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Application Note

waters_connect™-Enabled LipidQuan™ Workflow: A Tandem Quadrupole Method for Rapid Quantification of Bioactive Lipid in Plasma

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Published on September 4, 2026

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Abstract

Targeted lipidomics using tandem quadrupole mass spectrometry provides accurate quantitative information changes in biofluids and tissues lipid concentrations due to disease, environmental exposure, genetics etc. LipidQuan is a hydrophilic interaction liquid chromatography with tandem mass spectrometry (HILIC-MS/MS) method for the quantification of over 400 bioactive lipids in biofluids. This application note describes the acquisition and analysis of lipid concentration data using Xevo™ TQ Absolute XR Tandem Quadrupole Mass Spectrometer and waters_connect for Quantitation Software.

Benefits

- Quantitative method for the measurement of bioactive lipids in plasma to support translational biomarker studies
- Over 440 lipids measured in one simple, rapid (8-minute) HILIC method for polar and non-polar lipids
- Simple, intuitive data processing with exception focused review in waters_connect for Quantitation Software
- Multiple lipid classes including mono-, di-, triglycerides, FFA's, cholesterol esters. ceramides, hexosyl ceramides, PG, PC, SM, LPC, LPE, PS, PA, PI, LPA, and LPI in a single LC-MS/MS method

Introduction

Lipids perform a key role in multiple biological processes including cell signaling, energy storage, cell membranes creation, hormone level regulation, vital organs protection, and facilitate absorption of fat-soluble vitamins. The quantification of lipid in biofluids provides information on system health, pathophysiological processes, and disease staging.[1,2] LipidQuan is a validated method for the rapid quantification of over 400 bioactive lipids in biofluids, e.g., plasma and tissues[3] via HILIC-MS/MS operated in multiple reaction monitoring (MRM) mode. This application note describes the acquisition and data analysis bioactive lipid in rat plasma using ACQUITY™ Premier UPLC™ System coupled to Xevo TQ Absolute XR Tandem Quadrupole Mass Spectrometer and waters_connect Quantitation Software.

Experimental

Sample Preparation

A calibration line was prepared using the Avanti EquiSPLASH™ mix over the range of 0.334-167 ng/mL in protein precipitation solution. Rat plasma was prepared by protein precipitation with a pre-cooled acetonitrile:isopropanol (ACN:IPA) (2:1) at 4 °C (1:5, plasma:ACN/IPA) containing EquiSPLASH at 200 ng/mL. Samples were vortex-mixed for 1 minute and centrifuged at maximum of 10,300 g for 6 minutes at 4 °C before transferring the supernatant to glass vials for LC-MS analysis.

LC-MS/MS

The samples were analyzed using via a rapid (8-minute) HILIC-MS/MS method using an ACQUITY Premier UPLC System coupled to a Xevo TQ Absolute XR Tandem Quadrupole Mass Spectrometer operated in MRM mode with either +ve or -ve ESI ionization. The MRM precursor and product ion pair transitions, cone voltages and collision energies are described in Monjuma, et al.[3] A total of 431 transitions were acquired in +ve ESI and 446 transitions in -ve ESI. LC-MS/MS peak integration and lipid quantification was performed using the MS Quan Application in waters_connect for Quantitation Software.

LC Conditions

System:	ACQUITY Premier UPLC System
Column:	ACQUITY BEH™ Amide Column 1.7 µm, 2.1 × 100 mm
Column temperature:	45 °C
Flow rate:	0.6 mL/min
Mobile phase A:	95:5 Acetonitrile/water + 10 mM ammonium acetate
Mobile phase B:	50:50 Acetonitrile/water + 10 mM ammonium acetate
Gradient:	0.1% to 20.0% B for 2 minutes, then 20% to 80% B for 3 minutes followed by 3 minutes re-equilibration
Run time:	8 minutes
Injection volume:	1 µL

