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

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Shuichi Kawana[1], Yukihiko Kudo[1], Yuki Sakamoto[1], Katsuhiro Nakagawa[1], Laura Chambers[2], Richard Whitney[2], Haruhiko Miyagawa[1]
[1]Shimadzu Corporation.
1, Nishinokyo-Kuwabaracho Nakagyo-ku, Kyoto 604–8511, Japan,
[2]Shimadzu Scientific Instruments, Columbia, MD

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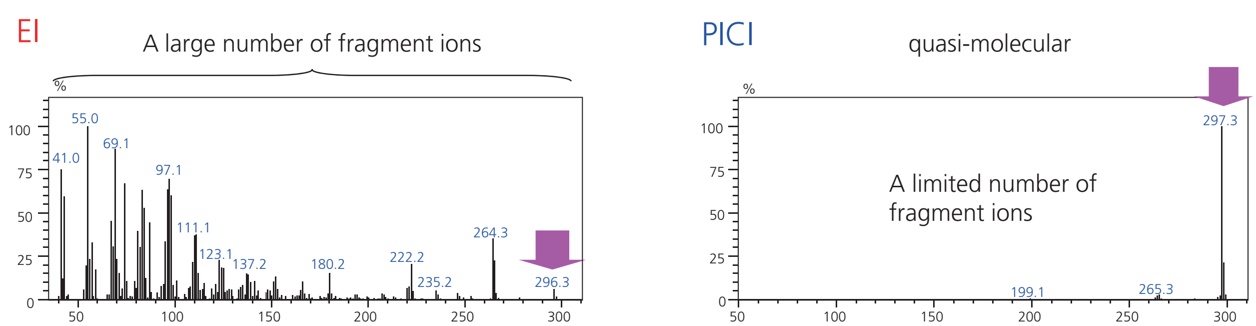
Introduction

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 EI mode because of the low abundance of fragment ions and the presence of co-eluting interferences. The more abundant quasi-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)



○ Easy selection of selective monitoring ions

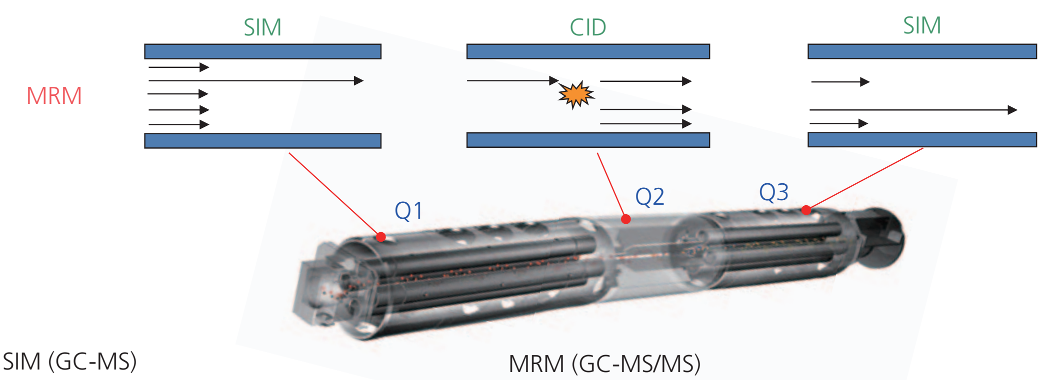
× Lower sensitivity

× Difficult selection of selective monitoring ions

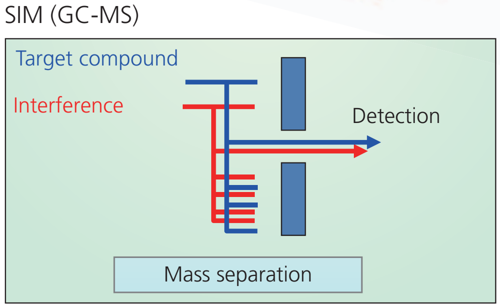
○ Higher sensitivity

Higher sensitive and selective method is required

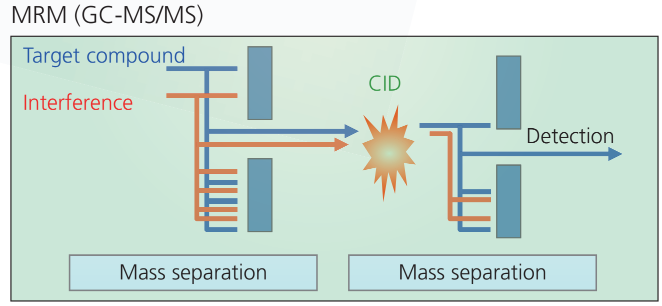
New approach of GC-MS/MS



SIM (GC-MS)



