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Comparing Automatic Identifications in the Macrolipidomic Profiles of Human Whole Blood Across UHPLC-MS/MS Platforms and Acquisition Modes

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Overview

- **Purpose**: To examine the total number and quality of positive lipid identifications in human whole blood samples across different MS platforms and acquisition modes, and analysis using automated software.
- **Methods**: Lipid extracts from technical replicate samples were analyzed by UHPLC-MS/MS using the same LC method, but different acquisition modes on a Waters Synapt G2S*i* QTOF (HDDIA [aka HDMS^E], HDDDA and DDA), and a Thermo Q-Exactive Orbitrap (DDA). Data was processed using SimLipid software.
- **Results**: The QTOF-HDDDA method resulted in the largest number of total lipid identifications, but the QTOF-DDA method resulted in the largest number of identifications with Full-Acyl IDentifications (FAID).

Introduction

Automated identifications of various lipid species are critical for untargeted or "macrolipidomic" profiling. Software can match analytical data to extensive libraries of spectra, but different instruments employing different technologies and different acquisitions modes can influence the ability to match spectra with library references. For this exercise, whole blood was examined due to its complex lipid profile and potential use of this matrix for biomarker discovery through dried blood spots.

Methods

Chemicals, Materials and Instruments

All chemicals and solvents, including chloroform, methanol, isopropanol, acetonitrile, formic acid and ammonium formate were purchased from Thermo-Fisher Scientific. The column was a Waters Aqcquity UPLC CSH C18 with dimensions 1.7um x 2.1mm x 150mm. The instruments used were a Waters Synapt G2S*i* QTOF mass spectrometer coupled to a Waters UPLC I-class system, and a Thermo Q-Exactive Orbitrap mass spectrometer coupled to a Dionex UltiMate 3000 UHPLC system.

Sample Preparation and UHPLC Settings

Lipids were extracted from the whole blood (50uL) of a healthy 26-year old male volunteer in quadruplicate using 2:1 chloroform:methanol containing 500pmol of diheptadecanoyl phosphatidylcholine as the internal standard. Extracts were dried and reconstituted in 100uL 65:35:5 isopropanol:acetonitrile:water +0.1% formic acid. The samples were analyzed using UHPLC-MS/MS on the Waters and Thermo/Dionex systems using a multi-step, reversed-phase gradient consisting of A: 60:40 acetonitrile:water and B: 90:10 isopropanol:acetonitrile, both with 10mM ammonium formate and 0.1% formic acid. The flow was set at 250uL/min, the column was kept at 45ºC, sample tray at 4ºC, and injection volume was 10uL.

Tandem Mass Spectrometry and Data Analysis

The Waters QTOF mass spectrometer was operated in positive ESI, spray voltage 2.5kV, high-resolution mode (continuum; approx. 55,000 res in HD, 42,000 res non-HD), scan range *m/z* 50→1000, under three different acquisition modes: 1) QTOF-HDDIA (HDMS^E) with scan frequency 0.2sec and Transfer Cell collision energy ramp 20V→50V; 2) QTOF-HDDDA for top-5 ions with scan frequency 0.1sec and transfer cell collision energy ramps 20V→30V at low mass, and 30V→50V at high mass; 3) QTOF-DDA for top-5 ions with the same scan frequency and collision energy ramps as HDDDA. All QTOF-MS data was lock-mass corrected using a dedicated spray infusing leucine enkephalin (*m/z* 556.2771).

The Thermo Q-Exactive mass spectrometer was operated in positive ESI, spray voltage 2.5kV, 35,000 resolution in MS and 17,500 resolution in MS/MS, scan range *m/z* 70→1000, DDA for top-5 ions and normalized collision energy of 17.5 (QE-DDA). QE-DDA MS data was lock-mass corrected using diisooctyl phthalate (*m/z* 391.28421).

An inclusion list was generated from the QTOF-HDDIA experiment and was used in QTOF-HDDDA, QTOF-DDA and QE-DDA experiments, which included *m/z* values for 758 precursor ions. Data analysis was completed using SimLipid software (PREMIER Biosoft, CA, USA) and Ad-Hoc analyses on peak area integration and repeatability were completed using Waters MassLynx and Thermo Xcalibur software.

