Identification of a Novel Lipid Family From Leukemia Cell Line

Seetaramanjaneyulu Gundimeda, Arunkumar Padmanaban.
Agilent Technologies India Pvt. Ltd, Bangalore, Karnataka, India

Agilent Technologies

Summary

Lipidomic studies can reveal aberrant lipid signatures in disease conditions. Discovery of novel lipids can lead to a better understanding of lipid biochemistry. We carried out lipid profiling studies of a leukemia cell line, THP-1. Lipids were extracted with methanol, water and chloroform (1:1:2 v/v) then the lipid extract was analyzed by RPLC on a Q-TOF-MS. Filtering the data using MassHunter prior to library search gave better results. Data filtered based on characteristic fragment ion or neutral loss in the MS/MS spectra identified lipid classes including PC, PE, PE, PI and PS. Several ether lipids are also identified from the lipid extract. Two phospholipids showed a novel fragment ion at *m/z* 111 and an uncommon neutral loss of 32 Da in the MS/MS spectra. This fragmentation behavior is attributed to a modification on the phosphate head group. This head group is associated with all kinds of hydrophobic chains similar to known classes of lipids.

Introduction

Lipid analysis can help us to better understand the metabolism. Dysfunctional lipid metabolism has been a signature in many metabolic disorders. Altered lipid metabolism has been reported in cancer, Alzheimer's, Parkinson's etc., Novel lipid identification has been a topical area globally and helped scientists in understanding lipid biosynthesis in a better way. Identification of a new class of oxidized phosphatidyl cholines (PC) helped scientists in identifying the mechanism of scavenging Low Density Lipoprotein (LDL) when it is oxidized. Along with accurate mass information, MS/MS information is also necessary for an unambiguous identification of lipids. Due to variations in ionization efficiencies among lipid classes, it may be essential to acquire the data both in positive and negative modes. Sometimes, this can severely hamper the detection of low ionizing species like ether lipids. To avoid this, lipid extract can be fractionated on a cartridge to reduce complexity.

Experimental

Lipids were extracted from cells/plasma using Folch's method involving methanol/water/chloroform (1:1:2 v/v). The total lipid extract was loaded on an Agilent Bond Elut aminopropyl cartridge to separate the mixture into individual classes of lipids by eluting them with suitable solvents. Ten different fractions were obtained each belonging to a different lipid class.

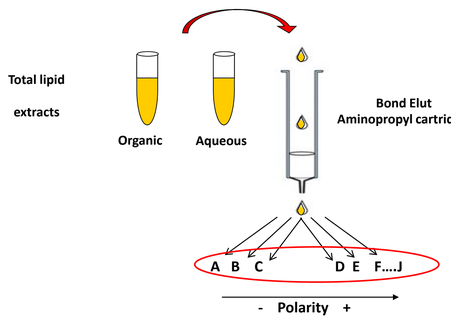
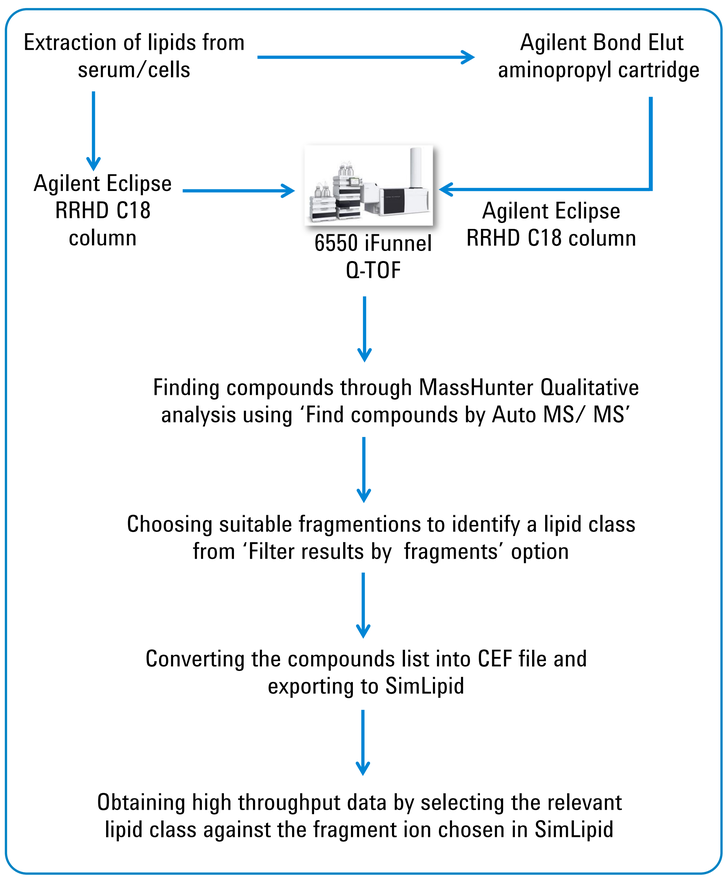


