Plasma Lipid Profiling Using High Resolution Mass Spectrometry and Complementary Fragmentation Strategies

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Overview

Purpose: A complete untargeted workflow for lipid profiling and identification of potential biomarkers in plasma samples.

Methods: MS profiling with high resolution mass spectrometry and a combination of CID and HCD fragmentation for high throughput lipid identification.

Results: More than 700 lipids identified and relatively quantified in plasma.

Introduction

Lipids are essential cellular constituents of mammalian cells that have many critical roles in cellular functions. Many lipid families such as glycerophospholipids, sphingolipids, glycerolipids and sterols are mainly present in outer layer of cell plasma membranes while other lipids are also involved in energy storage or participate in cell signalling. Due to the high structural diversity of lipids, a complete molecular profiling of biological matrices remains a challenge in lipidomics for qualitative method development. In this study, plasma lipid profiling was performed using RP LC-HRMS including MS^n experiments (CID and HCD) and Thermo Scientific™ LipidSearch™ software. Integrated data processing tools including peak detection, alignment algorithms and automatic annotation were added to increase the relevance of identified lipid species.

Methods

Sample Preparation

Plasma total lipid extract was prepared using modified Bligh and Dyer extraction. Plasma sample was diluted at 1/100 with a solution of MeOH / IPA / H2O (65:35:5) before injection. The sample was diluted by the following dilution factors: 1/2, 1/4, 1/8, 1/16 and 1/32.

Liquid Chromatography and Mass Spectrometry

Lipids were separated on a Hypersil Gold C8 150x2.1 mm column and detected with high resolution mass spectrometry (Orbitrap Elite™, Thermo Scientific) operated in positive and negative modes at resolution R=120,000 averaging 2 microscans. Data dependant tandem mass spectra were acquired either by resonance fragmentation in the ion trap (CID MS^n) or ion beam collision cell fragmentation (HCD) (Figure 1).

LC parameters:

Negative mode:

A: H2O/MeOH (60/40), 0.1 % formic acid.

B: IPA/MeOH (90/10), 0.1 % formic acid.

Positive mode:

A: H2O/MeOH (60/40), 0.1 % formic acid, 10mM ammonium formate.

B: IPA/MeOH (90/10), 0.1 % formic acid, 10mM ammonium formate.

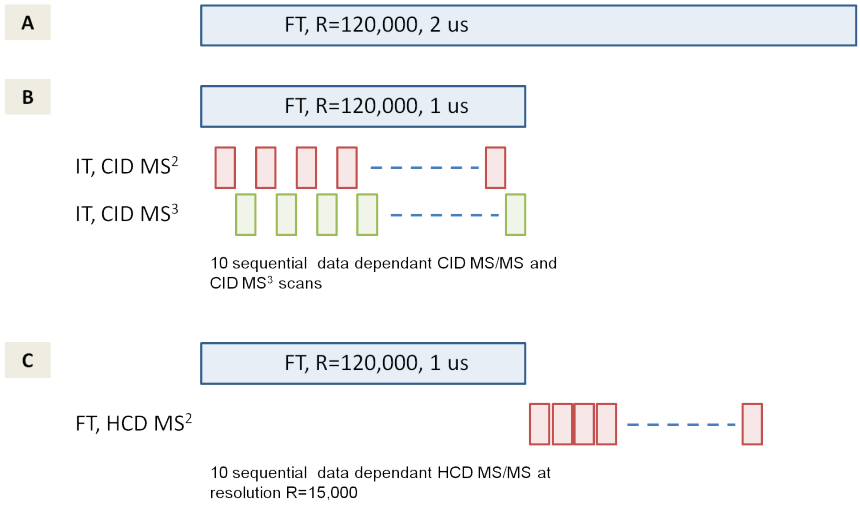
Flow rate: 0.4 mL/min

Column Hypersil Gold C8 150x2.1 mm, 3 μm particle size
Column T°C : 60°C
Injection volume : 10μL

MS parameters:

