Separation of Positional Isomers of PAHSAs Using LC-MS/MS Coupled with Differential Ion Mobility Mass Spectrometry

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ABSTRACT

Palmitic acid hydroxyl stearic acids (PAHSAs) are lipid isomers that only differ in the position of ester connection to the hydroxy-fatty acid, which makes the differentiation of these isomers by mass spectrometry (MS) extremely challenging. In this study, SelexION® differential mobility spectrometry (DMS) technology was used to improve the separation of these isomers by using different Compensation Voltages (CoV) for each isomer which will suppress the signal of other isomers. The result leads to a shorter LC-MS assay with better separation of the isomers and less background interference.

INTRODUCTION

PAHSAs were recently found to correlate highly with insulin sensitivity and are reduced in adipose tissue and serum of insulin-resistant humans. In animal study, they were found to exert several biological effects, such as improving glucose tolerance and ambient glycemia, stimulating insulin and GLP-1 secretion, enhancing insulin-stimulated glucose transport, and anti-Inflammatory effects. Different isomers have different distribution in tissues and exhibit different biological effects. Among these isomers, 9-position PAHSA is the most predominant one in brown adipose tissue (BAT) and white adipose tissue (WAT) and being the most upregulated one in mice. The unambiguous characterization and quantitation of these isomers are thus important in the diabetes research using these isomers as biomarkers. However, these lipid isomers only differ in the position of ester connection to the hydroxy-fatty acid, which makes differentiation of these isomers by mass spectrometry extremely challenging. The method reported in the literature used a long LC gradient (> 1 hr) in order to separate the isomers and to quantify them [1].

SelexION® DMS technology separates isomeric compounds based on their dipolar moment in an asymmetrically oscillating electrical field, which is orthogonal to the LC-MS separation. In this study, SelexION® technology coupling with LC-MS was used to effectively separate several PAHSA isomers based on their DMS properties. By using the SelexION® technology, the LC run time was shortened significantly with minimum background noise interference.

MATERIALS AND METHODS

Sample Preparation:
0.2 mL monkey serum was precipitated with 1.0 mL methanol mixed with 0.1% formic acid. Mixture was vortexed and centrifuged at 4000 rpm for 10min. The supernatant was dried down with nitrogen and reconstituted in 0.5 mL 93:7 MeOH:H_2O with 0.01% ammonium hydroxide and 5 mM ammonium acetate (pH 9.1).

HPLC Conditions:
Analytes were separated on a Shimadzu HPLC system and eluted from a Phenomenex Kinetex C18 (50 x 2.1 mm, 2.6 µm) column with a 3.5-min gradient run. The column temperature was controlled at 50°C by a column oven. The LC mobile phases composed of 5 mM NH_4OAc, 0.01% NH_4OH in 93% MeOH (pH 9.1) as solvent A, and acetonitrile as solvent B. The Flow rate was 0.4 mL/min and the injection volume was 20 µL.

MS/MS Conditions:
Analytes were detected by a SCIEX QTRAP® 6500+ MS/MS system using a SelexION® technology in negative electrospray ionization. Declustering potential: -120 V; Exit Potential: -10 V; Collision Energy: -34 eV; Collision Exit Potential: -14 V; Curtain gas: 20; CAD gas: 9; GS1&2: 70; Ion Spray voltage: -4500; Temperature: 450 °C.
SelexION® setting was: DMS temperature: Low, Modifier: 1-propanol, Modifier Composition: Low, DMS Offset: 3.0, Separation Voltage: 3900 V. DR: 45.
MRM transition for all isomers was 537.5 → 255.1. DW 50 ms. CoV for 5-, 9-, 13-PAHSAs are 5.0, 5.8, 6.3 V, respectively.

RESULTS

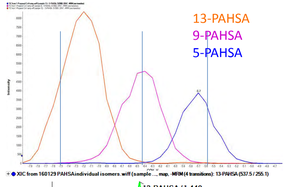


Fig. 1. CoV profiles of the PAHSA isomers. Each isomer has its unique CoV profile. Thus, by using different CoV values (as marked by the vertical lines) the signal of the other isomers will be suppressed by SelexION® technology.