Figure 1. Schematic diagram showing fractionation of lipid extract using solid phase extraction

Work flow



Results and Discussion

Total lipid extract without any fractionation was injected on to LC-MS and MS/MS data was obtained in negative mode. MS/MS data was acquired at low collision energy to register facile neutral losses and at high collision energy to get structural information. Using MassHunter, the MS/MS data was filtered to get spectra containing a fragment ion at *m/z* 140, a characteristic for phosphatidyl ethanolamines (PE). PCs are usually analyzed in positive mode. They can also be analyzed in negative mode in the form of a formate or acetate adduct. Upon MS/MS, PCs show a facile loss of 60 Da for the formate adduct and 74 Da for the acetate adduct. MS/MS data was filtered to identify this netural loss. Similarly, the data was filtered to identify phosphatidyl inositols (PI) using the fragment ion at *m/z* 241. The *m/z* 153 fragment ion was used to identify phosphatidic acids (PA) and a neutral loss of 87 Da was used to identify phophatidyl serines (PS). A partial list of PEs and PCs as identified by SimLipid are given in Tables 1 and 2.

Table 1. Partial list of lipids with fragment ion m/z 140 in the MS/MS spectra as identified by SimLipid

S.No.	m/z	No. of Hits	Main Class	Abbreviation/Common Name	Score	Lipid ID
1	724.53	5	PE	PE(P-16:0/20:3(8Z,11Z,14Z))	0.2726	LMGP02030027
2	724.53	5	PE	PE(P-16:0/20:3(8Z,11Z,14Z))	0.2726	LMGP02030027
3	726.5457	5	PE	PE(P-18:0/18:2(9Z,12Z))	0.2131	LMGP02030046
4	726.5457	5	PE	PE(P-16:0/20:2(11Z,14Z))	0.1798	LMGP02030026
5	726.5457	5	PE	PE(P-18:0/18:2(9Z,12Z))	0.2131	LMGP02030046
6	728.5612	5	PE	PE(P-18:0/18:1(9Z))	0.2429	LMGP02030004
7	728.5612	5	PE	PE(P-16:0/20:1(11Z))	0.2095	LMGP02030025
8	728.5612	5	PE	PE(P-18:0/18:1(9Z))	0.2429	LMGP02030004
9	742.5403	17	PE	PE(18:1(9E)/18:1(9E))	0.2238	LMGP02010039
10	744.556	19	PE	PE(18:0/18:1(9Z))	0.2772	LMGP02010036
11	744.556	19	PE	PE(18:0/18:1(9Z))	0.2772	LMGP02010036
12	744.556	19	PE	PE(18:0/18:1(9Z))	0.2772	LMGP02010036
13	746.5153	1	PE	PE(P-16:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	0.2625	LMGP02030001
14	748.5303	2	PE	PE(O-16:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	0.2417	LMGP02020020
15	748.5303	2	PE	PE(P-18:0/20:5(5Z,8Z,11Z,14Z,17Z))	0.2452	LMGP02030056
16	750.546	6	PE	PE(O-16:0/22:5(4Z,7Z,10Z,13Z,16Z))	0.1548	LMGP02020006
17	750.546	6	PE	PE(P-16:0/22:4(7Z,10Z,13Z,16Z))	0.2131	LMGP02030033
18	750.546	6	PE	PE(P-18:0/20:4(5Z,8Z,11Z,14Z))	0.2726	LMGP02030003
19	750.546	6	PE	PE(P-18:0/20:4(5Z,8Z,11Z,14Z))	0.2131	LMGP02030003
20	766.541	16	PE	PE(18:0/20:4(5Z,8Z,11Z,14Z))	0.3054	LMGP02010118
21	774.5468	1	PE	PE(P-18:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	0.275	LMGP02030005
22	776.5615	1	PE	PE(P-20:0/20:5(5Z,8Z,11Z,14Z,17Z))	0.0929	LMGP02030086

