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A High-Throughput Lipodomic Workflow Using the Waters Xevo MRT Mass Spectrometer

INTRODUCTION

The Xevo™ MRT Mass Spectrometer delivers an unrivaled combination of performance at speed, providing high resolution time-of-flight data independent of spectral acquisition rate. The MS specification of 100,000 (FWHM) resolution at scan rates of up to 100 Hz combined with <500 ppb mass accuracy enables confident identification of analytes at throughput levels that enable large cohort experiments.

The cutting-edge multi-reflecting ToF technology that Waters™ Corporation pioneered in the SELECT SERIES™ MRT, has been scaled into a compact benchtop platform to solve the most challenging problems in biomedical research and epidemiological studies.

In large scale metabolic phenotyping experiments performance is critical, from instrument stability over large studies to precision of measurement, generating high quality experimental outcomes. The novel acquisition system, with dual gain analogue-to-digital converter, delivers long term system stability over a high dynamic range, ensuring exceptional quality data with consistent robustness and reproducibility.

Here we describe, using an exemplar sample set, the use of the Xevo MRT Mass Spectrometer for the analysis of colorectal cancer human serum samples[1]. This combined with market leading chemistry and separations, using premier technology, and informatics including LipoStar2 from Mass Analytica™ (MassAnalytica.com) describes a complete lipidomic workflow.

EXPERIMENTAL CONDITIONS

SAMPLES

Samples	
Colorectal cancer (CRC) human serum samples	6 Healthy control plasma 4 Colon cancer plasma 2 Rectum cancer plasma NIST SRM 1950 plasma Study Reference/pooled (QC) A 100x dilution of EquiSPLASH

LC conditions	
LC System	Waters ACQUITY™ Premier
Column	ACQUITY Premier UPLC™ CSH™ C_{18} 2.1 x 50 mm, 1.7 µm
Column Temperature	55 °C
Sample temperature	8 °C
Injection volume	0.5 µL
Mobile phase A	50:25:25 H20:IPA:MeCN w/5 mM ammonium acetate and 0.05% acetic acid
Mobile phase B	50:50 IPA:MeCN w/5 mM ammonium acetate and 0.05% acetic acid
Flow rate	0.4 mL/min

Gradient table				
Time (min)	**Flow (mL/min)**	**%A**	**%B**	**Curve**
Initial	0.8	99.0	1.0	Initial
0.05	0.8	70.0	30.0	6
3.00	0.8	10.0	90.0	6
3.20	0.8	0.1	99.9	6
3.70	0.8	0.1	99.9	1
4.00	0.8	99.0	1.0	1

MS conditions	
Instrument	Xevo MRT Mass Spectrometer
Acquisition	Electrospray positive and negative ion mode
Capillary voltage	2.8 V/2.0 V
Desolvation temperature	500 °C
Source temperature	120 °C
Cone	40 V
Mass range	*m/z* 50–1200
Acquisition rate	10 Hz (for 200 injections) and 1, 5, 10 and 20 Hz
Acquisition mode	MS^E
Collision ramp in HE	20–45 eV
Acquisition and processing software	waters_connect™ platform with Sample Sub, AME and DATA Convert applications. LipidStar2, Mass Analytica

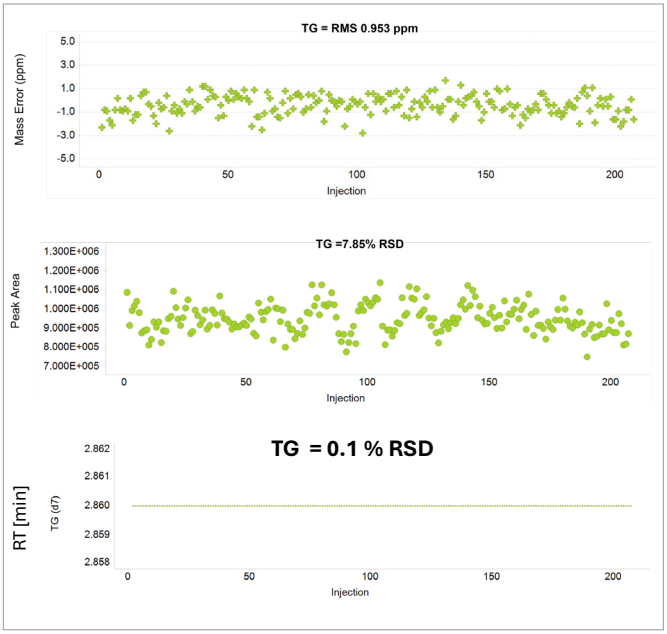

Figure 1: Mass measurement accuracy and peak area over 200 injections of a triglyceride lipid spiked into Plasma.

RESULTS

The Xevo MRT Mass Spectrometer was used to analyze a sample set of over 200 injections based on the Rapid Microbore Metabolic Profiling (RAMMP) methodology[2] with excellent analytical reproducibility observed for mass accuracy, retention time and peak area, and highlights the applicability of the system for complex biomedical research or epidemiological studies. The colorectal cancer human serum samples were prepared in duplicate and injected in triplicate in a randomized order, with EquiSPLASH spiked into the NIST plasma and the study QC samples injected every 6 sample injections.

Over the 200 injections, deuterated triglyceride d7 was observed with a mass accuracy of 0.95 ppm RSD and peak area measurement of 7.8% RSD (Figure 1). The excellent mass accuracy ensures analysts can use tight tolerances when performing compound identification reducing turnaround time from analysis to result. The exceptional tolerances on retention time and peak area measurement of 0.1% and 7.8% RSD, respectively allowed for high quality statistical analysis to be performed, and were demonstrated within the LipoStar2 software from Mass Analytica.

