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C146-E103

Identification of Phospholipid Molecular Species Using Neutral Loss Survey and MS^3 Analysis

Technical Report vol.2

1. Introduction

To elucidate the functions of phospholipids, it is important to conduct analysis not only of their classes and sub-classes, but of their molecular species as well. At Shimadzu, we found that electrospray ionization (ESI) MS^3 analysis is effective for more detailed and accurate annotation of each molecular species. We established a system for phospholipid molecular species analysis using a neutral loss (NL) survey experiment of the head-group-related mass values, and succeeding MS^3 analyses by selecting the resulting product ions as precursor ions for MS^3 analyses (Fig. 1). Using this new method, 34 different molecular species of phosphatidylcholine (PC) were identified even without pre-separation by LC. We then turned our attention to establishing a system for analysis of phosphatidylethanolamine (PE) and phosphatidylserine (PS) in a mixture of lipids.

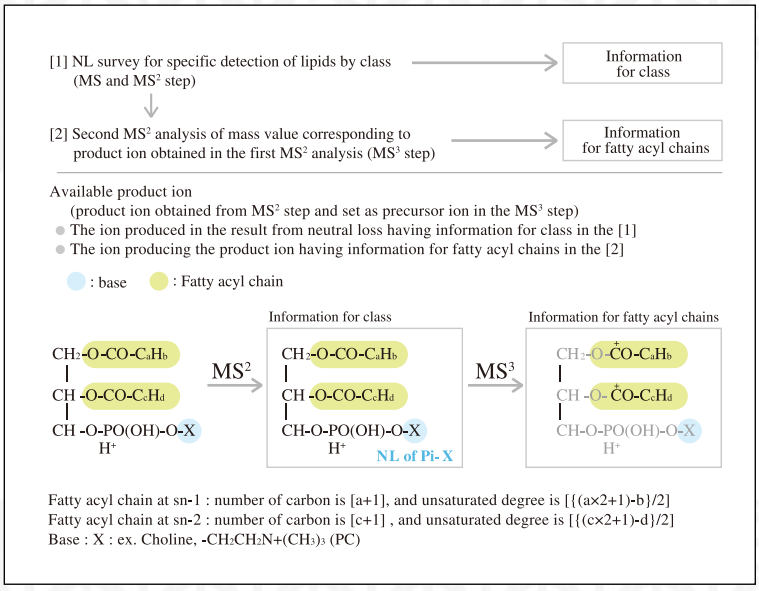

Fig. 1 System for Analysis of Phospholipid Molecular Species
- Neutral Loss (NL) Survey and MS^3 Analysis -

In the NL survey + MS^3 analysis, information for class and fatty acyl chains were obtained to make detailed analysis of diradyl phospholipids

2. Method

All of the phospholipids were extracted from rat brain (approx. 2 g), liver (approx. 5 g) and calf serum (100 μL) according to Bligh and Dyer's method. The ESI-MS analysis was conducted using a Shimadzu LCMS-IT-TOF (ion trap-time-of-flight mass spectrometer). The extracted phospholipids were directly subjected to ESI MS^2 and MS^3 analysis, using a Si60 column (1 x 100 mm, Nomura Chem., Japan). The mobile phase consisted of acetonitrile/ methanol (spiked with 0.1 % ammonium and 0.3 % acetate).

3. Results

The [M-phosphorylethanolamine] ([M-(Pi-EthN)]$^+$) ion corresponding to the [diglyceride-OH]$^+$ ion is generated by MS^2 analysis of the [M+H]$^+$ ion of PE in the positive mode (Fig. 2). By conducting MS^3 analysis, selecting this [M-(Pi-EthN)]$^+$ as the second precursor ion, [fatty acid (FA)-OH]$^+$ ions are generated through neutral loss of monoglyceride (MG) moieties, and [MG-H]$^+$ ions are generated by neutral loss of FA-related moieties, allowing effective identification of PE species fatty acyl chains. By conducting MS^2 of the [M-H]$^-$ ion in the negative mode, the [M-serine]$^-$ ion, corresponding to the [phosphatidic acid-H]$^-$ ion, was generated as a product ion (Fig. 3). By selecting this [M-serine]$^-$ ion as the second precursor ion, the fatty acyl chains of the PS species were effectively identified by MS^3 analysis, which generated the corresponding [FA-H]$^-$ ion.

