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, underaged MSI methods 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 utilizing a scanning quadrupole mass filter in a Q-ToF geometry 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 visualised and processed using a variety of informatics tools. The mode of operation has been implemented on a bench top 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 preserved using a cryostat and mounted on a standard microscope slide which was preserved in 95% ethanol until analysis by mass spectrometry. The tissues were directly mounted into the DESi source from the freezer, with all sample preparation in a permanent environment.

Mass spectrometry

MS imaging experiments were performed in negative ionisation mode using a Xevo G2 Q-ToF (Waters Corporation), figure 1, operating in SONAR™ mode. DESI matrix conditions were set at 2 µL/min, 50:50 MeOH:water with an ionisation source temperature of 100 °C. A 500 nL spray was delivered to the ion source, and a correlation of spatial and chemical information was provided directly using a Xevo G2 Q-ToF Mass Spectrometer instrument schematic. To demonstrate the specificity of SONAR™ in a DIA imaging workflow, figure 2 displays the example of lipid 786.54 and 788.54, which have significantly different ion images by MS imaging. The next step is the identification of potential biomarkers which is usually performed via a limited number of manual MS/MS experiments.

RESULTS

Comparison between Full SONAR and Hybrid DIA acquisition

A series of experiments were carried out to compare the sensitivity and applicability of SONAR™ vs the Hybrid DIA in a DESI imaging workflow. An hybrid SONAR DESI imaging experiment was acquired with a 5 Da quadrupole window, whereas the MS/MS data was acquired with the scanning quadrupole with elevated energy (HE). Full MS/MS mode is described in figure 1, where precursor and MS/MS data is acquired with the scanning quadrupole with low collision energy (LE) for the precursor and elevated collision energy (HE) for the product ion. Hybrid SONAR mode is presented in figure 2 where the quadrupole was set at 10 Da scan speed, whereas the MS/MS data was acquired with the scanning quadrupole with elevated energy (HE).

CONCLUSION

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