ADVANCES IN TARGETED OMICS QUANTITATION USING A NOVEL Q-TOF SCANNING QUADRUPOLE DIA METHOD

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INTRODUCTION

Targeted LC-MS based assays are increasingly applied in the post-discovery omics area with emphasis on validation, the first of many phases in translational analyses, or in studies that are aimed at gaining the understanding of biological systems, drug development and treatment. Context is driving current omics experiments, thereby driving the development of LC-MS acquisition methods that can provide both qualitative and quantitative information in a single experiment. A novel Data Independent Acquisition technique that satisfies both these requirements will be demonstrated.

In the method, a low-resolution quadrupole mass filter is scanned repetitively and both precursor and MS-MS data are acquired at spectral rates approaching 2000 spectra per second. This method produces a high duty-cycle, highly specific and unbiased two-dimensional data set which can be viewed and processed using readily available informatics. The mode of operation has been implemented on a benchtop quadrupole / ToF mass spectrometer and has been applied to targeted lipidomics and proteomics experiments involving the quantitation of both lipids and proteins in human plasma samples derived from normal, obese and diabetic donor cohorts.

METHODS

Sample preparation

Standards. A proteomics standard comprised a four protein digest mixture (Waters Corporation) spiked into 1 µg/µL *E. Coli* tryptic digest (Waters Corporation) matrix at a maximum concentration of 20 fmol/µL and then serially diluted to a lowest concentration of 100 amol/µL. A pre-mixed synthetic lipid standard mixture (Avanti Polar Lipids, Inc.) served as a lipidomics standard that was serially diluted in a human plasma extract.

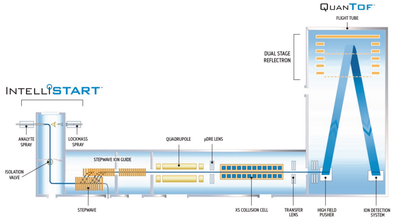


Figure 1. Xevo G2-XS QTof Mass Spectrometer instrument schematic.

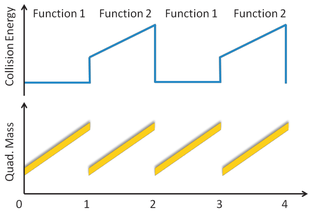
Biological samples. Proteins and lipids were extracted from human plasma (Innovative Research Inc), which originated from 6 control, 6 obese and 6 diabetic patients. The protein extracts were prepared with 1% RapiGest SF (Waters Corporation) prior to reduction, alkylation and overnight digestion with trypsin[1]. Lipid extractions were performed as previously described[2]. The extracts were centrifuged for phase separation and the lower fraction collected for LC-MS analysis.

LC-MS conditions

Label-free LC-MS was used for qualitative and quantitative peptide analyses. Experiments were conducted using a 90 min gradient from 5 to 40% acetonitrile (0.1% formic acid) at 300 nL/min using a M-Class system (Waters Corporation) and a HSS 1.8 µm C18 reversed phase 75 µm x 20 cm nanoscale LC column.

For lipid identification, the LC-MS experiments consisted of a 20 min gradient from 3 to 40% isopropanol:methanol (10 mM ammonium formate) at 500 µL/min using a ACQUITY UPLC system. Here, a BEH 1.7 µm C8 reversed phase 2.1 x 10 cm LC column was used.

A Xevo G2-XS QToF (Waters Corporation), Figure 1, was operated in SONAR^{TM} 2DMS mode. The optimized quadrupole window and the other parameters employed for lipid and peptide analyses are described in Figure 2.



Expt	Ion Mode	Quad Scan (Da)	Quad Window (Da)	ToF Scan (Da)	Function Integration Time (s)	Fn 1 CE (V)	Fn 2 CE (V)
Lipidomics	+	500 to 1200	10	50 to 1000	0.1	6	20 to 50
Lipidomics	-	500 to 1200	10	50 to 1000	0.1	6	25 to 55
Proteomics	+	400 to 900	23	50 to 2000	0.5	6	14 to 40

Figure 2. SONAR^{TM} acquisition method and DIA acquisition parameters used in the different experiments.

Bioinformatics

SONAR^{TM} DIA data were processed using Progeneseis QI, Progenesis QI for proteomics (Nonlinear Dynamics) and ProteinLynx Global Server (Waters Corporation) using optimized threshold and search parameters. Quantitative analysis was performed with Skyline (University of Washington) using libraries derived from PQI, PQIp and PLGS compound or protein database searches.

RESULTS

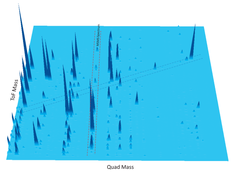


Figure 3. SONAR^{TM} DIA ToF vs. quadrupole m/z data, showing product ions (vertical bands) from peptides eluting over a 1 min window and the quadrupole sweep (diagonal line).

Specificity

Figure 3 represents typical SONAR^{TM} data and demonstrates that the data format is the same as other multi dimensional datasets, eg ion mobility; hence, exhibits improved specificity. When viewing these data, the drift time axis is replaced by quadrupole mass. The gain in acquisition specificity *vs.* a non resolving first mass analyser DIA (MS^E) based method is described in Figure 4.