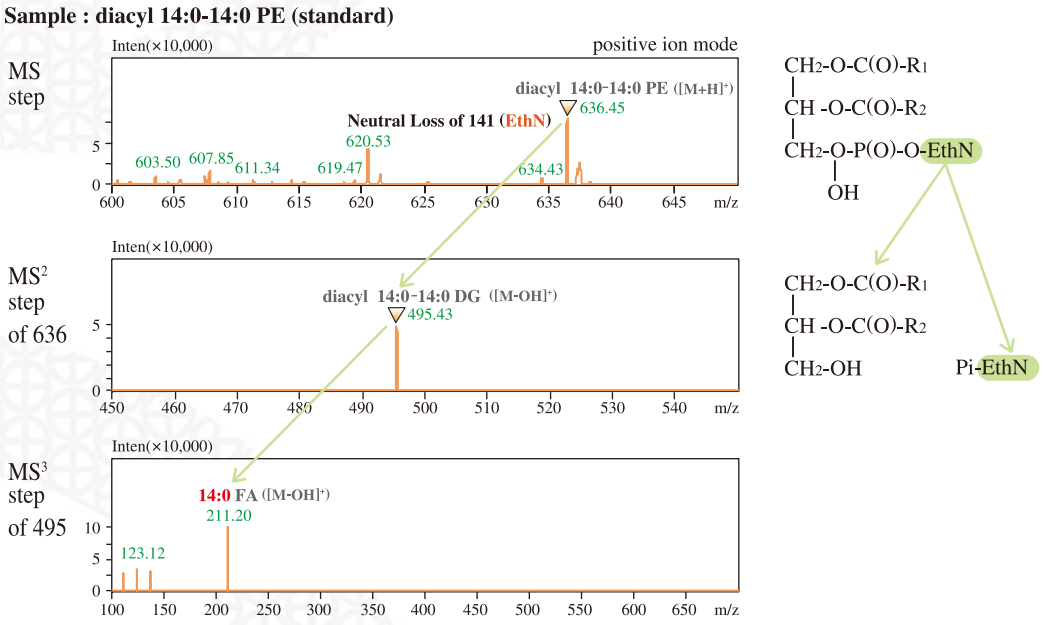

Fig. 2 Neutral Loss Survey and MS^3 Analysis of PE
Two fatty acyl chains of PE were identified using a combination of NL survey of 141 u ($C_2H_3NH_2$+H) and MS^3 of DG (MS^2 product ion).

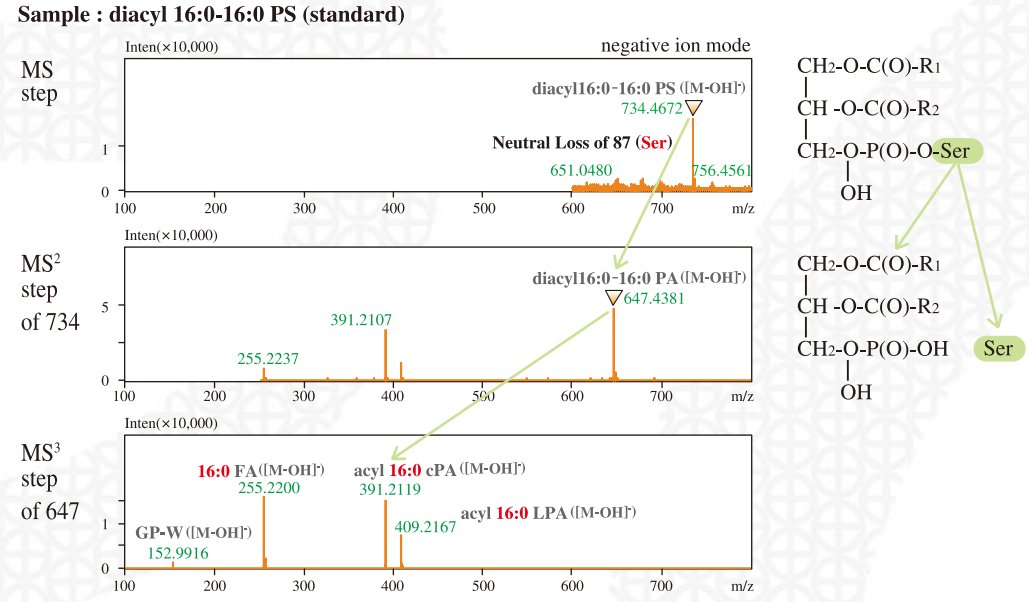

Fig. 3 Neutral Loss Survey and MS^3 Analysis of PS
Two fatty acyl chains of PS were identified using a combination of NL survey of 87 u [$C_2H_3(NH_2)COOH$+H] and MS^3 of PA (MS^2 product ion).