MS Conditions

System:	Xevo TQ Absolute XR Tandem Quadrupole Mass Spectrometer
Ionization mode:	ESI (+/-)
Capillary voltage:	2.8 kV (+)/1.9 kV (-)
Acquisition mode:	MRM
Source temperature:	120 °C
Desolvation temperature:	500 °C
Cone gas flow:	150 L/hr
Desolvation flow:	1000 L/hr
Nebulizer gas:	7 bar
Ion guide offset 1:	3 V
Ion Guide offset 2:	0.3 V

Data Management

Chromatography software:	waters_connect Software
MS software:	waters_connect Software
Data processing:	MS Quan Application

Results and Discussion

Quantitative lipidomics can provide insight into relative and quantitative changes in lipid abundance due to phenotypic variation, e.g., age, gender, disease state, and environmental exposure. Analysis of large data sets require a high-throughput method. LipidQuan Workflow facilitates the analysis of polar and non-polar lipid classes biofluids by employing a HILIC-based lipid class separation and MRM MS quantification.[3,4] The LipidQuan Methodology contains a total of 2,041 MRM transitions for 16 polar and non-polar lipid classes. The lipids were chromatographed using a rapid (8 minute) HILIC separation which facilitates classed-based separation based on the polar head groups. This facilitated classed-based quantification using the EquiSPLASH mix (Avanti Research) as it contains stable isotopically labeled (SIL) for 13 lipid classes. Representative chromatograms of the +ve & -ve ESI analysis of control rat plasma are shown in Figure 1.

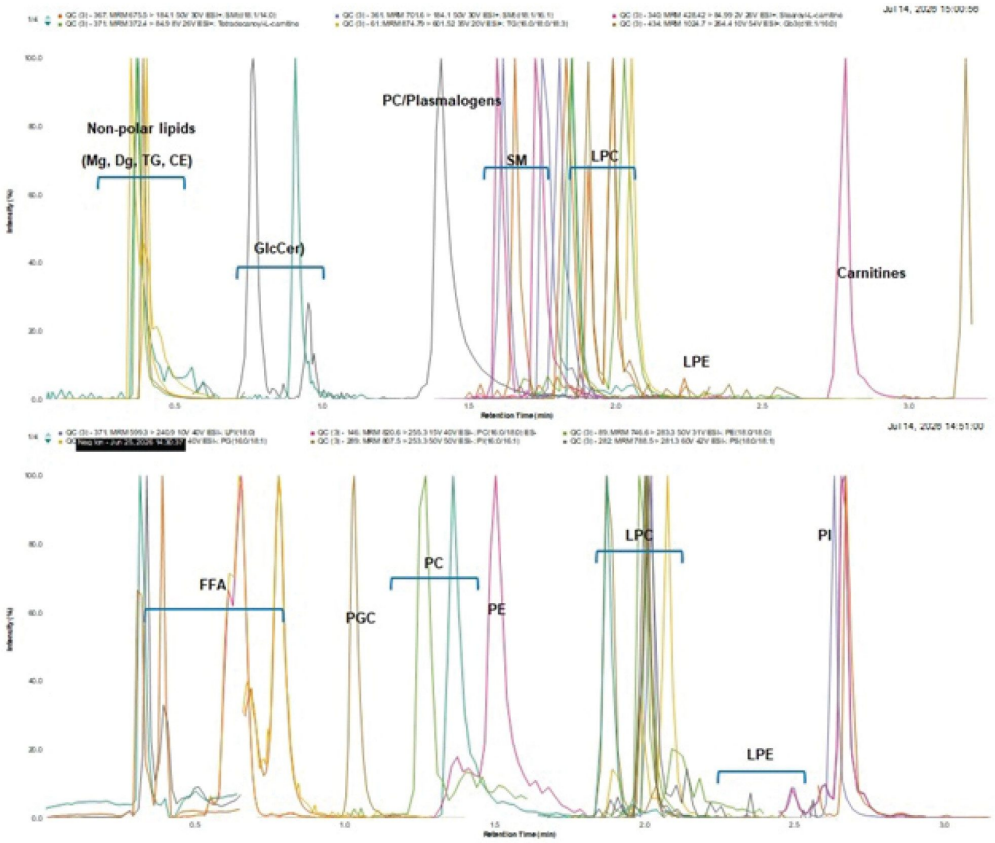
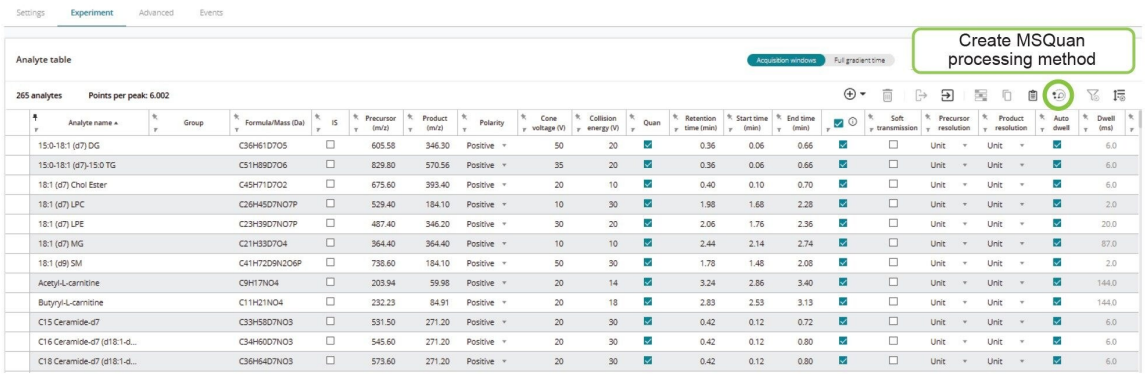


