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Application News

Phospholipid Profiling Method in Human Plasma Using the Shim-pack Scepter™ Claris C18

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User Benefits

- The MRM Library for Phospholipid Profiling enables the reliable identification of peaks of actual samples without the use of reference standards, allowing users to develop their own analytical methods.
- With the Shim-pack Scepter Claris C18 column, analysis of phospholipids in human plasma can be performed in 20 minutes.
- It enables comprehensive quantitative profiling of 162 human plasma phospholipid compounds.
- Minimizing the number of MRM transitions enables quantitative profiling of human plasma phospholipids with good repeatability.

■ Introduction

Quantitative profiling of phospholipids in biological samples is a promising technique for discovering biomarkers and analyzing disease mechanisms. Phospholipids have diverse structures, so the number of phospholipid compounds detected varies depending on the sample. The MRM Library for Phospholipid Profiling contains a total of 1969 MRM transitions to monitor phospholipid polar head groups and fatty acids as product ions, so users can select the MRMs required for phospholipid analysis of samples. Moreover, by monitoring the product ions of both polar head groups and fatty acids, it is possible to obtain a reliable estimate of phospholipid structures even without the use of a reference standard, enabling users to create their own phospholipid profiling methods.[1)]

In this article, we describe the development of a human plasma phospholipid profiling method using the Shim-pack Scepter Claris C18. Using this method, we detected 162 phospholipid compounds in a human plasma sample. By minimizing the number of MRM transitions, we also improved the repeatability of peak area values.

■ Samples and Sample Preparation

Pooled human plasma was purchased from Kohjin Bio. LC/MS was performed using the Nexera UHPLC ultra high performance liquid chromatograph and the LCMS-8060NX triple quadrupole mass spectrometer (from Shimadzu Corporation). An aqueous solution of 20 mM ammonium formate and a mixed solution of acetonitrile and isopropanol (1:1) were used for mobile phases A and B, respectively. The Shim-pack Scepter Claris C18, 2.1 × 100 mm, 1.9 µm was used as the analytical column. For the MS parameters, dwell time and pause time for each MRM transition were both set at 1 msec for the method using 721 MRM transitions and 2 msec for the method using 399 MRM transitions. A 20 µL sample of human plasma was spiked with 1 mL of methanol containing 0.1 % formic acid, and then it was mixed. The phospholipids were extracted and centrifuged, and 3 µL of supernatant was injected into the LC-MS system.

Table 1 Analysis Conditions

HPLC Conditions (Nexera™)	
Column:	Shim-Pack Scepter Claris C18-120*1 (100 mm × 2.1 mm, 1.9 µm)
Column Oven:	50 °C
Solvent A:	20 mM Ammonium formate - water
Solvent B:	Acetonitrile/2-Propanol (1/1, v/v)
Flowrate:	0.3 mL/min
Injection Volume:	3 µL
MS Conditions (LCMS-8060NX)	
Ionization:	ESI, Positive/Negative
Mode:	MRM
Nebulizing Gas Flow:	2.5 L/min
Drying Gas Flow:	10.0 L/min
Heating Gas Flow:	10.0 L/min
DL Temp.:	250 °C
Block Heater Temp.:	400 °C
Interface Temp.:	240 °C
CID Gas Pressure:	230 kPa
Dwell Time/Pause Time:	1 msec/1 msec
Dwell Time/Pause Time:	2 msec/2 msec

