Development of polar lipid profiling method by supercritical fluid chromatography/mass spectrometry

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Introduction

Recently, the interest in lipidomics has been increased by various roles of lipids in the cell, such as energy store, membrane components, cellular signaling, cell–cell interactions, etc. In order to study the various functions of lipids, effective analytical methods are attempted to develop for lipid profiling.

Lipids have high complexity and diverse polarity according to the attached hydrophilic molecules and the combination of hydrophobic acyl chain species. Therefore, it is a very challenging work to analyze simultaneously complex lipids in a mixture.

We developed a simultaneous profiling method based on supercritical fluid chromatography /mass spectrometry (SFC/MS) for targeting polar lipids by applying trimethylsilyl (TMS) derivatization.

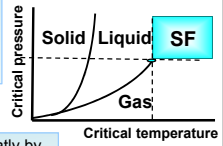
Application of TMS derivatization

In our previous study, 14 types of diverse lipids were analyzed simultaneously by SFC/MS. However, the analysis of several polar lipids was not sufficient due to the peak tailing and low sensitivity. Therefore, in this research, TMS derivatization was applied to improve these problems. By TMS derivatization, the character of polar lipids was changed in the SFC packed column and the peak shapes and sensitivity might be advanced.

Supercritical fluid (SF)

SF has suitable properties for mobile phase of chromatography.

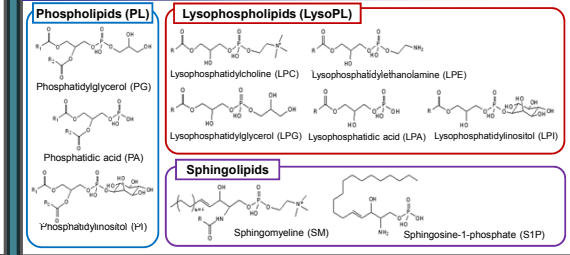
- Temperature and pressure above critical points
- Low viscosity
- High diffusivity
- Density is greatly transmutable continuously



Supercritical carbon dioxide (SC-CO_2)

Non-polar, its polarity can also be varied greatly by adding a modifier such as methanol

Target polar lipids



Experiments

Sample preparation

Each standard sample of PG, PI, PA, LPC, LPE, LPI, LPG, LPA, SM, and S1P was prepared by dissolving in methanol.

10 µL of sheep plasma was added into 160 µL of MeOH and 0.5 µg/mL of LPC (17:0) used as the internal standard. After vortexing and incubating on ice for 10 min, the mixture was centrifuged (10000 g, 5 min) to get 140 µL of supernatant.

< TMS derivatization >

1. Several polar lipid standards and sheep plasma were desolvated by N_2 gas.
2. 60 µL of *N*-Trimethylsilylimidazole (TMSI) and 40 µL of pyridine were added and reacted at 37°C for 30 min.
3. The derivatized lipids were subjected to SFC/MS/MS analysis.

SFC/MS/MS conditions

(a) SFC conditions

(Analytical SFC method station, Waters)

Mobile phase : Carbon dioxide (CO_2, 99.9% grade)
Modifier : Methanol with 0.1% (w/w) $HCOONH_4$
Flow rate : 3 mL/min
Oven temperature : 37 ℃
Back pressure : 10 MPa

(b) MS/MS conditions (Xevo TQ, Waters)

Ionization method : Electrospray ionization
Ion mode : positive
Capillary voltage : 3.00 kV
Desolvation temperature : 450 ℃
Desolvation gas flow : 800 L/hr
Cone gas flow : 60 L/hr
Collision gas flow : 13.2 mL/hr
MS collision energy : 6 V
Source temperature : 150 ℃
Extractor voltage : 3 V

Results and Discussion

Polar lipid profiling based on SFC/MS/MS by TMS derivatization

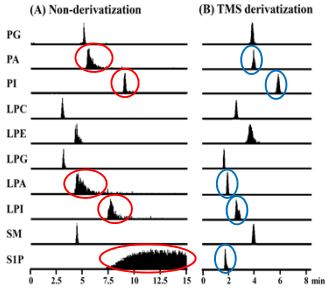
Optimized MS/MS method by TMS derivatization