	Positive mode:	Negative mode:
Spray voltage:	3.7 kV	3.1 kV
Sheath gas:	15	20
Auxiliary gas:	10	5
S-Lens RF:	70%	70%
Capillary Temperature:	320	320
Mass range:	150-2000	150-2000
Full Scan Resolution:	120,000	120,000
OT HCD resolution:	15,000	15,000
Full Scan AGC:	3E6	3E6
MS^n AGC:	5E4	5E4
MSn fragmentation energy:	35%	35%
HCD fragmentation energy:	35%	35%

FIGURE 1. LC-MS/MS methods



Data Analysis

Full scan spectra were processed using a software tool developed from XCMS / Cent Wave allowing exact mass information and retention time to be matched with existing databases. In addition a newly develop lipid database search algorithm (LipidSearch) was used to match the different types of MS^n spectra with precursor and predicted fragments databases for lipid identification.

Results

Lipids from plasma were extracted and analyzed by high resolution mass spectrometry including a survey scan at resolution R=120,000 with Orbitrap detection (Figure 1A). Lipids were identified based on retention time alignment between unique lipid species, their corresponding ^{13}C and $^{13}C_2$ isotopes and their isotopic ratios. More than 700 isotopic patterns were detected from obtained full scan experiments (Figure 2 and 3). Reproducibility of the analytical workflow was evaluated by repeating the sample LC/MS analysis 3 times. An overall variation of less than 25% (coefficient of variation) was observed for more than 80% of the detected signals.

FIGURE 2. Data processing : application to human plasma samples.

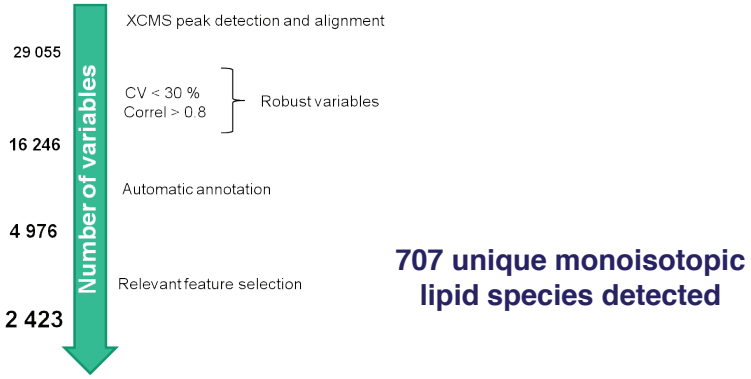
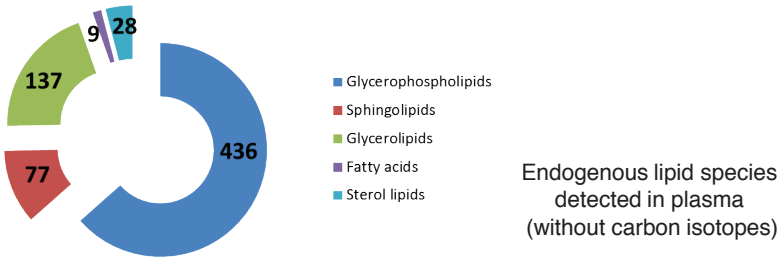


FIGURE 3. Lipid mapping based on Full Scan MS information.



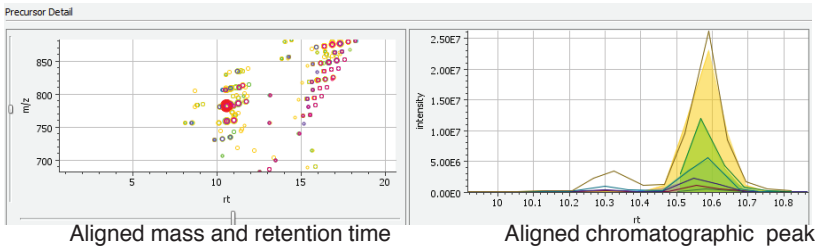
Unambiguous lipid identification requires tandem mass spectrometry due to the heterogeneity of structures and a multiplicity of isomers. Fragments from several stages and types of fragmentation were collected in order to obtain a variety of information which can be subsequently matched to databases for lipid identification. LipidSearch software contains a database of lipids and associated fragments. Correlating this information with our experimental data resulted in 491 lipid species identified by CID MS^n and 302 by HCD which can be classified following their polar head and structure (Table 2).