Using this new method in conjunction with the MS^2 method in rapid analysis allowed identification of 7 molecular species of PS (Table 1, Figs. 4, 5). Using MS^3 in combination with NL survey allowed highly accurate identification of two fatty acyl chains of the phospholipids. In addition to the NL survey information obtained in MS^2 analysis, the LCMS-IT-TOF proved very useful in assuring reliable identification of the two fatty acyl chains by providing excellent mass accuracy for the MS^3 product ions (Table 2). 132 (one hundred thirty-two) phospholipids (including PC, sphingomyelins, lysophosphatidylcholine, PE, lysoPE, PS, phosphatidylinositol, phosphatidylglycerol, and triglyceride) were identified in the lipid mixture derived from the rat liver, as summarized in Table 3. The possibility of using this method for quantitative analysis of the metabolome was also investigated. A 1.3-fold increase or 20% decrease in detection was obtained using this method. It is expected that this new method will be effective for comprehensive lipid metabolome analysis.

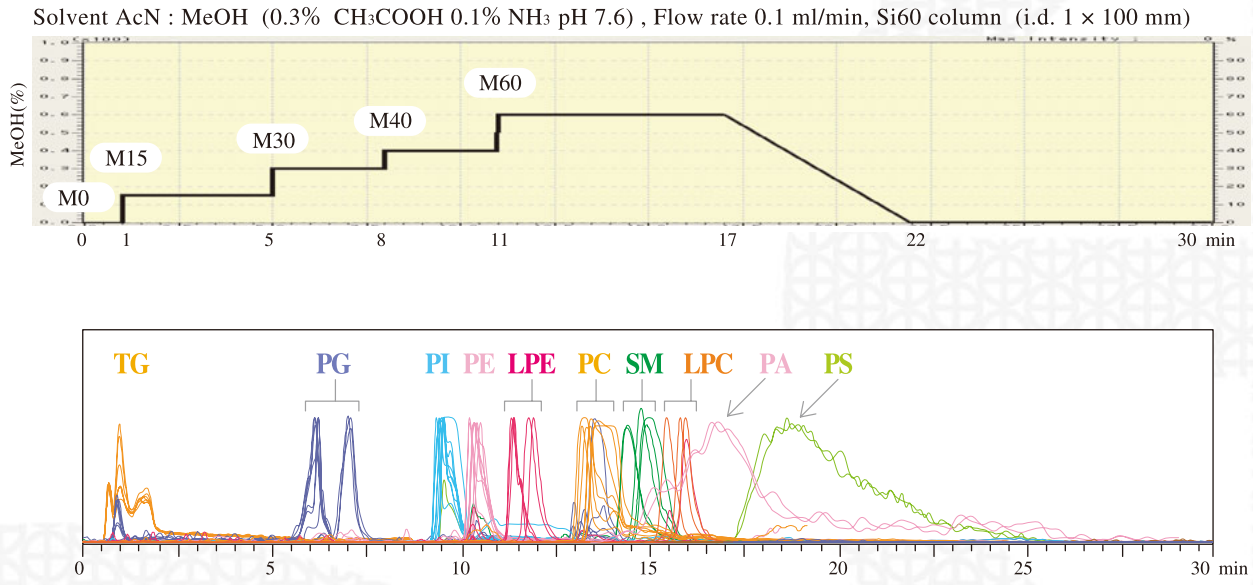

Fig. 4 Retention Times of Respective Lipid Classes Using a Si Column

	Molecular Species	Theoretical m/z	Difference (Da)	Mass Accuracy (ppm)
1	diacyl 38:4 PS (18:0–20:4)	810.5285	0.0017	2.1
2	diacyl 40:6 PS (18:0–22:6)	834.5285	0.0020	2.4
3	diacyl 40:5 PS (18:0–22:5)	836.5442	0.0034	4.0
4	diacyl 36:1 PS (18:0–18:1)	788.5442	0.0019	2.4
5	diacyl 36:2 PS (18:0–18:2)	786.5285	0.0069	8.8
6	diacyl 36:4 PS (16:0–20:4)	782.4972	0.0037	4.7
7	diacyl 38:6 PS (16:0–22:6)	806.4972	0.0015	1.8