MRM (GC-MS/MS)

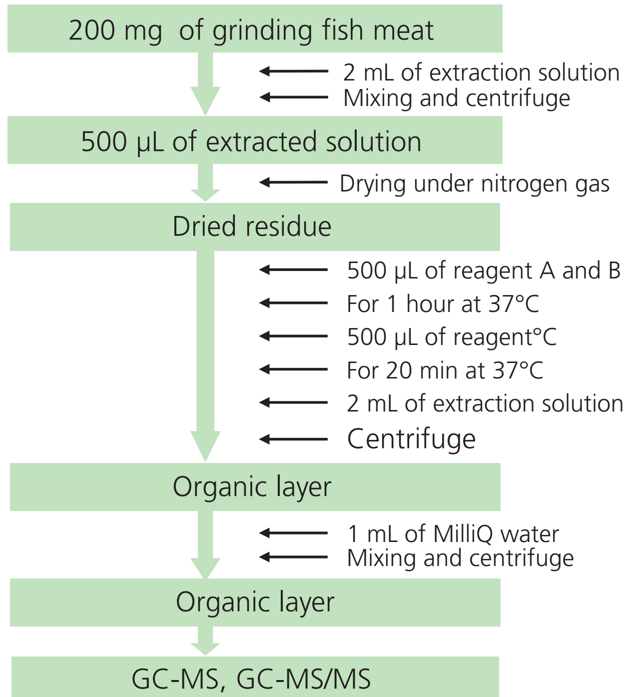


Methods and Materials

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 µm)

[GC]
Injection temp. :250°C
Column oven temp. :40°C (2 min)→(4°C/min)→240°C(15 min)
Injection mode :Split (1:10)
Carrier gas flow control :Constant linear velocity (20.0 cm/sec)

[MS]
Interface temp. :250°C
Ion source temp. :200°C
Measuring mode :SIM, MRM
Collision gas :Ar (200 kPa)
Collision energy (CE) :An optimal value for each compound

Ionization mode
EI :70eV
PICI :Iso-butane (70 kPa)

Monitoring *m/z*
EI-SIM :the most intense ion above *m/z* 100 (below *m/z* 100 for C4:0 and C6:0)
EI-MRM
Precursor ions :the same as the ions used in EI-SIM mode.
Product ions :the most intense ion above *m/z* 100.
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.

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Results

3-1. Limit of Quantitation (LOQs)

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

Black: Saturated fatty acids   Red: Unsaturated fatty acids   (highlighted) more than two times more sensitive

