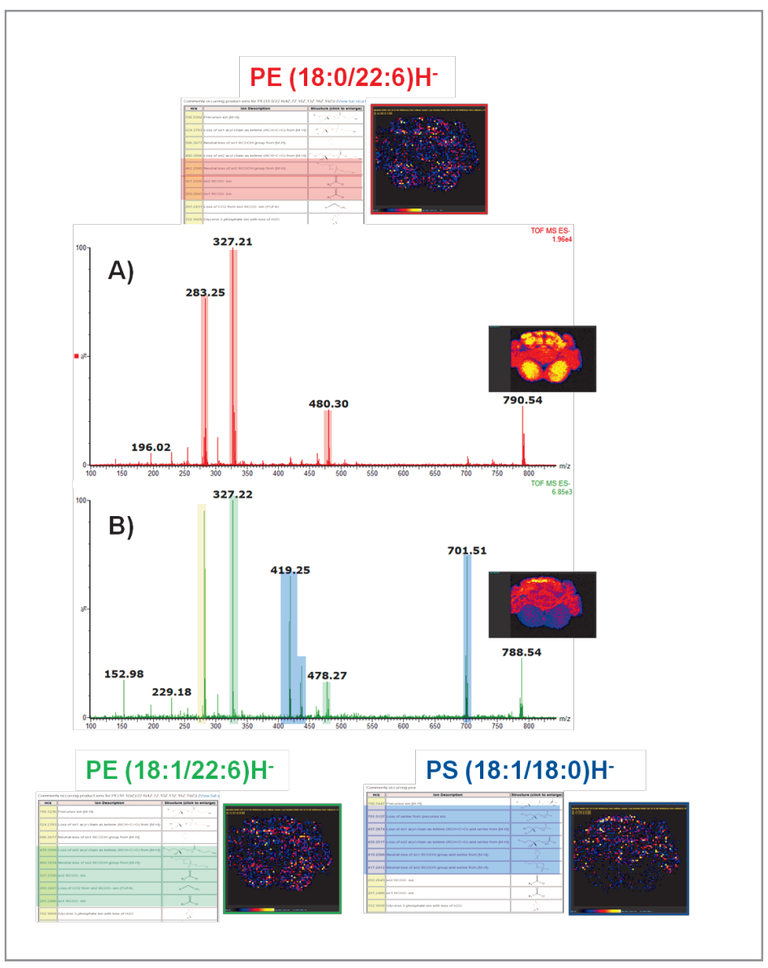
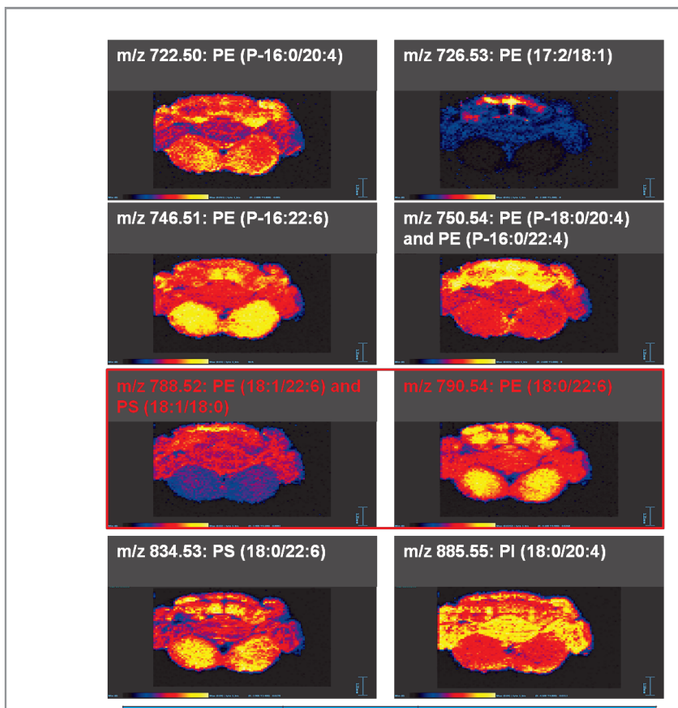


Figure 3. Specific MS/MS spectra of m/z 788.53 and m/z 790.53 with potential lipid identification as respectively PE (18:/22:6)H$^-$ and PS (18:1/18:0)H$^-$ for m/z 788.53 and PE (18:0/22:6)H$^-$ for m/z 790.53



Experimental m/z	Theorical m/z	Lipid ID
700.53	700.53	PE (P-16:0/18:1)
716.52	716.52	PE (16:0/18:1)
722.51	722.51	PE (P-16:0/20:4)
726.53	726.51	PE (17:2/18:1)
738.51	738.51	PE (16:0/20:4)
742.54	742.54	PE (18:1/18:1)
746.51	746.51	PE (P-16:0/22:6)
750.55	750.54	PE (P-16:0/22:4)
750.55	750.54	PE (P-18:0/20:4)
760.5	760.51	PS (16:0/18:1)
760.5	760.49	PE (16:1/22:6)
762.5	762.53	PS (16:0/18:0)
762.5	760.51	PE (16:0/22:6)
766.53	766.54	PE (18:0/20:4)
772.51	766.49	PE (17:2/22:6)
774.53	774.51	PE (17:1/22:6)
788.53	788.52	PE (18:1/22:6)
788.53	788.54	PS (18:1/18:0)
790.54	790.54	PE (18:0/22:6)
794.55	794.57	PE (18:0/22:4)
810.52	810.53	PS (18:0/20:4)
834.53	834.53	PS (18:0/22:6)
857.52	857.52	PI (16:0/20:4)
885.55	885.55	PI (18:0/20:4)

Figure 4. Full MS scan ion images of lipids with potential identification using the SONAR ^{TM} fragmentation information

CONCLUSION

- **SONAR^{TM} DIA acquisition provides multi dimensional data sets exhibiting precursor and fragment information using a Q-Tof based mass spectrometer.**
- **Full SONAR^{TM} and Hybrid SONAR ^{TM} were compared using different quadrupole window to demonstrate the specificity vs sensitivity characteristics**
- **Hybrid SONAR^{TM} was successfully implemented in a DESI imaging workflow**
- **High Energy SONAR^{TM} allowed the identification of nominally isobaric species by displaying specific fragment ion images.**

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