*1 P/N:227-31210-02

■ Development of Method for Analysis of Human Plasma Phospholipids

Methanol extracts of human plasma were analyzed using 422 MRM transitions to monitor the product ions derived from polar head groups in the MRM Library for Phospholipid Profiling. A total of 721 MRM transitions of polar head groups and fatty acids were extracted using the MRM Event Link Editor.[2)] In the analysis conditions (Table 1), a 20-minute analytical cycle was selected for chromatography. The 11 MRM chromatograms for PC 38:6 are shown in Fig. 1. The black trace at the top of the figure is the MRM chromatogram monitoring polar head group-derived *m/z* 184 as a product ion. The red portions of this trace indicate saturation of the detector. The other 10 MRM chromatograms are MRMs for monitoring deprotonated fatty acids as product ions. Using fast polarity switching, the MRMs monitoring the choline portion of polar head groups operated in positive ion mode, whereas the MRMs monitoring fatty acids operated in negative ion mode. The peaks detected at 10.15 min, 10.25 min, and 10.45 min were identified as PC 18:2_20:4, PC 18:1_20:5, and PC 16:0_22:6, respectively. Conversely, no peaks were detected in the MRM chromatograms of PC 16:1_22:5 and PC 18:3_20:3 (the top part of Fig. 1). As shown in the bottom part of Fig. 1, by removing the undetected MRM transitions, the total number of MRM transitions could be narrowed down to 399. The profiling method using 721 MRM transitions allocated a monitoring time of 2 msec per MRM transition, with a maximum loop time of 1.28 sec (10.94 min), whereas the profiling method using 399 MRM transitions allocated a monitoring time of 4 msec per MRM transition, with a similar loop time of 1.11 sec (10.92 min).

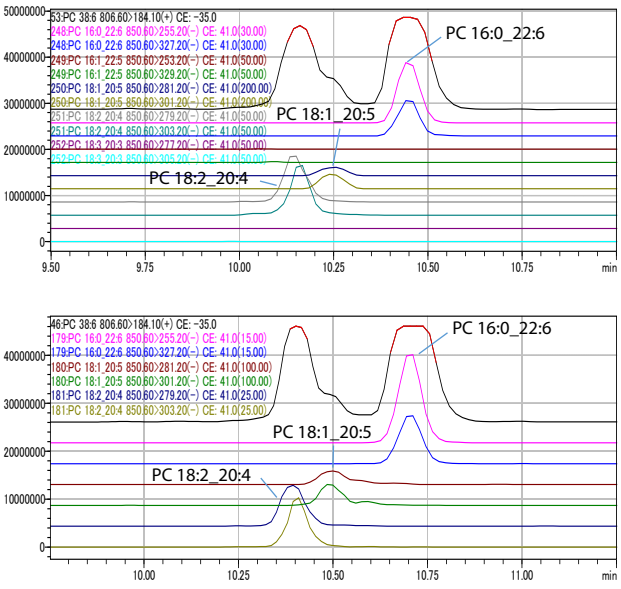

Fig. 1 MRM Chromatograms of PC 38:6 Obtained from Analysis of Plasma Extract
Top figure = 721 MRM transitions at 2 msec/MRM;
Bottom figure: 399 MRM transitions at 4 msec/MRM

Table 2 Listing of 162 Phospholipid Compounds Detected in Methanol Extracts of Plasma by Profiling Method Using 399 MRM Transitions (4 msec/MRM), Average Area Values for 50 Repeated Analyses, and Repeatability (CV%)
(All peak areas are detected by MRM monitoring of fatty acids as product ions. Phospholipid compounds with ≥ CV 20 % [14 compounds] are highlighted in gray.)