Figure 1. Positive (A) and negative (B) ion chromatograms of control rat plasma.

Data Acquisition

LC-MS/MS method was defined in the waters_connect Acquisition Manager. Time-based scheduling of the MRM transitions was employed to maximize MS dwell time and ensure that >8 points per peak were acquired for each lipid MRM transition, as shown in Figure 2. Data acquisition was controlled by waters_connect Sample Submission Application. The samples were queued in two separate batches, one for +ve ESI and one for -ve ESI mode, with the two data collected sequentially. A total of 239 and 232 lipids were measured in +ve ESI and -ve ESI modes, respectively. Robust data acquisition over the course of the study was ensured by the Stepwave™ XR Ion Guide of the Xevo TQ Absolute XR Tandem Quadrupole Mass Spectrometer, which prevents high mass ions contaminating the resolving quadrupole of the mass spectrometer.[4]



Analyte name	Group	Formula/Mass (Da)	IS	Precursor (m/z)	Product (m/z)	Polarity	Cone voltage (V)	Collision energy (V)	Quan	Retention time (min)	Start time (min)	End time (min)		Soft transmission	Precursor resolution	Product resolution	Auto dwell	Dwell (ms)
15:0-18:1 (d7) DG		C36H61D7O5	☐	605.58	346.30	Positive	50	20	☑	0.36	0.06	0.66	☑	☐	Unit	Unit	☑	6.0
15:0-18:1 (d7)-15:0 TG		C51H89D7O6	☐	829.80	570.56	Positive	35	20	☑	0.36	0.06	0.66	☑	☐	Unit	Unit	☑	6.0
18:1 (d7) Chol Ester		C45H71D7O2	☐	675.60	393.40	Positive	20	10	☑	0.40	0.10	0.70	☑	☐	Unit	Unit	☑	6.0
18:1 (d7) LPC		C26H45D7NO7P	☐	529.40	184.10	Positive	10	30	☑	1.98	1.68	2.28	☑	☐	Unit	Unit	☑	2.0
18:1 (d7) LPE		C23H39D7NO7P	☐	487.40	346.20	Positive	30	20	☑	2.06	1.76	2.36	☑	☐	Unit	Unit	☑	20.0
18:1 (d7) MG		C21H33D7O4	☐	364.40	364.40	Positive	10	10	☑	2.44	2.14	2.74	☑	☐	Unit	Unit	☑	87.0
18:1 (d9) SM		C41H72D9N2O6P	☐	738.60	184.10	Positive	50	30	☑	1.78	1.48	2.08	☑	☐	Unit	Unit	☑	2.0
Acetyl-L-carnitine		C9H17NO4	☐	203.94	59.98	Positive	20	14	☑	3.24	2.86	3.40	☑	☐	Unit	Unit	☑	144.0
Butyryl-L-carnitine		C11H21NO4	☐	232.23	84.91	Positive	20	18	☑	2.83	2.53	3.13	☑	☐	Unit	Unit	☑	144.0
C15 Ceramide-d7		C33H58D7NO3	☐	531.50	271.20	Positive	20	30	☑	0.42	0.12	0.72	☑	☐	Unit	Unit	☑	6.0
C16 Ceramide-d7 (d18:1-d...		C34H60D7NO3	☐	545.60	271.20	Positive	20	30	☑	0.42	0.12	0.80	☑	☐	Unit	Unit	☑	6.0
C18 Ceramide-d7 (d18:1-d...		C36H64D7NO3	☐	573.60	271.20	Positive	20	30	☑	0.42	0.12	0.80	☑	☐	Unit	Unit	☑	6.0