Table 1 Identification of PS in Rat Liver

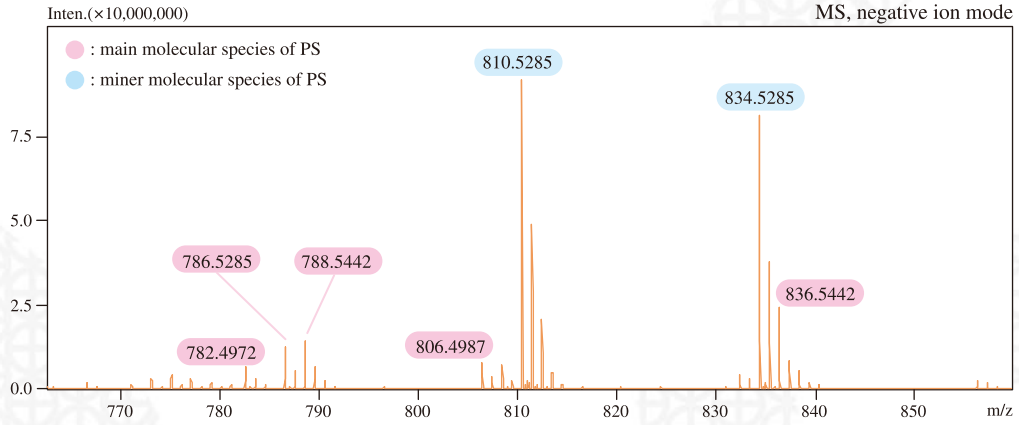

Fig. 5 Mass Spectrum of PS in Rat Liver

Number of possible candidates : m/z of MS

	Possible candidates for peak at m/z 810		theoretical m/z	difference (Da)	mass accuracy (ppm)
1	diacyl 38 : 4 PS	-H	810.5285	0.0017	2.1 ↑10ppm
2	alkacyl 41 : 10 DiMePE	-H	810.5438	0.0170	20.9
3	alkacyl 43 : 10 PE	-H	810.5438	0.0170	20.9 ↑20ppm
4	diacyl 40 : 10 DiMePE	-H	810.5074	0.0194	24.0
5	diacyl 42 : 10 PE	-H	810.5074	0.0194	24.0
6	alkacyl 39 : 4 PS	-H	810.5649	0.0381	47.0
7	alkacyl 40 : 11 PS	-H	810.4710	0.0558	68.9
8	diacyl 39 : 3 DiMePE	-H	810.6013	0.0745	91.9
9	diacyl 41 : 3 PE	-H	810.6013	0.0745	91.9
10	diacyl 39 : 11 PS	-H	810.4346	0.0922	113.8 ↑130ppm
11	alkacyl 40 : 3 DiMePE	-H	810.6377	0.1109	136.8
12	alkacyl 42 : 3 PE	-H	810.6377	0.1109	136.8

Reduction in number of possible candidates
10ppm : 1
20ppm : 3
130ppm : 10

percentage of reduction
10ppm : 100% (=1/1*100)
20ppm : 33% (=1/3*100)
130ppm : 10% (=1/10*100)

Number of possible candidates : m/z of MS^3

	Possible candidates for peak at m/z 283		theoretical m/z	difference (Da)	mass accuracy (ppm)
1	acyl 18 : 0 FA	-H	283.2637	0.0000	0.0 ↑10ppm
2	alk 19 : 0 FA	-H	283.3001	0.0364	128.5
3	alk 20 : 7 FA	-H	283.2062	0.0575	203.1
4	acyl 19 : 7 FA	-H	283.1698	0.0939	331.6

Reduction in number of possible candidates
10ppm : 1

	Possible candidates for peak at m/z 303		theoretical m/z	difference (Da)	mass accuracy (ppm)
1	acyl 20 : 4 FA	-H	303.2324	0.0028	9.3 ↑10ppm
2	alk 21 : 4 FA	-H	303.2688	0.0392	129.2