TABLE 2. Lipid identification with LipidSearch software

Class	HCD Positive	CID Positive	HCD Negative	CID Negative
LPC	*24*	*3*	*14*	*9*
PAF	*0*	*0*	*2*	*8*
PC	*52*	*51*	*40*	*47*
LPE	*0*	*0*	*4*	*5*
LdMePE	*0*	*0*	*8*	*9*
PE	*0*	*9*	*11*	*9*
dMePE	*0*	*1*	*13*	*12*
PS	*0*	*0*	*0*	*1*
PG	*2*	*0*	*0*	*0*
LPI	*0*	*0*	*1*	*4*
PI	*0*	*0*	*2*	*4*
PEt	*0*	*0*	*0*	*1*
PMe	*0*	*1*	*0*	*0*
SM	*30*	*0*	*22*	*38*
MG	*0*	*30*	*0*	*0*
DG	*7*	*100*	*0*	*0*
TG	*116*	*155*	*0*	*0*
FA	*0*	*0*	*0*	*2*
OAHFA	*0*	*0*	*0*	*1*
GM3	*0*	*0*	*1*	*0*
ChE	*4*	*5*	*0*	*0*
SiE	*1*	*0*	*0*	*0*
DGDG	*0*	*0*	*1*	*2*
Total	236	355	119	152

Several dilutions were performed to access linearity and limit of detection. LipidSearch software was used to align the identified lipid species after perform peak detection and integration. Integrated peaks are displayed and the corresponding MS^2 spectra are overlaid for easy comparison and validation. Samples can be grouped and ratios against a control experiment are calculated (Figure 4).

FIGURE 4. Alignment of PC (16:0/20:4)+H across dilutions: 1/2, 1/4, 1/8, 1/16 and 1/32. Reported ratios are 0.45, 0.26, 0.11, 0.045, 0.022.



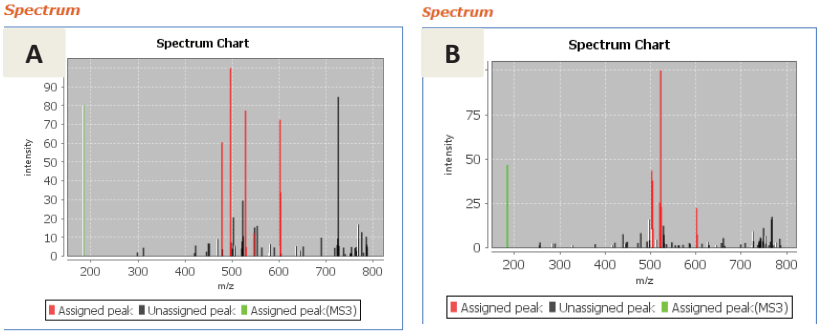
Aligned mass and retention time

Aligned chromatographic peak

Aligned MS^2 spectra

In many cases CID MS^2 produces fragments which contribute to the identification of the aliphatic chain and allow to discriminate several isobaric species (Figure 5).

FIGURE 5. Fragmentation of PC (36:3)+H by CID MS^n. A: Identification of PC (16:0/20:3)+H. B: Identification of PC (18:1/18:2)+H.

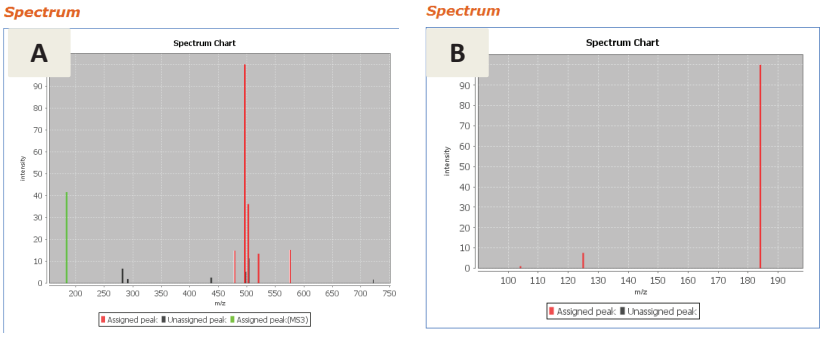


ProductIon	Type	ObsMz	Intensity
LPC(16:0)-OH	MS2	478.365	60.5
LPC(16:0)+H	MS2	496.473	100
LPC(16:0)+H [isotope]	MS2	497.454	6.9
LPC(20:3)-OH	MS2	528.42	77.4
LPC(20:3)-OH [isotope]	MS2	529.425	4.9
LPC(20:3)+H	MS2	546.449	12.2
NL[PC]	MS2	601.686	72.5
NL[PC] [isotope]	MS2	602.656	33.9
NL[PC,H]	MS2	602.656	33.9
(P-Cho)+H	MS3	184.028	80.2