#	Compound name	Area average, N=50	CV%	#	Compound name	Area average, N=50	CV%	#	Compound name	Area average, N=50	CV%
1	LPC 18:0	6177041	3.0	40	PC 18:0_18:3	375140	9.5	11	PE 18:2_18:2	223178	9.8
2	LPC 16:0	9802674	3.1	41	PC 14:0_18:2	386116	9.8	12	PE 18:1_18:2	1010339	9.8
3	LPC 18:1	2421854	3.7	42	PC 16:0_16:0	3928665	10.0	13	PE 18:0_22:5	175023	10.3
4	LPC 20:0	32267	5.5	43	PC 20:0_20:3	48442	10.1	14	PE 16:0_22:6	437192	12.1
5	LPC 20:1	52626	5.8	44	PC 16:1_20:3	322785	10.4	15	PE 16:0_22:5	165195	12.4
6	LPC 20:3	225141	6.0	45	PC 16:0_22:5	997157	10.4	16	PE 18:1_20:4	485760	12.8
7	LPC 18:2	3108221	7.0	46	PC 16:1_16:1	116440	10.6	17	PE 16:0_20:3	198385	13.2
8	LPC 20:4	310999	7.2	47	PC 16:1_18:0	314093	10.7	18	PE 16:0_22:4	109268	15.5
9	LPC 16:1	209038	7.7	48	PC 18:2_18:3	291473	10.8	19	PE 18:0_20:5	95307	17.8
10	LPC 20:2	53345	9.9	49	PC 18:1_20:0	87167	11.0	20	PE 16:0_18:0	34051	18.0
11	LPC 14:0	76323	10.8	50	PC 18:1_22:4	191357	11.0	21	PE 16:0_16:1	76007	19.2
12	LPC 22:4	17209	13.5	51	PC 16:0_16:1	1058488	11.2	22	PE 18:2_20:4	49800	22.9
13	LPC 22:5	13866	17.7	52	PC 18:2_20:2	523119	11.5	23	PE 18:0_18:3	29847	23.5
15	LPC 22:6	11759	19.7	53	PC 20:4_20:4	183577	11.5				
16	LPC 18:3	18299	22.6	54	PC 14:0_20:3	163028	12.0	1	LPI 18:2	62147	7.9
18	LPC 20:5	3198	29.6	55	PC 14:0_16:0	366978	12.5				
				56	PC 20:0_20:4	66559	13.0	1	PI 16:0_20:4	1625149	6.3
1	PC 18:0_18:1	17593612	5.1	57	PC 20:1_20:3	102885	13.1	2	PI 18:1_18:2	749066	7.8
2	PC 18:0_20:1	287132	5.7	58	PC 14:0_18:1	391625	13.2	3	PI 18:1_20:4	623478	8.0
3	PC 18:0_20:3	6382676	5.8	59	PC 14:0_20:4	113338	13.2	4	PI 18:0_18:2	4686413	8.4
4	PC 18:0_20:2	1686430	5.8	60	PC 18:1_20:5	153987	13.5	5	PI 18:0_18:1	1871696	8.4
5	PC 18:2_20:0	358777	6.0	61	PC 18:2_22:6	122179	13.7	6	PI 16:0_16:1	269652	8.9
6	PC 16:0_18:1	40586458	6.1	62	PC 18:2_20:5	56038	14.3	7	PI 18:1_18:1	1024022	9.5
7	PC 18:0_18:0	212889	6.3	63	PC 20:1_20:4	135411	14.3	8	PI 16:0_18:2	1656760	9.7
8	PC 16:1_18:2	892681	6.4	64	PC 20:2_20:3	87456	15.0	9	PI 16:1_18:0	308673	9.7
9	PC 18:0_22:4	1724774	6.5	65	PC 18:2_22:1	33901	16.0	10	PI 16:0_22:6	155112	10.0
10	PC 18:1_20:3	1026152	6.5	66	PC 14:0_14:0	45553	16.6	11	PI 16:0_18:1	1168268	10.0
11	PC 18:0_22:6	1623970	6.6	67	PC 20:3_20:4	94010	16.8	12	PI 18:0_20:4	5623662	10.1
12	PC 16:0_20:5	1387372	6.7	68	PC 18:1_22:5	74129	17.5	13	PI 16:0_22:5	154892	10.9
13	PC 18:0_20:4	17979487	6.8	69	PC 14:0_16:1	66093	18.7	14	PI 16:0_20:3	318089	11.0
14	PC 18:0_18:2	20178291	6.8	70	PC 16:1_18:3	33010	20.8	15	PI 18:0_20:3	1682281	11.1
15	PC 16:0_20:4	19682444	6.9	71	PC 14:0_22:6	31688	21.2	16	PI 18:0_22:6	481170	11.1
16	PC 16:0_18:0	738134	7.0	72	PC 14:0_18:0	32908	21.6	17	PI 18:0_20:2	140801	11.9
17	PC 18:1_18:1	5939682	7.0	73	PC 20:2_20:4	88613	22.8	18	PI 16:0_16:0	197577	12.5
18	PC 18:1_18:2	13429414	7.2	74	PC 18:3_20:4	20516	23.3	19	PI 18:0_22:5	249879	13.0
19	PC 18:2_18:2	10517941	7.2	75	PC 14:0_18:3	22805	34.3	20	PI 18:2_18:2	45797	13.1
20	PC 16:0_18:3	2074384	7.5					21	PI 18:1_20:3	106444	14.0
21	PC 16:0_20:3	19949822	7.5	1	LPE 18:0	626578	4.5	22	PI 16:1_18:1	79563	14.7
22	PC 16:1_20:4	444816	7.6	2	LPE 16:0	452433	5.6	23	PI 18:0_22:4	116850	15.2
23	PC 18:1_20:1	230508	7.7	3	LPE 18:1	425138	8.0	24	PI 16:0_20:2	57118	16.4
24	PC 18:2_20:3	448077	7.7	4	LPE 20:3	52213	9.8	25	PI 16:0_22:4	71594	16.5
25	PC 18:1_20:4	3054182	7.8	5	LPE 20:4	143917	11.0	26	PI 18:0_18:3	63795	17.3
26	PC 18:2_20:4	1568280	7.8	6	LPE 18:2	512823	11.4	27	PI 18:0_20:5	59845	21.3
27	PC 16:0_20:1	556459	7.9	7	LPE 16:1	11641	19.8				
28	PC 18:1_22:6	403052	7.9	8	LPE 22:6	15264	22.7	1	SM 40:1	8684535	2.7
29	PC 16:1_18:1	1190137	8.0					2	SM 40:2	4381336	4.2
30	PC 18:2_20:1	461432	8.4	1	PE 18:0_18:1	1280573	5.1	3	SM 38:1	3561874	5.3
31	PC 16:0_18:2	23272211	8.4	2	PE 18:0_18:2	1586992	6.2	4	SM 32:1	1604389	7.7
32	PC 16:0_22:6	5037442	8.9	3	PE 16:0_18:1	346496	6.3	5	SM 36:1	3097597	8.2
33	PC 18:0_20:5	718250	8.9	4	PE 18:1_18:1	785699	6.7	6	SM 38:2	728823	8.3
34	PC 18:2_22:0	36347	9.0	5	PE 18:0_20:3	693877	6.9	7	SM 34:2	2833614	9.1
35	PC 18:1_20:2	303152	9.0	6	PE 18:0_20:4	4361704	7.0	8	SM 34:1	12204767	9.3
36	PC 18:1_18:3	299341	9.1	7	PE 16:0_18:2	1921149	8.3	9	SM 36:3	139144	9.6
37	PC 16:0_20:2	2000827	9.3	8	PE 18:0_22:6	586692	8.6	10	SM 36:2	1359938	13.1
38	PC 16:0_22:4	2014140	9.4	9	PE 18:0_22:4	162582	8.9	11	SM 38:3	28054	36.7
39	PC 18:0_22:5	809705	9.5	10	PE 16:0_20:4	1324845	9.2	12	SM 36:4	3510	45.8