Reduction in number of possible candidates
10ppm : 1

Table 2 Possible Peak Identifications for MS and MS^3 Ions

Class of phospholipid	Identification summary
PC	10
SM	3
LPC	8
PE	7

Class of phospholipid	Identification summary
LPE	9
PS	7
PI	2
LPI	3

Class of phospholipid	Identification summary
PG	11
LPG	5
TG	67
Total	132

Table 3 Summary of Identified Phospholipids in a Lipid Mixture from Rat Liver

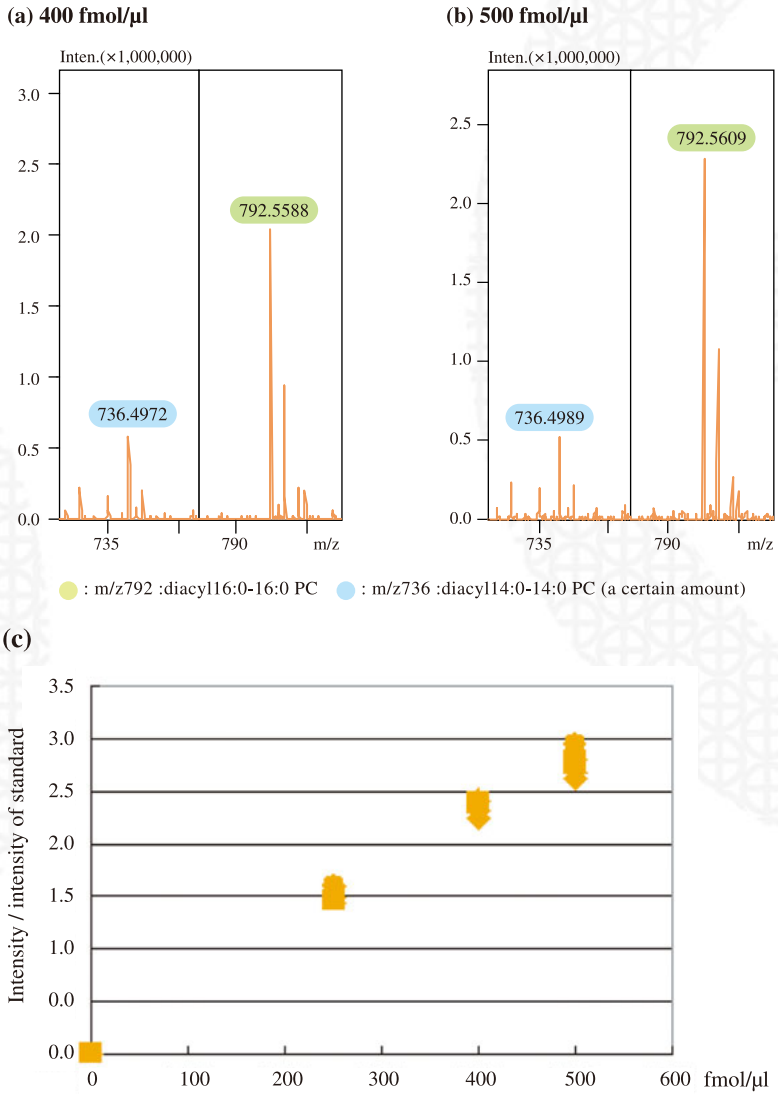

Fig. 6 A possibility of this method for use in quantitative analysis of a metabolome.

4. Conclusion

1) By selecting the proper conditions for neutral loss scanning of 141 u or 87 u, PE or PS species were identified separately from other phospholipids (Figs. 2, 3).
2) The new systematic analysis of individual classes of phospholipids by conditional NL survey (MS & MS^2) combined with subsequent MS^3 has been shown to be a very effective method (Fig. 6). This method will be useful for lipidome (lipid metabolome) analysis.
3) When using the IT-TOF, very high mass accuracy was obtained in MS, MS^2 and MS^3 without using internal standards (Table 2), demonstrating that NL survey in combination with MS^3 provides high mass accuracy identification of a set of two FA of phospholipids.

LCMS-IT-TOF LIQUID CHROMATOGRAPH MASS SPECTROMETER

Mayuko Ishida[1,2], Shinichi Yamaguchi[3], Junichi Taniguchi[3], Junko Iida[3], Kozo Miseki[3], Osamu Nishimura[1], Takao Shimizu[2], Ryo Taguchi[2,4]
[1]Life Science Laboratory, Life Science Business Unit, Analytical and Measuring Instruments Division, Shimadzu Corporation, Japan, [2]Graduate School of Medicine, The University of Tokyo; [3]MS/GC Business Unit, Analytical and Measuring Instruments Division, Shimadzu Corporation, Japan; [4]Crest JST

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SHIMADZU CORPORATION. International Marketing Division
3. Kanda-Nishikicho 1-chome, Chiyoda-ku, Tokyo 101-8448, Japan
Phone: 81(3)3219-5641 Fax. 81(3)3219-5710
URL http://www.shimadzu.com

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