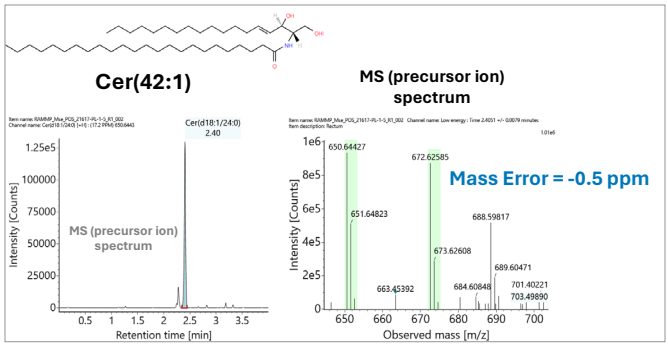

Figure 2: Identification of the ceramide lipid in the CRC study.

The ppm measurement accuracy, retention time and peak area for all of the components in the EquiSPLASH were 1.2 ppm RSD, <1 % RSD and 8.1 % RSD, respectively. Highlighted in this study was the use of the DATA Convert application to generate the universal file format, mzML, at the point of acquisition, which were then processed within LipoStar2. LipoStar2 is designed to process, perform statistical analysis to establish sample groupings and identify lipids through database searching and pathway profiling[3], enabling high quality scientific interpretation. Through the pathway profiling functionality within LipoStar2 the endogenous ceramide lipid Cer 18:1/24:0 (Figure 2) was identified within the Colon vs Rectum cancer sample sets, an up-regulated lipid in CRC dysregulation[4].

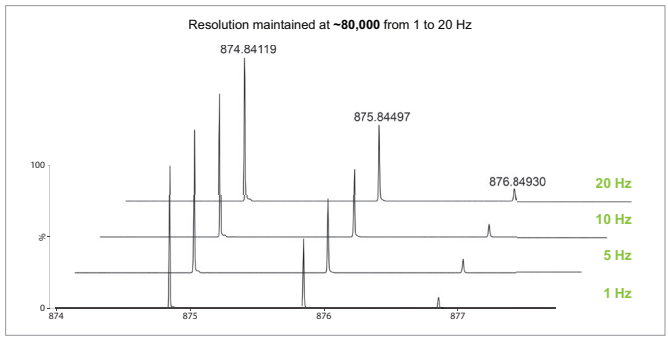

Figure 3: Mass resolution of 80,000 FWHM maintained at scan rates of 1, 5, 10 and 20 Hz.

The high quality data generated from multi reflecting time-of-flight technology delivers mass resolution consistent over a broad range and independent of acquisition rate. This was demonstrated on the endogenous lipid TG(52:3) measured at 1, 5, 10 and 20 Hz at a resolution of 80,000 FWHM (Figure 3).

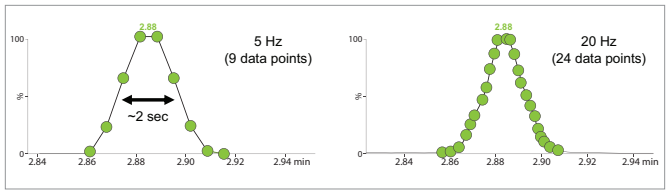

Figure 4: Extracted ion chromatogram of a triglyceride lipid from a 4 min LC/MS acquisition measured at 5 and 20 Hz.

The system can performed both qualitative and quantitative analysis. Figure 4 shows the impact of acquisition rate on the number of points across, both at the modest scan speed of 20 Hz, and at the lower rate of 5 Hz. The Xevo MRT Mass Spectrometer can operate up to 50 Hz in MS mode and 100 Hz in MS/MS mode and highlights the potential to acquire at faster scan speeds for increased throughput and lab productivity.

SUMMARY

The exceptional system performance and reproducibility of the Xevo MRT Mass Spectrometer enabled an extensive lipidomic study to be performed, and highlights the importance of system stability when looking for statistically significant biological differences within samples. The waters_connect platform applications were used to acquire and then export samples via .mzML for data processing, interpretation and library searching within LipoStar2. The study highlights a high-throughput lipidomic workflow that empowers scientists to make meaningful scientific discoveries, combining column chemistries, separations, and informatics with the exceptional data quality of the Xevo MRT Mass Spectrometer.

Unlock a new level of confidence with the Xevo MRT Mass Spectrometer

References

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2. Rapid Microbore Metabolic Profiling (RAMMP) with Ion Mobility for the Lipidomic Investigation of Plasma from Breast Cancer Patients, Adam King, Lee A. Gethings, Robert S. Plumb. Waters Application Note 720006321, June 2018.
3. Lipostar, a Comprehensive Platform-Neutral Cheminformatics Tool for Lipidomics, Laura Goracci, Sara Tortorella, Paolo Tiberi, Roberto Maria Pellegrino, Alessandra Di Veroli, Aurora Valeri, Gabriele Cruciani. Anal. Chem. 2017, 89, 11, 6257–6264
4. Ceramide Metabolism Enzymes – Therapeutic Targets against Cancer, Ana Gomez-Larrauri, Upasana Des Adhikari, Marta Aramburu-Nunez, Anita Custodia, Alberto Ouro. Medicina, 2021-07, 57(7), Article 729

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