Figure 2. LipidQuan Acquisition Method (+ve ESI) in waters_connect Software.

Lipid Quantification

Lipid concentrations were quantified using the MS Quan Application in the waters_connect Software Platform; the acquisition method was created in the software. The individual concentrations of the EquiSPLASH calibration line were input into LEVELS tab in the MS Quan Application method, as shown in Figure 3a, and the response curve created from the EquiSPLASH curve was used to determine the concentration of the plasma lipids from the same class, as shown in Figure 3b. Quantification was performed via the construction of calibration curves using linear regression and 1/x weighting. A total of nine concentration levels were constructed to define the quantification range of 0.334-167 ng/mL. Representative calibration curve obtained for the lipids 18:1 (d7) LPE, 18:1 (d9) SM, 15:0 18:1 (d7) PE and 18:1 (d7) LPC are shown in Figure 4.

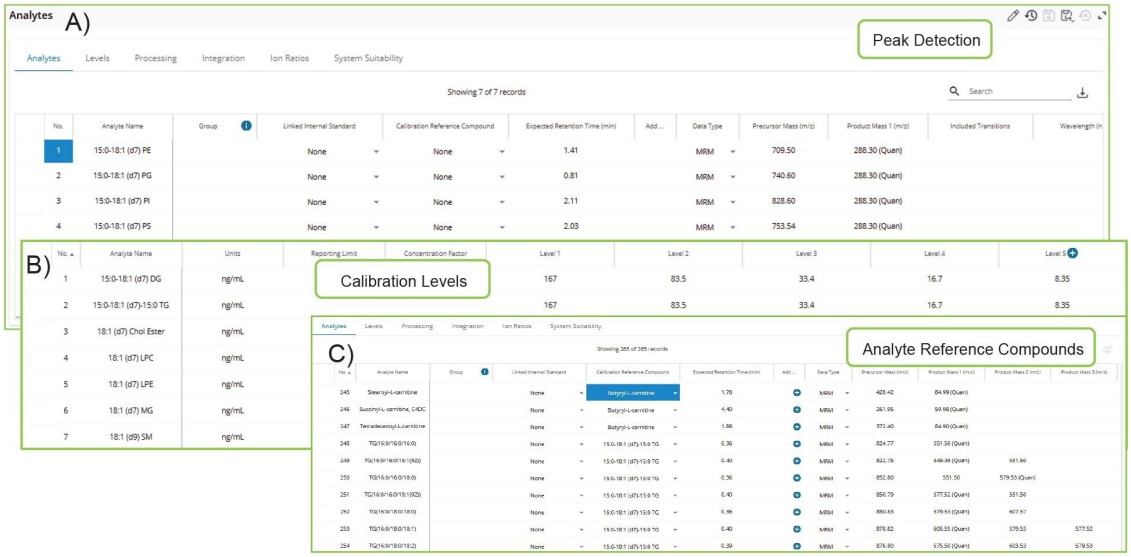


Figure 3. MS Quan Application analysis method for +ve ESI A) Analyte peak detection parameters, B) Calibration levels for SIL EquiSPLASh mix, C) Analyte reference compounds for individual lipids.