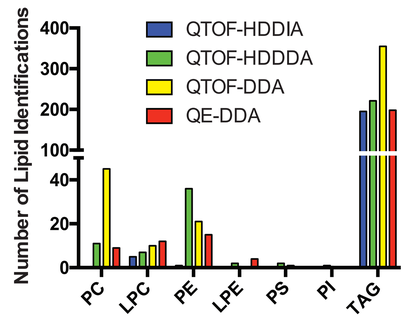
Results

Table 1. Lipid Identifications from the Four MS Acquisition Modes

Lipid Class	Sub Class	Acquisition Mode: QTOF-HDDIA	QTOF-HDDDA	QTOF-DDA	QE-DDA
PC	Diacylglycerophosphocholines	69	268	400	104
PC	1-alkyl,2-acylglycerophosphocholines	38	77	114	42
PC	Monoacylglycerophosphocholines	14	17	21	14
PC	1-(1Z-alkenyl),2-acylglycerophosphocholines	7	44	67	7
PC	Dialkylglycerophosphocholines	5	16	22	4
PC	1-acyl,2-(1Z-alkenyl)-glycerophosphocholines	0	4	7	1
PC	1-acyl,2-alkylglycerophosphocholines	1	2	7	0
PC	1Z-alkenylglycerophosphocholines	1	2	3	0
PC	Monoalkylglycerophosphocholines	2	2	2	0
PC	Oxidized glycerophosphocholines	0	0	3	0
PE	Diacylglycerophosphoethanolamines	16	164	95	34
PE	1-(1Z-alkenyl),2-acylglycerophosphoethanolamines	6	35	8	10
PE	Monoacylglycerophosphoethanolamines	0	9	0	6
PE	1-alkyl,2-acylglycerophosphoethanolamines	1	35	0	1
PE	Dialkylglycerophosphoethanolamines	0	2	0	0
PE	1-acyl,2-alkylglycerophosphoethanolamines	1	1	0	0
PE	Oxidized glycerophosphoethanolamines	4	4	5	5
PS	Diacylglycerophosphoserines	7	25	25	12
PI	Diacylglycerophosphoinositols	0	7	0	0
PG	Dialkylglycerophosphoglycerols	1	3	0	0
PG	Diacylglycerophosphoglycerols	1	0	0	0
PA	Diacylglycerophosphates	4	67	0	0
PA	Monoacylglycerophosphates	0	1	0	0
Sterols	Steryl esters	6	4	5	5
TAG	Triacylglycerols	631	906	474	433
Total		**815**	**1695**	**1258**	**678**

PC, glycerophosphocholines; PE, glycerophosphethanolamines; glycerophosphoserines; PI, glycerophosphinositols; PG, glycerophosphoglycerols; PA, glycerophosphates; TAG, triradylglycerols.

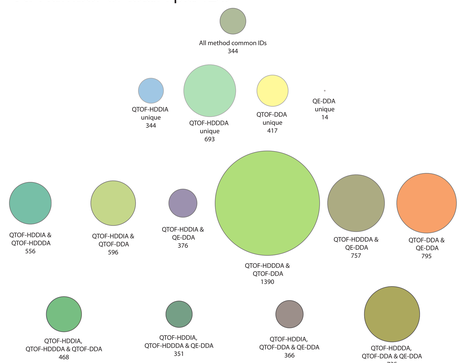
Figure 1. Lipid IDs with Full-Acyl IDentifications (FAID)



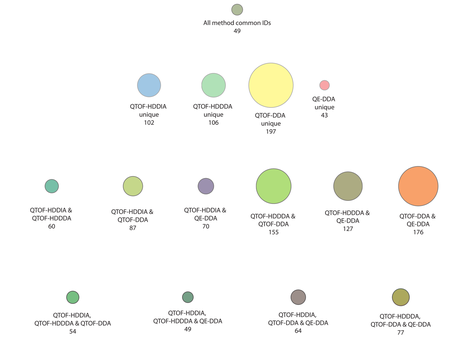
PC, diacylglycerophosphocholines; LPC, Monoacylglycerophosphocholines; PE, Diacylglycerophosphoethanolamines; LPE, Monoacylglycerophosphoethanolamines; PS, Diacylglycerophosphoserines; PI, Diacylglycerophosphoinositols; TAG, Triacylglycerols. FAID is achieved when three acyl fragments are identified for TAG, two for PC, PE, PS and PI, and one for LPC and LPE in the MS/MS spectra.