ProductIon	Type	ObsMz	Intensity
LPC(18:2)-OH	MS2	502.473	43.4
LPC(18:2)-OH [isotope]	MS2	503.431	10.4
LPC(18:1)-OH	MS2	504.468	37.9
LPC(18:1)-OH [isotope]	MS2	505.393	10.9
LPC(18:2)+H	MS2	520.533	25.4
LPC(18:2)+H [isotope]	MS2	521.624	9.3
LPC(18:1)+H	MS2	522.431	100
LPC(18:1)+H [isotope]	MS2	523.516	22.8
NL[PC]	MS2	601.767	22.3
NL[PC] [isotope]	MS2	602.819	7.5
NL[PC,H]	MS2	602.819	7.5
(P-Cho)+H	MS3	184.024	46.7

However the low mass cut-off of CID experiments often prevent the confirmation of the polar head group. This limitation is overcome by the use of CID MS^3 (Figure 6A) or HCD fragmentation (Figure 6B). Figure 3 displays an example where both strategies give access to the phosphocholine (P-Cho) group and identify PC (16:0/18:2).

FIGURE 6. Identification of PC (16:0/18:2)+H with CID MS^n(A) and HCD(B) fragmentation

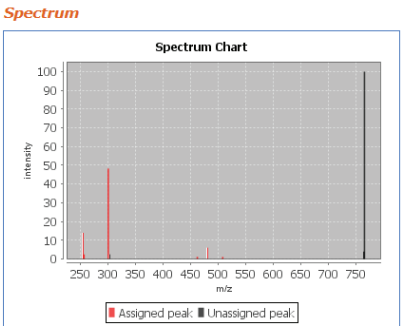


ProductIon	Type	ObsMz	Intensity
LPC(16:0)-OH	MS2	478.442	14.8
LPC(16:0)+H	MS2	496.386	100
LPC(18:2)-OH	MS2	502.454	36.2
LPC(18:2)+H	MS2	520.515	13.5
NL[PC]	MS2	575.581	15.2
(P-Cho)+H	MS3	184	41.6

ProductIon	Type	ObsMz	Intensity
C5H14N1O1	MS2	104.107	1
C2H6O4P1	MS2	124.999	7.5
(P-Cho)+H	MS2	184.073	100

Beam type HCD fragmentation does not suffer from low mass cut-off and produces complementary fragment ions as demonstrated in Figure 7 where dMePE(16:0/20:5)-H is fragmented by HCD to provide both neutral loss fragments of each fatty acid loss and the corresponding fatty acid ion in negative mode.

FIGURE 7. Identification of dMePE(16:0/20:5)-H with HCD fragmentation.



ProductIon	Type	ObsMz	Intensity
FA(16:0)-H	MS2	255.233	14
FA(20:5)-H-CO2	MS2	257.227	2.4
FA(20:5)-H	MS2	301.217	48.2
NL[FA(20:5]	MS2	462.297	1.2
NL[FA(20:5]+H2O	MS2	480.309	6.1
NL[FA(16:0]	MS2	508.282	1.1

Conclusion

- A complete profiling strategy for detection of lipids based on accurate mass, isotope profile, retention time and MS^n information.
- More than 700 lipids identified in plasma with LipidSearch for identification based on MS^n information.
- Sample alignment for differential analysis and relative quantification available in LipidSearch.
- Acquisitions in positive and negative polarity provides complementary identification across different lipid classes.
- CID MS^3 fragmentation is often necessary to confirm lipid class identification based on polar head group detection.
- HCD fragmentation is a good alternative and provides fragment information across the entire mass range.

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