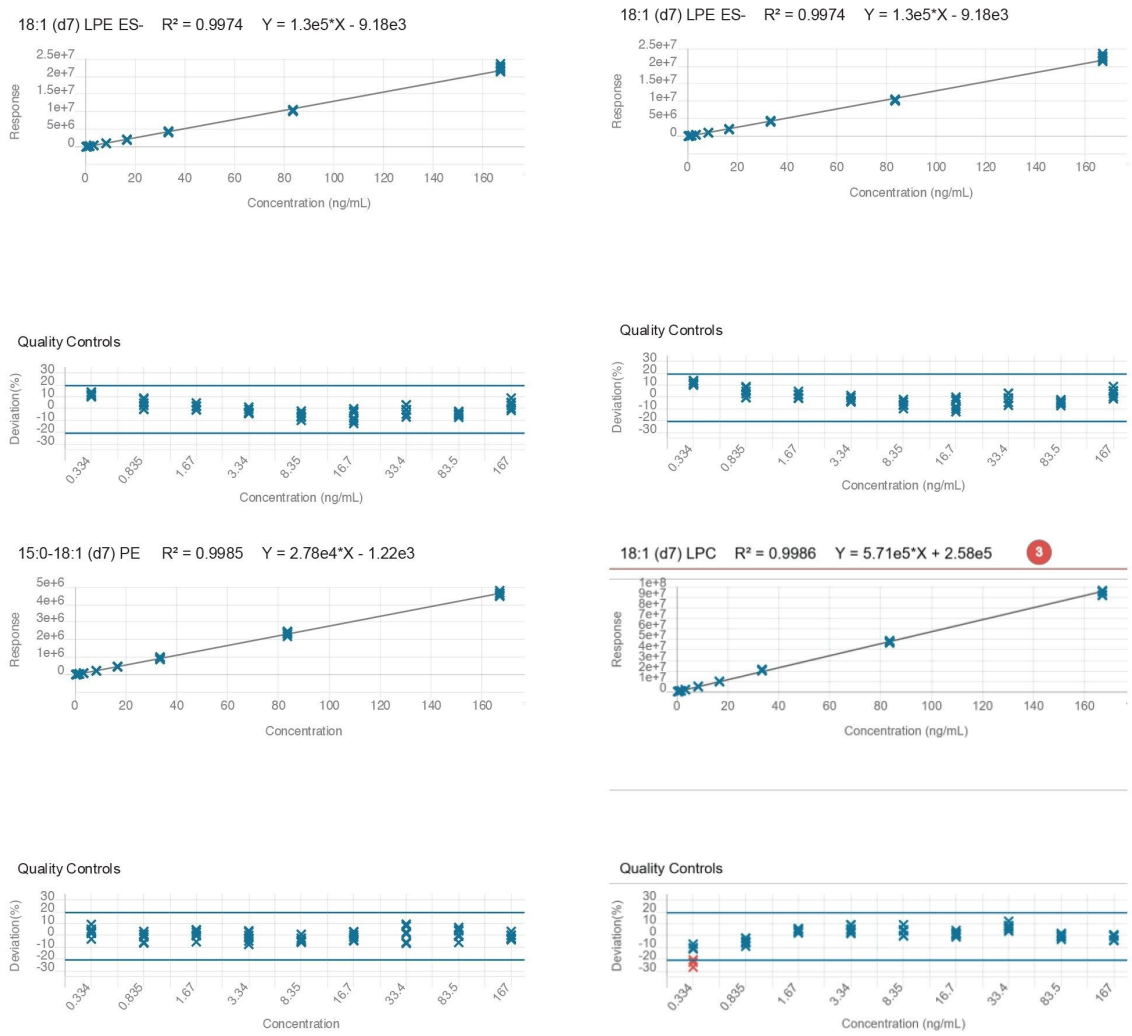


Figure 4. Representative calibration of lipids 18:1 (d7) LPE, 18:1 (d9) SM, 15:0 18:1 (d7) PE, and 18:1 (d7) LPC in rat plasma extract over the concentration range 0.334–167 ng/mL.