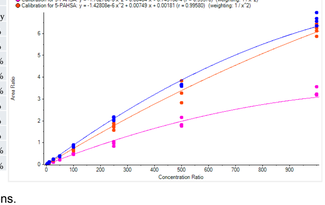
Isomer	MRM transition	% Contribution
5-PAHSA	13-PAHSA	0.00%
	9-PAHSA	0.00%
	5-PAHSA	--
9-PAHSA	13-PAHSA	2.22%
	9-PAHSA	--
	5-PAHSA	0.00%
13-PAHSA	13-PAHSA	--
	9-PAHSA	3.77%
	5-PAHSA	0.00%

Fig. 2 & Table 1. Selective LC traces of 13-, 9- and 5-PAHSA. When injecting each individual isomers, the other two isomers were also monitored and the contributions from the other isomers were calculated to be less than 4% at most.

Table 2. Accuracy and Precision of 13-, 9- and 5-PAHSA.

Std Conc	13-PAHSA		9-PAHSA		5-PAHSA	
(ng/mL)	CV%	Accuracy	CV%	Accuracy	CV%	Accuracy
1	7.26	98.45%	8.49	100.06%	9.94	99.37%
2.5	9.07	100.34%	9.97	97.38%	9.69	96.42%
5	7.11	99.52%	9.22	97.99%	1.08	107.22%
10	7.79	115.68%	2.07	114.90%	6.19	107.39%
25	5.31	108.37%	7.9	100.07%	3.46	100.09%
50	6.25	82.74%	4.5	95.24%	4.15	98.75%
100	5.83	96.43%	4.22	105.49%	5.7	91.25%
250	4.34	99.70%	9.69	86.49%	8.05	97.56%
500	1.08	92.61%	12.09	94.39%	14.85	100.44%
1000	8.08	110.47%	2.33	110.53%	5.03	102.12%

Fig. 3. Calibration of 13-, 9-, 5-PAHSA. LDR is from 1 to 1000 ng/mL



Std curve statistics are from 4 replicates of injections.

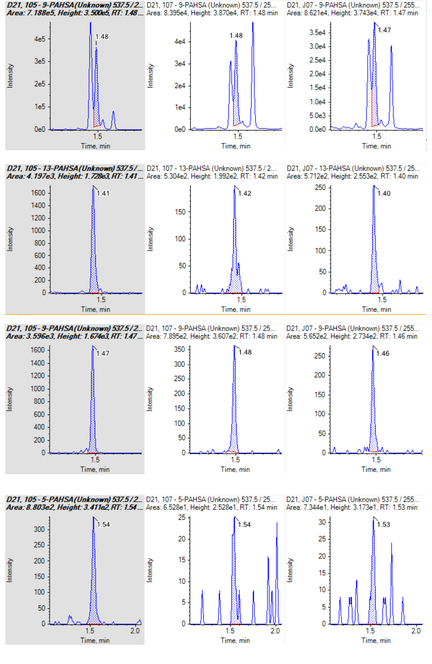


Fig. 4. Chromatograms of PAHSAs in three monkey serum samples. Top panel showed the MRM XIC with DMS off. All PAHSA isomers showed up and interfered with each other. The low panels are the XICs of 13-, 9-, 5-PAHSA with DMS on. Interferences from other isomers are minimized.

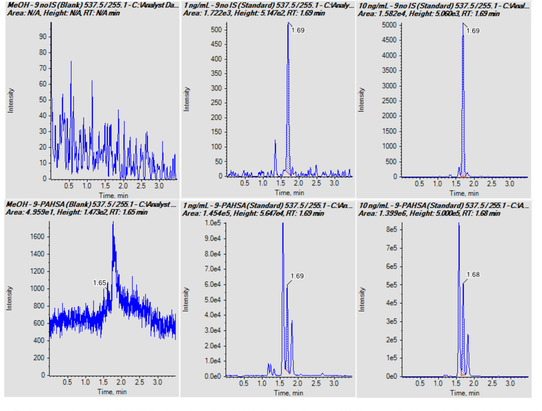


Fig. 5. Comparison of PAHSA signals with DMS On (top) and Off (bottom). From left to right are XICs of blank, LLOQ at 1 ng/mL, and 10 ng/mL standards. By using difference CoV values, adjacent 5- and 13-PAHSA signals are almost completely suppressed, which makes the integration of 9-PAHSA much easier.

CONCLUSIONS

With the orthogonal separation power of DMS with SelexION® technology, PAHSA isomers in the monkey serum samples can be better resolved with minimum background interferences in a shorter LC-MS method.

REFERENCES

[1] Yore MM, et. al., *Cell*. 2014 Oct 9;159(2):318-32.

TRADEMARKS/LICENSING

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