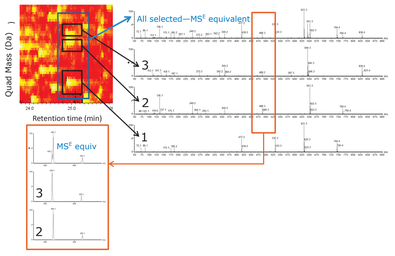


Figure 4. SONAR^{TM} DIA specificity improvement showing a small product ion region (top left) with 3 co-eluting peptides. Regions can be extracted to produce individual spectra (right, bottom 3 traces), along with DIA (MS^E) equivalent spectra (top). Bottom left demonstrates that in this small region where 2 fragments of about the same m/z (488 amu) are present, they can be separated and the interference removed.

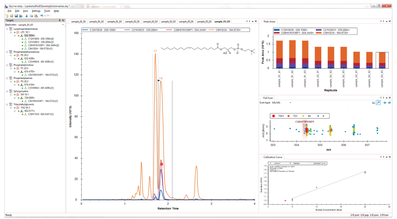


Figure 5a. Multiple dimension ion extraction using retention time, quadrupole (precursor) m/z and ToF (product ion) m/z for a synthetic lipid mixture spiked into human plasma extract.

Concept

Shown in Figures 5a and 5b is the conceptual use of the SONAR^{TM} 2DMS DIA data whereby precursor and product ions are extracted in multiple dimensions, *i.e.* retention time and first (quadrupole) and second (ToF) mass analyser *m/z*. As illustrated, the method is amenable to both metabolomics/ lipidomics (a) and proteomics applications (b). The following sections will demonstrate that this data can be utilized for both qualitative and quantitative analysis of (multi) omics data.

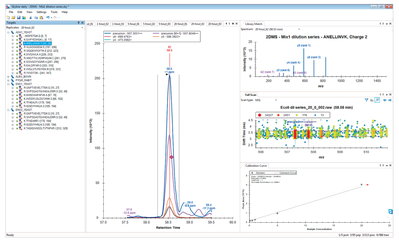


Figure 5b. Multiple dimension ion extraction using retention time, quadrupole (precursor) m/z and ToF (product ion) m/z for a 4 protein digest mixture spiked and serially diluted into a tryptic digest of cytosolic E.coli.

Discovery omics

The ability to search and quantify (relatively) SONAR^{TM} DIA omics data is shown in Figures 6 and 7, highlighting disease specific compounds, either lipids, peptides or proteins that show similar regulation trends.

Figure 6. Discovery lipidomics DIA 2DMS analysis illustrating identification, compound distribution, and grouped lipid quantitation/hierarchical clustering.

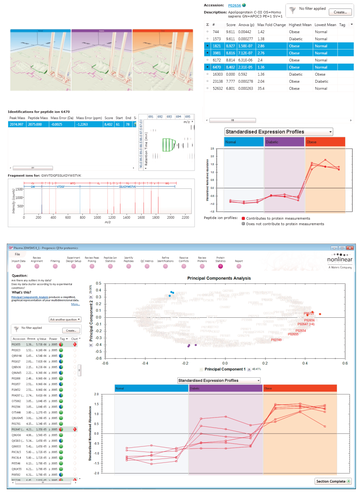


Figure 7. Discovery proteomics DIA SONAR^{TM} analysis strategy showing peak detection, identification, grouped quantitative peptide analysis, and protein quantitation/resolution for pathway associated proteins (apolipoprotein plasma/serum complement).

Targeted omics

A number of analytes, both lipids and peptides, were selected for targeted analysis of the data, Figure 8. In both instances, precursor/product ions list were provided to the software, along with quadrupole (precursor) *m/z* extraction information. Both examples show the presence of specific and a-specific product ions which can be readily identified and discarded for further down-stream quantitative analysis.

Application results validation

Application of SONAR^{TM} to discovery omic studies investigating obesity and diabetes has identified key targets including a variety of lipid classes, apolipoprotein alleles and a range of Ig proteins, all involved in metabolic syndrome related pathways.

Apolipoproteins in particular affect lipid metabolism by inhibition of lipoprotein lipase. Increased apolipoprotein CI and CIII are associated with increased triglycerides concentrations and decreased visceral adipose tissue area[3]. Obesity is also closely linked with inflammation, causing variation with immune system based pathways, such as phagocytosis. Quantitative profiles observed for a subset of the detected phospholipids and Ig-kappa proteins map to the phagocytosis pathway and correspond with previous findings linking B1 B cell phagocytosis with high fat diets[4].

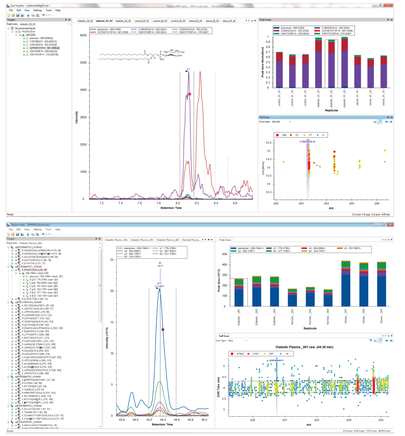


Figure 8. Targeted analysis of potential/reported disease specific lipid (Phosphatidylinositol) and protein (Apolipoprotein C1) markers of interest related to either obesity or diabetes.

CONCLUSION

- **SONAR^{TM} DIA acquisition provides multi dimensional data sets exhibiting improved specificity and over other DIA methods**
- **SONAR^{TM} data acquired in multi omics experiments shows excellent qualitative and quantitative characteristics and can be applied in discovery, targeted and translational omics studies**
- **Application of SONAR^{TM} detected and quantitatively confirmed known diabetes/ obesity proteins and lipid indicators**

References

1. M'Basu et al. Advances in Quadrupole and Time-of-Flight Mass Spectrometry for Peptide MRM based Translational Research Analysis. Proteomics, in press
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