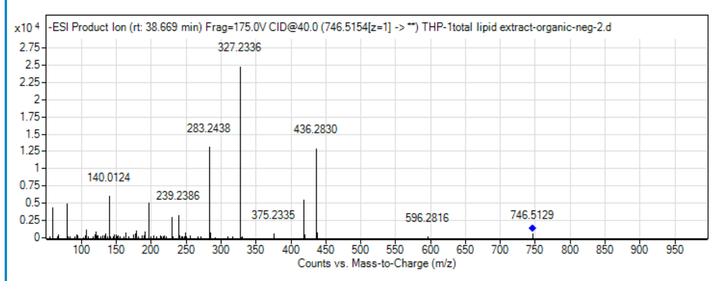


Figure 2. MS/MS spectra of ion *m/z* 746 identified as PE(p-16:0/22:6)

Table 2. Partial list of lipids with a neutral loss of 60 Da in the MS/MS spectra as identified by SimLipid

S.No.	m/z	Abbreviation/Common Name	Matched Ions
1	526.3517	PC(O-8:0/O-8:0)	168.0435(23.0416), 377.2464(17.81), 395.2573(3.1002), 466.3299(100.0), 526.3474(0.4178)
2	776.5465	PC(16:0/16:1(9Z))	168.0441(2.9968), 480.3106(5.0248), 645.4488(0.4044), 716.5253(50.669), 776.5482(4.6354)
3	776.5823	PC(O-16:0/17:1(9Z))	168.0452(2.3301), 448.3182(4.6106), 466.3298(7.3257), 716.5608(62.2532), 776.5807(15.1877)
4	778.5622	PC(16:0/16:0)	168.0439(2.3442), 462.299(1.5275), 480.3105(3.3262), 718.541(38.1114), 778.5632(4.3062)
5	788.5822	PC(O-16:0/18:2(9Z,12Z))	448.3186(3.5495), 466.3304(9.928), 728.5604(62.1327), 788.5846(6.7691)
6	790.5988	PC(O-16:0/18:1(9Z))	448.3195(3.4953), 466.3304(7.997), 730.577(64.9164), 790.5985(8.1877)
7	812.5831	PC(O-16:0/20:4(5E,8E,11E,14E))	285.2216(0.3348), 466.3311(12.5816), 752.561Z(65.9954), 812.5814(9.1799)
8	814.5982	PC(O-16:0/20:3(8Z,11Z,14Z))	448.3174(2.2539), 466.3311(12.2134), 754.5764(62.968), 814.5897(11.3579)
9	832.6091	PC(18:0/18:1(11Z))	168.0441(3.5128), 490.3301(1.5409), 508.3405(5.2542), 772.5873(67.9147), 832.609(10.6638)
10	846.6613	PC(O-16:0/22:1(11Z))	448.3215(5.008), 466.3305(5.9868), 786.6396(100.0), 846.6601(20.2847)
11	856.6093	PC(18:0/20:3(5Z,11Z,14Z))	168.0437(5.646), 490.326(2.5278), 508.3364(4.2435), 796.5878(80.0761), 856.6054(15.5549)

Figure 3. MS/MS spectra of ion *m/z* 776, identified as PC (16:/16:1) by SimLipid

Identification of a Novel Lipid

Phospholipids typically give fragment ions at *m/z* 153, 97 and 79. Among these ions 97 and 79 arise from phosphate moiety. MS/MS spectra of two precursor ions at *m/z* 409 and 437 showed ion at *m/z* 111, at a shift of 14 Da from the expected ion at *m/z* 97. This can be correlated to the presence of an additional CH_2 in the head group. The spectra also showed another uncommon neutral loss of 32 Da from the precursor. These two observations strongly indicated the presence of a P-OMe group in the phosphate head. The spectra did not show any other intense ion related to the hydrophobic chain. Acyl and alkenyl chains give dominant fragment ions in the MS/MS spectra of lipids. $RCOO^-$ ions generated from acyl chains are very stable and hence dominate the MS/MS spectra. Vinylic ion, $R\text{-}C{=}C\text{-}O^-$ generated from alkenyl chains also shows a reasonably intense peak in the MS/MS spectra. In the absence of any such dominant peak corresponding to the hydrophobic chain, precursor ions 409 and 437 may contain an ether linked hydrophobic chain. The rigidity to fragment may be correlated to the formation of a stable cyclized structure after the loss of methanol. A similarity in the fragmentation behavior made both precursors 409 and 437 fall in the same class of lipids. with a difference of 28 Da, these two lipids can be expected to differ in the length of the hydrophobic chain.

Characterization of the Head Group

Accurate mass measurement confirmed the proposed structure with a methyl hydrogen phosphate head group and C16 ether linked hydrophobic chain for ion 409 and C18 chain for ion 437. The MS/MS spectra of these compounds is given in figures 4 & 5.