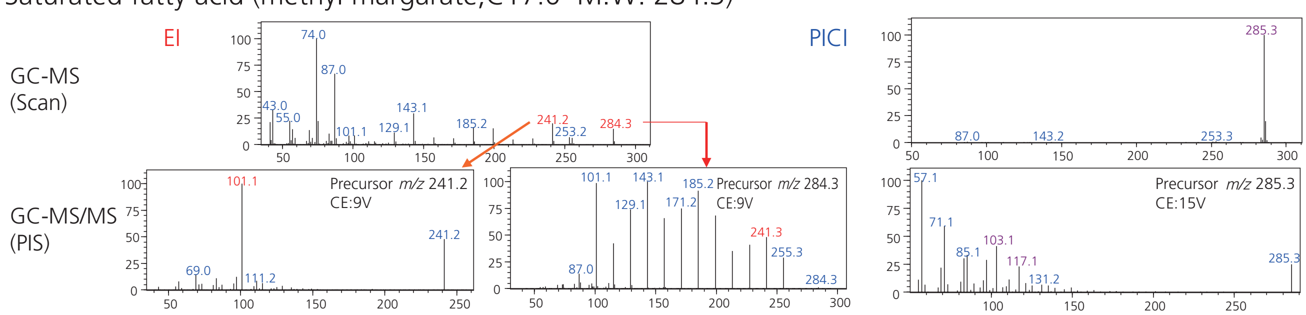
		CI MRM LOQ (pg)	CI SIM LOQ (pg)	EI MRM LOQ (pg)	EI SIM LOQ (pg)
1	Methyl butanoate;4:0	1.9	8.3	2.8	26.0
2	Methyl caproate;6:0	3.6	5.9	3.5	39.2
3	Methyl caprylate;8:0	4.8	4.8	10.5	53.9
4	Methyl caprate;10:0	8.8	7.2	32.9	36.8
5	Methyl undecanoate;11:0	20.6	30.1	22.0	44.0
6	Methyl laurate;12:0	10.9	6.6	56.5	74.4
7	Methyl tridecanoate;13:0	13.2	5.2	48.7	42.2
8	Methyl myristate;14:0	10.1	5.5	5.6	69.7
9	Methyl myristoleate;(Z)14:1n-5	2.7	4.7	178.3	134.0
10	Methyl pentadecanoate;15:0	36.6	4.8	29.3	32.1
11	Methyl cis-10-pentadecenoate;(Z)15:1n-5	4.6	4.0	225.3	33.7
12	Methyl palmitate;16:0	15.2	7.3	12.6	74.5
13	Methyl palmitoleate;(Z)16:1n-7	16.2	19.0	36.0	249.5
14	Methyl margarate;17:0	29.2	20.0	5.6	22.0
15	Methyl cis-10-heptadecenoate;(Z)17:1n-7	14.5	22.1	215.7	245.9
16	Methyl stearate;18:0	35.3	8.9	10.2	11.6
17	Methyl elaidate;(E)18:1n-9	19.8	5.5	180.7	173.9
18	Methyl oleate;(Z)18:1n-9	9.8	6.4	353.2	58.1

		CI MRM LOQ (pg)	CI SIM LOQ (pg)	EI MRM LOQ (pg)	EI SIM LOQ (pg)
19	Methyl linolelaidate;(E)18:2n-6	23.2	28.8	253.9	52.0
20	Methyl linoleate;(Z)18:2n-6	16.5	23.2	297.9	160.7
21	Methyl arachisate;20:0	58.5	11.5	9.1	11.6
22	Methyl ganma-linolenate;(Z)18:3n-6	81.2	17.6	167.8	349.8
23	Methyl cis-11-icosenoate;(Z)20:1n-9	36.0	22.0	45.1	145.1
24	Methyl linolenate;(Z)18:3n-3	135.7	23.9	414.6	213.1
25	Methyl heneicosanoate;21:0	108.5	25.5	33.7	41.0
26	Methyl cis-11,14-Icosadienoate;(Z)20:2n-6	36.5	13.4	282.1	238.4
27	Methyl behenate;22:0	279.0	23.3	29.6	7.0
28	Methyl eicosa-8,11,14-trienoate;20:3n-6	220.3	31.7	405.2	140.5
29	Methyl erucate;22:1n-9	96.1	31.2	387.8	143.3
30	Methyl cis-11,14,17-Icosatrienoate;(Z)20:3n-3	284.5	24.3	-	446.0
31	Methyl tricosanoate;23:0	357.2	19.3	54.2	24.8
32	Methyl arachidonate;(Z)20:4n-6	151.7	45.5	181.2	292.2
33	Methyl cis-13,16-Docosadienate;(Z)22:2n-6	128.1	315.7	335.3	283.2
34	Methyl lignocerate;24:0	503.8	41.8	52.6	10.3
35	Methyl cis-5,8,11,14,17-Eicosapentaenoate;(Z)20:5n-3	184.9	54.9	286.5	437.4
36	Methyl nervonate;(Z)24:1n-9	99.4	56.5	445.2	230.7
37	Methyl cis-4,7,10,13,16,19-Docosahexaenoate;(Z)22:6n-3	255.7	304.2	161.5	281.7