Figure 2. Lipid Identifications Within and Between MS Acquisition Methods

A. Number of total lipid IDs

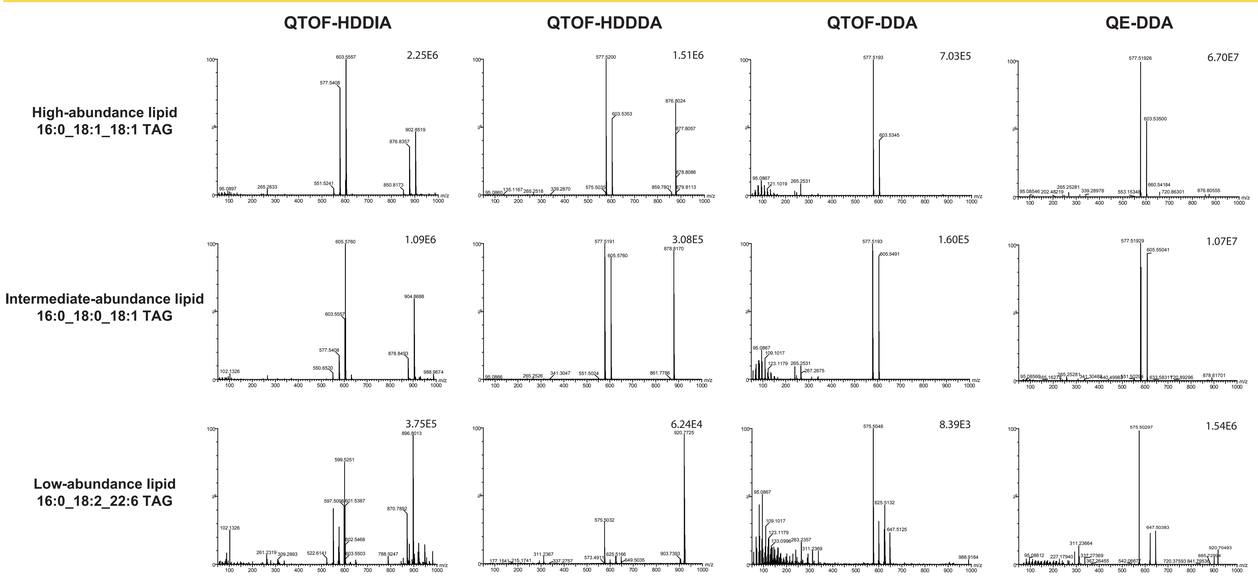


B. Number of lipid IDs with FAID



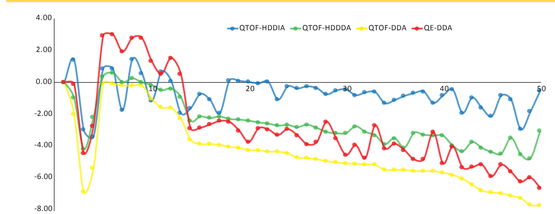
The size of the circles is proportional to the number of identifications with the size of the circles in **B** scaled up 300% relative to **A**.

Figure 3. MS/MS Spectra for Selected High-, Intermediate-, and Low-Abundant Lipid Species in Whole Blood Across MS Platforms and Acquisition Modes



The underscore in the compound name indicates that there is no explicit regiospecific information given in terms of *sn*-1, *sn*-2 or *sn*-3 localization on the glycerol backbones of these structures.
The QTOF-HDDIA method resulted in a convoluted MS/MS spectra (likely fragments from other lipid species that co-eluted and were not properly assigned to the precursor ions of interest).
The QTOF-HDDDA and QTOF-DDA were similar but there was greater fragmentation in QTOF-DDA despite the same collision energy ramps.
The QTOF-DDA and QE-DDA spectra showed similar fragmentation patterns and relative ion abundances despite being done on different instruments.

Figure 4. Log2-ratio transformation of normalized abundances of PC species



The repeatability of quantitation of 50 PC species (x-axis), where fragment ion absolute abundances from MS/MS spectra were summed and divided by the sum of fragment ions from the internal standard. Coefficients of variability were below 5% for all methods after integration of peak areas from extracted ion profiles using precursor ion accurate masses.

Conclusions

- QTOF-HDDDA resulted in the largest number of lipid IDs, but QTOF-DDA resulted in the largest number of species with FAID.
- There were 344 species in total that were identified in consensus across the four methods, and 49 with FAID. QTOF-HDDDA and QTOF-DDA had the highest consensus while QTOF-HDDIA tended to have the lowest.
- Fragmentation patterns were similar across methods, but parent ion abundances were much lower in QTOF-DDA and QE-DDA than QTOF-HDDDA suggesting that colision energy optimization may be necessary to maximize lipid identifications.
- The quantitative ability of QTOF-HDDDA, QTOF-DDA and QE-DDA was similar using summed absolute abundances of fragment ions.