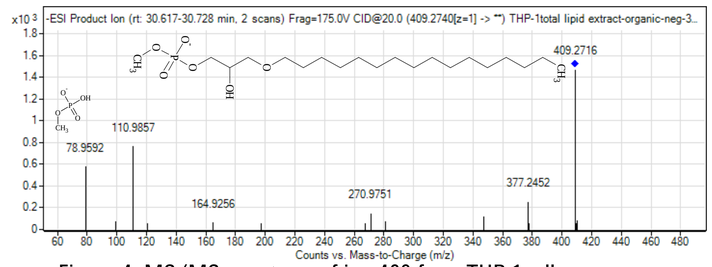


Figure 4. MS/MS spectrum of ion 409 from THP-1cells

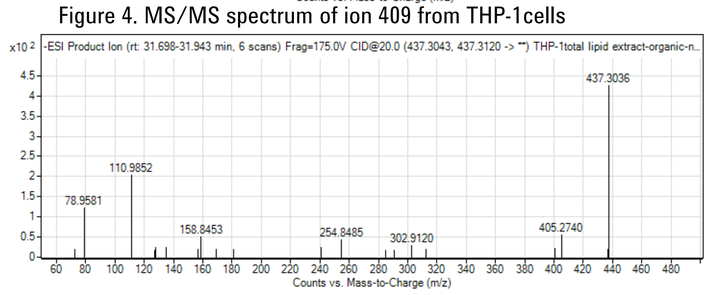


Figure 5. MS/MS spectrum of ion 437 from THP-1cells

Table 3. Fragment ions from MS/MS spectra of Phosphatidic acid

m/z	Ion Description	Structure (click to enlarge)
395.26	Precursor ion [M-H]-	
171.01	Glycerol-3-phosphate ion	
153.00	Glycerol-3-phosphate ion with loss of H2O	
96.97	H2PO4- ion (from phosphate)	
78.96	PO3- ion (from phosphate)	

Peak list from the MS/MS spectrum of an ether linked phosphatidic acid with an unmodified head group showed an ion at *m/z* 97 corresponding to phosphate moiety which is missing in the spectra of ion 409 and 437. The peak list also indicated that ion *m/z* 111 is not common to phosphatidic acids confirming the modification on the head group in the structure.

Identification of Modified Lipids From Human Plasma

The total lipid extract from plasma was loaded onto an aminopropyl cartridge and was separated into ten different fractions each containing different lipid class. All these fractions were subjected to LC-MS analysis. The modified lipids identified in THP-1 cells were observed in human plasma also. Apart from C16 and C18, hydrophobic chains with unsaturation were also found to contain the methyl hydrogen phosphate head group. MS/MS spectra of ion 435 with a C18:1 hydrophobic chain was given as an example in figure 6. Lipids with ester linked hydrophobic chains were also found to contain this modified head group. MS/MS spectra of ion 451 was given as an example of this class of lipids in figure 7. Due to the high charge carrying capacity, ester fragment ion dominated the spectra.

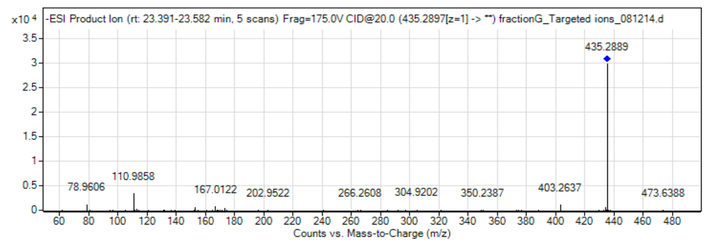


Figure 6. MS/MS spectrum of ion 445 from human plasma

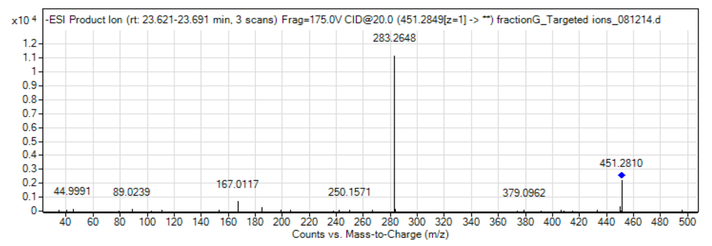


Figure 7. MS/MS spectrum of ion 437 from THP-1cells

Conclusions

- SimLipid could be used to identify the lipids from their MS/MS spectra
- Low ionizing lipids such as ether lipids were identified from the total lipid extract using the Agilent 6550 iFunnel Q-TOF mass spectrometer without any fractionation
- A novel lipid with a modification on the phosphate head group was identified
- Lipids with this modified head group were found to contain different classes of hydrophobic chains similar to known classes of lipids
- A deep understanding of the biochemical pathway of this lipid class can enhance knowledge about the functions of lipids in health and disease