Table 2 lists the 162 phospholipid compounds detected in human plasma by the profiling method using 399 MRM transitions, the average area values for 50 repeated analyses, and the repeatability of the method (CV%). CV% was calculated using the peak areas detected by MRM monitoring (negative ion mode) of fatty acid-derived product ions. Among these 162 phospholipid compounds, 148 compounds (91 %) had a CV of ≤ 20 %. When the repeatability was confirmed using 721 MRM methods, 108 compounds (64 %) had a CV of ≤ 20 %. These results demonstrate that minimizing the total number of MRM transitions and doubling the monitoring time allocated to each MRM transition (from 2 to 4 msec) significantly improved repeatability.

While the above-mentioned results were obtained using the LCMS-8060NX system, we have confirmed that similar results can also be obtained using the LCMS-8060RX system.

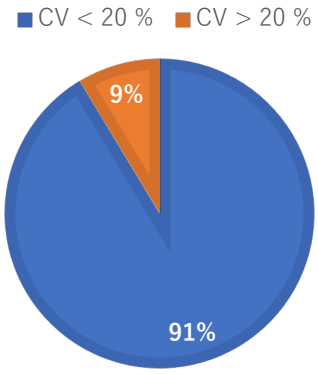

Fig. 2 Repeatability of Analysis of 162 Phospholipid Compounds Detected in Human Plasma by a Profiling Method Limited to 399 MRM Transitions (4 msec/MRM)

■ Conclusion

- In this study, we developed a method for profiling phospholipids in human plasma using the Shim-pack Scepter Claris C18.
- Using this method, we detected 162 phospholipid compounds in a human plasma sample.
- By minimizing the number of MRM transitions and doubling the monitoring time allocated to each MRM transition, we significantly improved the repeatability of the profiling method.
- This method enables quantitative comparisons of the conjugated fatty acids for these 162 human plasma phospholipid compounds.

■ References

1) Shimadzu Review 77 (3-4), 125-134 (2020)

2) Japan Patent Registration No. 6611009

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