

Determination of Fatty Acids in Foods Using Gas Chromatography with Positive-ion Chemical Ionization Tandem Mass Spectrometry

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Introduction

A GC-MS with electron ionization mode (EI) is widely used for qualitative and quantitative determination of multi-component fatty acids in foods. However, selection of an appropriate monitoring ion is not always easy in El mode because of the low abundance of fragment ions and the presence of co-eluting interferences. The more abundant guasi-molecular ion of positive ion chemical ionization

mode (PICI) can be used instead, but it is also subject to overlapping co-elutants, in which case neither EI nor PICI mode produces ideal results. The tandem mass spectrometry (MS/MS) is one of choices available to solve this problem. In this study, the following four methods, GC-EI-MS, GC-PICI-MS, GC-EI-MS/MS, and GC-PICI-MS/MS, are evaluated.

EI and PICI Oleic acid methyl ester (C18:1n-9) (M.W. 296.3)





New approach of GC-MS/MS



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Methods and Materials

Sample Preparation

Brevoort



A fatty acid methylation kit and a fatty acid methyl ester purification kit. (Nacalai Tesque, Inc.)



Analytical Conditions

GC-MS: GCMS-TQ8030 Column: SP-2560 (L=100 m, 0.20 mm I.D., df=0.25 $\mu m)$



PICI-SIM :M+H+ PICI-MRM

Precursor ions : the same as the ions used in PCI-SIM mode.

Product ions : the most intense ion above m/z 100.

200 mg of grinding fish meat
200 mg of grinning har mean 2 mL of extraction solution Mixing and centrifuge
500 µL of extracted solution
Drying under nitrogen gas
Dried residue
 ← 500 µL of reagent A and I ← For 1 hour at 37°C ← 500 µL of reagent°C ← For 20 min at 37°C ← 2 mL of extraction solutio ← Centrifuge
Organic layer
← 1 mL of MilliQ water ← Mixing and centrifuge
Organic layer
+
GC-MS, GC-MS/MS

PICI MRM

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Results 3-1. Limit of Quantitation (LOQs)

LOQs= s x t (n-1, 0.0 1) where n=8, s: standard deviation, t (n-1, 0.01): Student's t value

Bl	ack: Saturated fatty acids	more than two times more sensitive					
		CIMRM	CI SIM	EI MRM	EI SIM	CIMRM CISIM EIMRM EIS	SIM
		LOQ (pg)	LOQ (pg)	LOQ (pg)	LOQ (pg)	LOQ (pg) LOQ (pg) LOQ (pg) LOQ	(pg)
1	Methyl butanoate;4:0	1.9	8.3	2.8	26.0	19 Methyl linolelaidate;(E)18:2n-6 23.2 28.8 253.9 5	52.0
2	Methyl caproate;6:0	3.6	5.9	3.5	39.2	20 Methyl linoleate;(Z)18:2n-6 16.5 23.2 297.9 16	60.7
3	Methyl caprylate;8:0	4.8	4.8	10.5	53.9	21 Methyl arachisate;20:0 58.5 11.5 9.1	11.6
4	Methyl caprate;10:0	8.8	7.2	32.9	36.8	22 Methyl ganma-linolenate;(Z)18:3n-6 81.2 17.6 167.8 34	49.8
5	Methyl undecanoate;11:0	20.6	30.1	22.0	44.0	23 Methyl cis-11-icosenoate;(Z)20:1n-9 36.0 22.0 45.1 14	45.1
6	Methyl laurate;12:0	10.9	6.6	56.5	74.4	24 Methyl linolenate; (Z)18:3n-3 135.7 23.9 414.6 21	13.1
7	Methyl tridecanoate;13:0	13.2	5.2	48.7	42.2	25 Methyl heneicosanoate;21:0 108.5 25.5 33.7 2	41.0
8	Methyl myristate;14:0	10.1	5.5	5.6	69.7	26 Methyl cis-11,14-icosadienoate;(2)20:2n-6 36.5 13.4 282.1 23	38.4
9	Methyl myristoleate;(Z)14:1n-5	2.7	4.7	178.3	134.0	27/Methyl benenate;22:0 279.0 23.3 29.6	/.0
10	Methyl pentadecanoate: 15:0	36.6	4.8	29.3	32.1	28 Methyl erusete 221 p. 0	40.5
11	Methyl cis-10-pentadecenoate:(Z)15:1n-5	4.6	4.0	225.3	33.7	29 Methyl cic 11 14 17 Jecestrioporte/(7)20:2p 2 294 5 24 2	43.3
12	Methyl palmitate: 16:0	15.2	7 3	12.6	74 5	30 Methyl tricosepoete: 23:0 264.5 24.3 - 44	10.0
13	Methyl palmitoleate:(7)16:1n-7	16.2	19.0	36.0	249.5	37 Methyl arachidonate; 23:0 357.2 19.5 34.2 2	<u>24.0</u> 02.2
14	Methyl margarate: 17:0	29.2	20.0	5.6	270	33 Methyl cis-13 16-Docosadienate: (7)22:2n-6 128 1 315 7 335 3 28	92.2 83.7
15	Methyl cis-10-heptadecenoate;(Z)17:1n-7	14.5	22.1	215.7	245.9	34 Methyl lignocerate;24:0 503.8 41.8 52.6	10.3
16	Methyl stearate;18:0	35.3	8.9	10.2	11.6	35 Methyl cis-5,8,11,14,17-Eicosapentaenoate;(Z)20:5n-3 184.9 54.9 286.5 43	37.4
17	Methyl elaidate;(E)18:1n-9	19.8	5.5	180.7	173.9	36 Methyl nervonate;(Z)24:1n-9 99.4 56.5 445.2 23	30.7
18	Methyl oleate;(Z)18:1n-9	9.8	6.4	353.2	58.1	37 Methyl cis-4,7,10,13,16,19-Docosahexaenoate;(Z)22:6n-3 255.7 304.2 161.5 28	81.7
Measuring mode		Total num	nber		Saturated	Unsaturated	
	EI SI	7			6	1	
	EI MRM	10			9	 PICI was the most sensitive mode, especial 	lly in
	PICI SIM	32			13	19 the analysis of unsaturated fatty acids	-