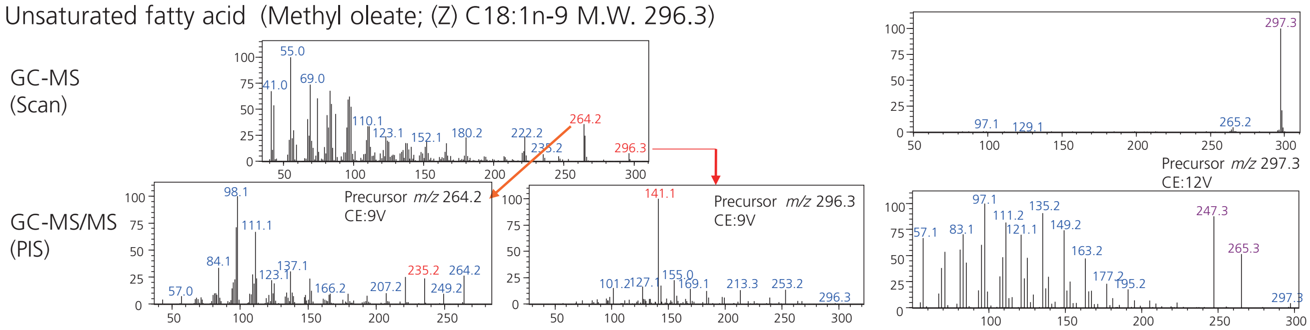
Measuring mode	Total number	Saturated	Unsaturated
EI SI	7	6	1
EI MRM	10	9	1
PICI SIM	32	13	19
PICI MRM	18	7	11

PICI was the most sensitive mode, especially in the analysis of unsaturated fatty acids

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



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



EI

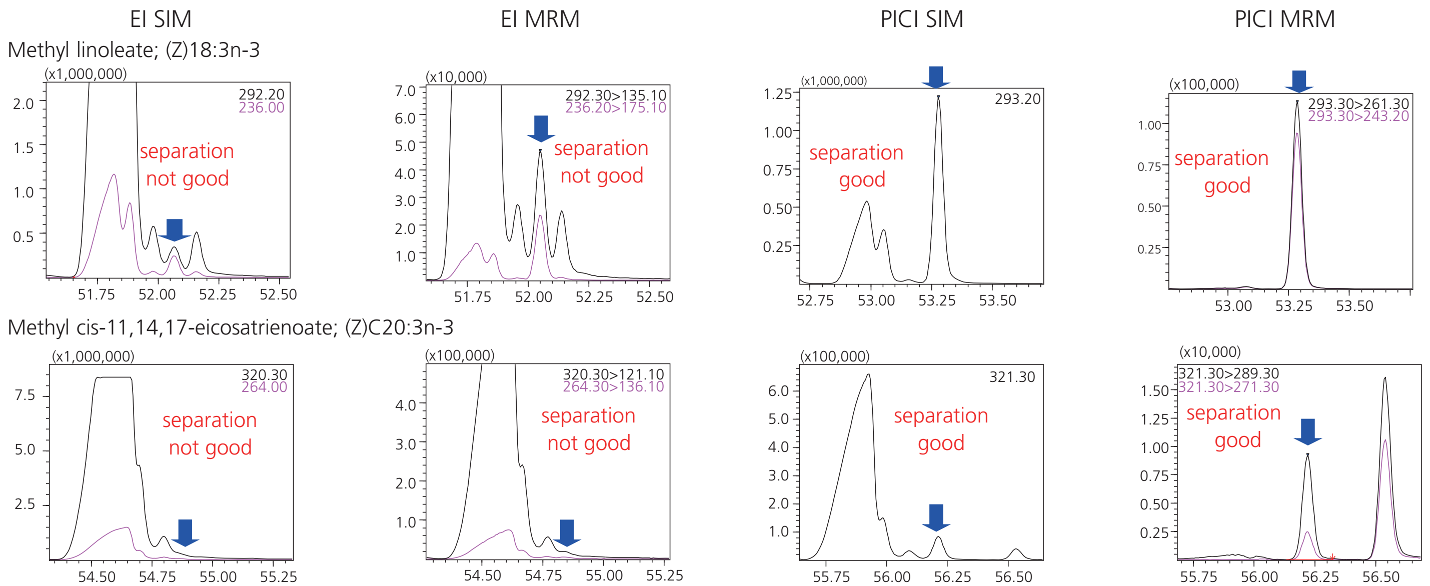
A variety of fragment ions Intensity of each ion is low (especially unsaturated fatty acids)