Polar lipids	Ion mode	SRM transitions	CV	CE	Number of adducted TMS
PG	Positive	[M+H] > [M-315]	20	30	2
PA	Positive	[M+H] > [M-169]	25	25	1
PI	Positive	[M+H] > [M-619]	35	30	5
LPC	Positive	[M+H] > 184	45	35	1
LPE	Positive	[M+H] > [M-140]	30	15	1
LPG	Positive	[M+H] > [M-315]	30	20	3
LPA	Positive	[M+H] > [M-169]	30	15	2
LPI	Positive	[M+H] > [M-547]	45	25	5
SM	Positive	[M+H] > 184	45	30	1
S1P	Positive	[M+H] > 264	25	20	2

CV: Cone voltage, CE: MS/MS collision energy

By TMS derivatization, different number of TMSs was adducted to polar lipids. SRM condition was optimized to analyze TMS derivatized polar lipids. Additionally, Inertsil ODS-EP column (250 x 4.6 mm ID; 5 µm, GL Sciences) was selected to polar lipid profiling.

Profiling of 10 polar lipid standards



By TMS derivatization, the peak tailing and intensity of PA, PI, LPA, LPI and S1P were advanced comparing to non-derivatization.

Validation study

Polar lipids	RT (min)	RSD (n=6) (%) RT	RSD (n=6) (%) Peak area	Correlation (R^2)	Linear range (fmol)	LOD (fmol) Derivatized	LOD (fmol) Non-derivatized
PG	3.65	0.7	5.9	0.9999	66 – 33000	66	820
PA	3.75	0.7	5.5	0.9928	630 – 31000	310	630
PI	5.58	1.7	14.8	0.9982	75 – 19000	23	940
LPC	2.40	0.4	3.1	0.9984	9.4 – 47000	7.2	9.4
LPE	3.43	0.7	7.1	0.9988	170 – 8600	140	86
LPG	1.59	1.0	8.7	0.9993	220 – 11000	220	110
LPA	1.84	1.6	9.0	0.9912	95 – 24000	47	24000
LPI	2.51	1.1	5.2	0.9901	290 – 7200	140	5400
SM	3.69	0.8	6.3	0.9989	9.8 – 4900	9.8	25
S1P	1.72	1.7	8.4	0.9972	13 – 13000	5.3	7900

Validation study of derivatized lipid analysis was performed to estimate its analytical performance for the quantitative analysis of polar lipids. LOD of derivatized lipids was also compared with non-derivatized lipids. In conclusion, the developed method showed high reproducibility and high reliability. And the LOD of PG, PI, LPA, LPI, and S1P was enhanced by 12, 40, 510, 39 and 1490-fold respectively.