11

Saturated fatty acid (methyl margarate;C17:0 M.W. 284.3)

18



Unsaturated fatty acid (Methyl oleate; (Z) C18:1n-9 M.W. 296.3)





the analysis of unsaturated fatty acids



A variety of fragment ions Intensity of each ion is low (especially unsaturated fatty acids)

Unsaturated fatty acids produced intense product ions

more than two times more sensitive

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Brevoort EI SIM EI MRM PICI SIM PICI MRM Methyl linoleate; (Z)18:3n-3 (x1.000.000) (x10,000) (x1,000,000 (x100,000) 7.0 1.25 292.20 292.30>135.10 236.20>175.10 293 20 2.0 293.30>261.30 293.30>243.20 6.0 1.00 1.00 5.0 separation 1.5 separation separation separation 0.75 0.75 4.0 not good not good good good 1.0 3.0 0.50 0.50 2.0 0.5 0.25 0.25 1.0 51 75 52.00 52.25 52.50 51.75 52.00 52.25 52.50 53.00 53.25 53.50 52.75 53.25 53.50 53.00 Methyl cis-11,14,17-eicosatrienoate; (Z)C20:3n-3 (x10,000) (x1,000,000) (x100,000) (x100,000) 320.30 320.30>121.10 321.30 321.30>289.30 321.30>271.30 6.0 1 50 7.5 4.0 separation separation separation separation 5.0 -1.25 good good not good not good 3.0. 4.0 1.00 5.0 0.75 3.0 2.0 2.5 2.0 0.50 10 1.0 0.25 54.50 54.75 55.00 55.25 54.50 54.75 55.00 55.25 55.75 56.00 56.25 56.50 55.75 56.00 56.25 Methyl erucate; C22:1n-9 Methyl cis-11,14,17-eicosatrienoate; (Z)C20:3n-3 EI-GC-MS C22:1n-9 (x1,000,000) 125 320.30 125 7.5 100-100 75 75 5.0 50 50 25 25 2.5 C20:3n-3 264.3 291.3320 Ω 250 100 150 200 250 300 350 150 200 300 100 54.50 54.75 55.00 55.25 EI-GC-MS/MS C22:1n-9 (x100,000) 125 320.30>121.10 Precursor m/z 320.3 100 Precursor m/z 320.3 CE:9V 4.0 100 CE:9V 94 75 -75 3.0 50 50 2.0 25 C20:3n-3 320.3 25 2/19 3 1.0 0 150 300 50 100 150 200 250 300 54.50 54.75 55.00 55.25 PICI-GC-MS C22:1n-9 (x100,000) 125 321.30 125 *m/z* 321 6.0 353.3 321.3 100-100 Fragment ion 5.0 75 -75 4.0 50 -3.0 50 25 -C20:3n-3 25 2.0 289. 0 1.0 0 100 150 200 250 300 350 100 150 200 250 300 350 56.00 56.25 56.50 55.75 Precursor m/z 353.3 Precursor m/z 321.3 PICI-GC-MS/MS CE-9V CE:9V (x10,000) 100 303.3 321.30>289.30 321.30>271.30 100 . 1.50 C20:3n-3 321.3 75 1.25 75 -1.00

0.75

0.50

0.25

55.75 56.00 56.25

56.50

3-2. Mass Separation in Food Sample

50

25

0

50

100

150

200

250

300

. 350

400

5

400

321.3 289.3

300

350 400

250

50

25

0

50

100

150

200

56.50



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Conclusions

- The PICI-SIM and PICI-MRM modes showed better LOQs compared to EI-SIM and EI-MRM in the analysis of 37 fatty acids, especially the unsaturated fatty acids.
- Since El mass spectrum patterns of the fatty acids were similar, it was not easy to choose characteristic ion to separate from other fatty acids, especially in real samples.
- Although PICI-SIM was more selective compared to EI-SIM and EI-MRM, only several ions could be chosen for monitoring.
- Since PICI-MRM was more selective than PICI-SIM due to MS/MS, the fatty acids were selectively detected, which improved the reliability of identification.





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