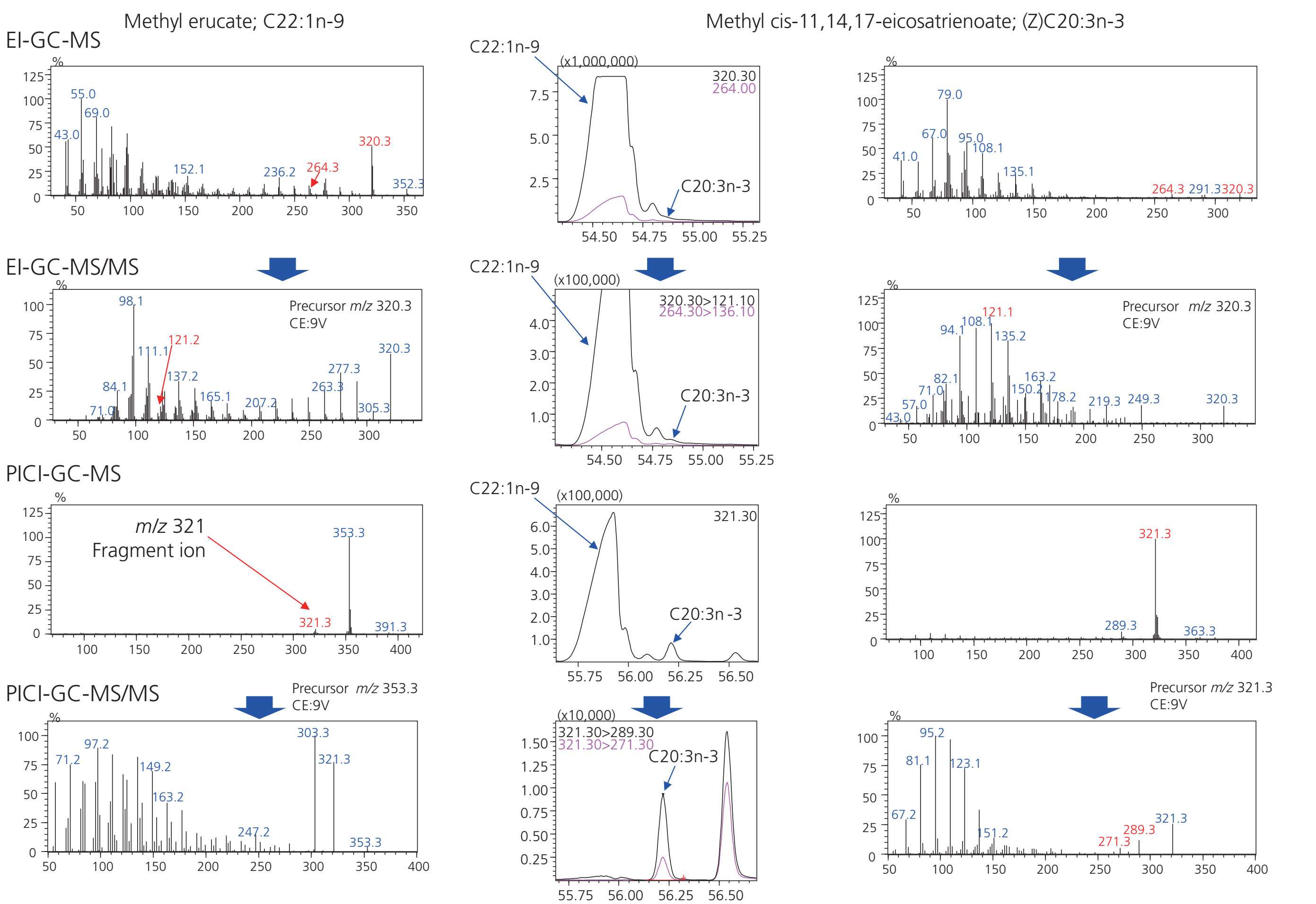
PICI

Unsaturated fatty acids produced intense product ions

3-2. Mass Separation in Food Sample

Brevoort





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 EI 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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