Polar lipid profiling of sheep plasma

Polar lipids	Cn_Un	TMS derivatization RT (min)	TMS derivatization Relative ratio of molecular species (peak area/total area)	Non-derivatization RT (min)	Non-derivatization Detection
PI	40:4	6.43	0.06 ± 0.11		N.D.
	40:5	6.23	1.2 ± 0.6		N.D.
	40:6	6.07	1.7 ± 0.3	8.82	
	38:2	6.21	0.2 ± 0.2		N.D.
	38:3	5.92	5 ± 2.	8.97	
	38:4	5.71	64 ± 2.	8.71	
	38:5	5.47	2.7 ± 0.5	8.56	
	38:6	5.24	0.7 ± 0.4		N.D.
	36:1	5.82	0.1 ± 0.1		N.D.
	36:2	5.53	4.3 ± 0.5		N.D.
	36:3	5.16	1.9 ± 0.5	8.16	
	36:4	4.91	11 ± 1.	8.13	
	36:5	4.87	0.02 ± 0.04		N.D.
	34:1	4.97	0.7 ± 0.4		N.D.
	34:2	4.76	6 ± 2.		N.D.
	32:1	4.41	0.02 ± 0.03		N.D.
LPC	24:4	2.86	0.0008 ± 0.0013		N.D.
	24:5	2.77	0.002 ± 0.004	3.33	
	24:6	2.61	0.002 ± 0.003	3.19	
	22:1	3.01	0.03 ± 0.01	3.62	
	22:2	2.83	0.001 ± 0.001	3.41	
	22:5	2.34	0.62 ± 0.05	2.99	
	22:6	2.28	1.8 ± 0.2	2.93	
	20:0	2.71	0.22 ± 0.07	3.38	
	20:1	2.52	0.8 ± 0.1	3.12	
	20:2	2.39	0.7 ± 0.1	2.99	
	20:3	2.21	1.3 ± 0.1	2.81	
	20:4	2.10	4.2 ± 0.2	2.72	
	18:0	2.31	38.8 ± 0.4	2.95	
	18:1	2.14	10.13 ± 0.04	2.72	
	18:2	2.01	11.4 ± 0.2	2.59	
	18:3	1.97	0.05 ± 0.02	2.51	
	16:0	1.94	29.4 ± 0.1	2.54	
	16:1	1.83	0.52 ± 0.04	2.40	
	16:2	1.75	0.002 ± 0.002		N.D.
	14:0	1.72	0.11 ± 0.03	2.18	
LPE	20:4	3.05	4 ± 3.		N.D.
	18:0	3.72	54 ± 3.	4.62	
	18:1	3.57	5 ± 2.		N.D.
	18:2	3.42	2 ± 2.	4.21	
	16:0	3.12	35 ± 2.	3.86	
LPA	20:4	1.74	15.1 ± 0.3		N.D.
	18:0	1.89	4 ± 7.		N.D.
	18:1	1.71	6 ± 5.		N.D.
	18:2	1.69	70 ± 3.		N.D.
	16:0	1.62	5 ± 4.		N.D.
LPI	18:0	2.61	100.		N.D.
SM	26:0	6.70	0.11 ± 0.05	7.12	
	26:1	6.26	0.38 ± 0.07	6.83	
	26:2	5.96	0.06 ± 0.04	6.59	
	26:3	5.63	0.001 ± 0.002		N.D.
	26:4	5.32	0.003 ± 0.003		N.D.
	26:5	5.02	0.004 ± 0.006	6.21	
	24:0	5.88	18.1 ± 0.4	6.49	
	24:1	5.48	29.3 ± 0.3	5.99	
	24:2	5.24	7.2 ± 0.4	5.75	
	24:3	5.02	0.53 ± 0.02	5.55	
	24:4	4.57	0.06 ± 0.02	5.27	
	24:5	4.36	0.13 ± 0.08	5.15	
	24:6	4.19	0.08 ± 0.03	4.96	
	22:0	5.13	7.1 ± 0.4	5.71	
	22:1	4.85	2.7 ± 0.2	5.40	
	22:2	4.50	0.40 ± 0.08	5.16	
	22:3	4.31	0.01 ± 0.01	4.86	
	22:4	4.04	0.003 ± 0.004		N.D.
	22:5	3.90	0.05 ± 0.03		N.D.
	22:6	3.74	0.06 ± 0.03		N.D.
	20:0	4.68	2.3 ± 0.1	5.07	
	20:1	4.21	0.33 ± 0.03	4.88	
	20:2	3.85	0.009 ± 0.004	4.62	
	20:3	3.70	0.003 ± 0.001		N.D.
	20:4	3.61	0.004 ± 0.006	4.32	
	18:0	3.80	2.4 ± 0.1	4.50	
	18:1	3.64	0.63 ± 0.07	4.33	
	18:2	3.32	0.011 ± 0.008	4.07	
	16:0	3.26	25.8 ± 0.5	3.98	
	16:1	3.12	2.1 ± 0.1	3.81	
	14:0	2.79	0.20 ± 0.02	3.52	
S1P	-	1.79	100.		N.D.

Simultaneous profiling of diverse polar lipids in sheep plasma was carried out successfully. By TMS derivatization, diverse polar lipids that consist of 16 PIs, 20 LPCs, 5 LPEs, 5 LPAs, 1 LPI, 31 SMs, and 1 S1P were analyzed. Comparing to non-derivatization, 20 minor species of PI, LPC, LPE and SM and 7 molecular species of LPA, LPI and S1P were analyzed in addition.

Summary

- A high-throughput and high-resolution analytical method based on SFC/MS was established for the simultaneous profiling of polar lipids in a mixture.
- By TMS derivatization, the peak tailing and intensity of polar lipids were advanced successfully. In validation study, high reproducibility, reliability, and sensitivity were proved.
- Qualitative and quantitative analysis of polar lipids in sheep plasma were performed and it showed the usefulness of this method based on SFC/MS by TMS derivatization.