Data Analysis

The large number of lipids monitored (>440), combined with the complex nature of lipids, the HILIC separation mechanism, and number of lipid isomers isobaric results in the close elution of lipid species. These factors make baseline allocation review a complex and time-consuming process. MS Quan Application offers a rapid approach to data analysis allowing the scientist to review the data in one simple interface, as shown in Figure 5. Using a review by exception approach, allows scientist to quickly check calibration, QCs, and blanks for the whole data

set or for individual compounds (Figure 5).

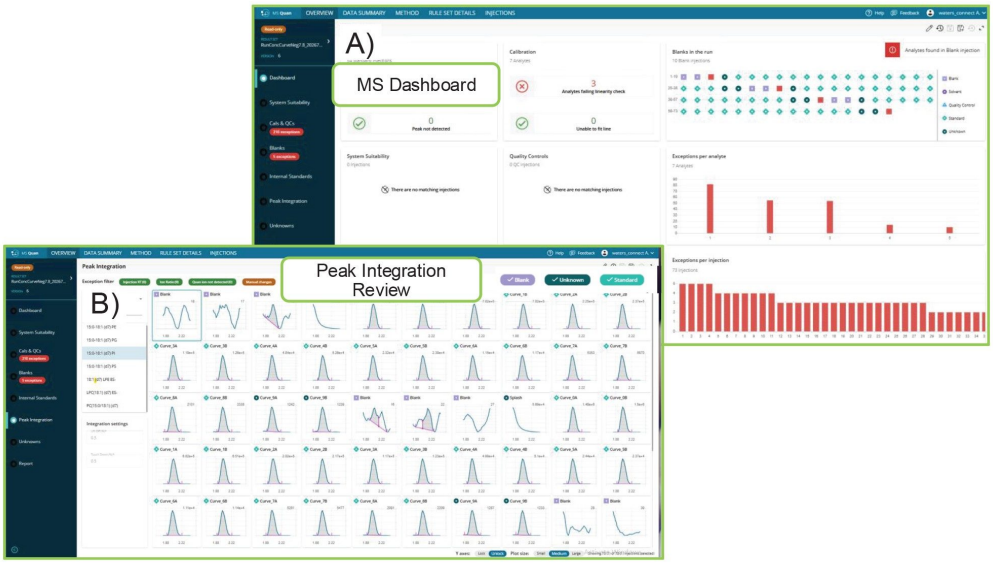


Figure 5. MS Quan Application dashboard and peak integration review.

Figure 6. Peak integration review and baseline modification.

Conclusions

The generation of accurate quantitative measurements of lipids provides insight into lipid dysregulation as a result of pathophysiological process, genetics, environmental exposure, and other factors. LipidQuan is a validated HILIC-MS/MS-based method for the quantification of over 400 bioactive lipids in biofluids and tissues. The Xevo TQ Absolute XR Tandem Quadrupole Mass Spectrometer combined with waters_connect

Software has been employed to streamline the acquisition and data analysis lipid concentrations in rat plasma. The high-speed MRM acquisition capability of the Xevo TQ Absolute XR Tandem Quadrupole Mass Spectrometer allowed for the collection of over 430 MRM transitions in just 5 minutes while the robustness of the StepWave XR Ion Guide ensured robust performance for the duration of the study. Data acquisition, peak integration, and lipid quantification was simplified by waters_connect for Quantitation Software and the MS Quan Application.

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720009559, September 2026

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