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Application Data Sheet

No.85

GC-MS
Gas Chromatograph Mass Spectrometer

Analysis of Fatty Acids Using PCI-GC-MS/MS

While some fatty acids, such as the n-3 fatty acids EPA and DHA, are beneficial to human health because they lower the amount of blood-borne neutral fat, too much intake of saturated fatty acids raises the risk of some diseases. For this reason, there is a need for the batch analysis of these fatty acids in the life sciences and food engineering sectors. Despite requiring methylation, GC-MS has gained attention because of its suitability for multicomponent batch analyses.

In fatty acid analyses utilizing GC-MS, the EI (electron ionization) method is used for ionization. With the EI method, there are many types of fragment ions, making it easy to select an *m/z* to enable separation by mass from impurities. However, because of the large number of fragment ions, the sensitivity of the individual ions is reduced, making it difficult to detect trace quantities of fatty acids. In contrast, with the PCI (positive chemical ionization) method, protonated molecular ions can be detected, from which molecular weight data can be obtained. Since there is only a small number of fragment ion types, the sensitivity is increased. This means, however, that the ion types that can be selected for monitoring are limited and there may not be any ions that can be separated by mass from impurities.

This application data sheet introduces the results of an investigation of sensitivity based on the EI-SIM, PCI-SIM, EI-MRM, and PCI-MRM methods. In addition, in Application Data Sheet No. 86, we introduce the results of an investigation of separation from impurities in the analysis of fatty acids in foods.

Analysis Conditions

The Supelco® 37 Component FAME Mix (P/N: 47885-U, SIGMA-ALDRICH) was utilized as the standard sample. The standard sample was diluted in stages with dichloromethane, and used for sensitivity evaluation. Table 1 shows the analysis conditions. Analysis methods included in the GC/MS Metabolite Database Ver. 2 were used for the monitoring *m/z* and for the EI-SIM, PCI-SIM, EI-MRM, and PCI-MRM methods.

Table 1: Analysis Conditions

GC-MS:	GCMS-TQ8030
Column:	SP-2560 (Length 100 m; 0.20 mm I.D.; df = 0.25 μm)
Glass insert:	Splitless insert with wool (P/N: 221-48876-03)

[GC]	
Sample injection unit temp.:	250 °C
Column oven temp.:	40 °C (2 min) → (4 °C /min) → 240 °C (15 min)
Injection mode:	Split
Split ratio:	10
Carrier gas control:	Linear velocity (20.0 cm/sec)
Injection volume:	1 μL

[MS]	
Interface temp.:	250 °C
Ion source temp.:	200 °C
Measurement mode:	
GC-MS:	SIM
GC-MS/MS:	MRM
Ionization method:	EI and PCI methods
PCI reagent gas:	Isobutane
PCI reagent gas pressure:	70 kPa

Analysis Results

Fig. 1 shows mass chromatograms for 100 pg methyl cis-10-heptadecenoate (Z, 17:1n-7) obtained in the analysis modes. Sensitivity was evaluated by calculating the lower limit of quantitation using a t-test on the area reproducibility results for which the %RSD was 20 % or less with the analysis repeated 8 times. The lower limits of quantitation in each analysis mode are shown in Table 2. Table 3 shows the efficacy of each analysis mode for saturated and unsaturated fatty acids, with analysis methods having a sensitivity difference within twice the lower limit of quantitation for the analysis method offering the highest sensitivity taken as advantageous. For unsaturated fatty acids, the PCI method was superior to the EI method.

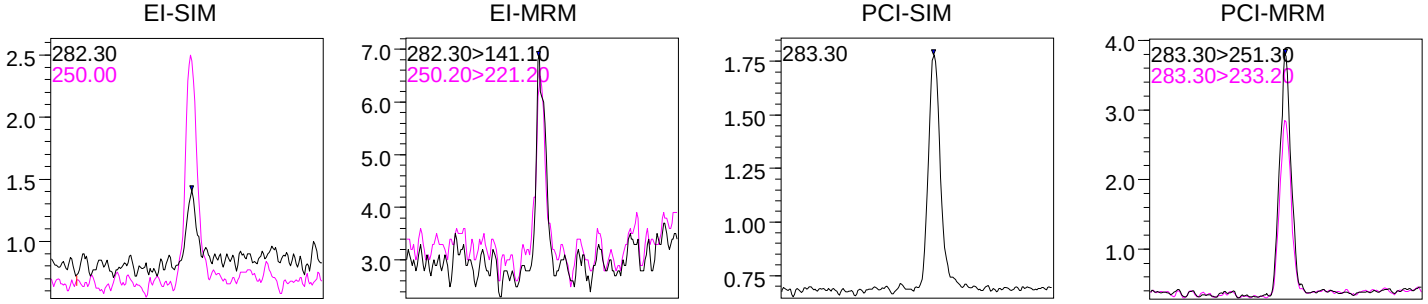

Fig. 1: Mass Chromatograms for Methyl Cis-10-Heptadecenoate (Z, 17:1n-7) Measured in Individual Analysis Modes

Table 2: Lower Limits of Quantitation in Each Mode for 37 Fatty Acid Methyl Esters

		EI SIM LOQ (pg)	EI MRM LOQ (pg)	PCI SIM LOQ (pg)	PCI MRM LOQ (pg)
1	Methyl butanoate;4:0	26.0	2.8	8.3	1.9
2	Methyl caproate;6:0	39.2	3.5	5.9	3.6
3	Methyl caprylate;8:0	53.9	10.5	4.8	4.8
4	Methyl caprate;10:0	36.8	32.9	7.2	8.8
5	Methyl undecanoate;11:0	44.0	22.0	30.1	20.6
6	Methyl laurate;12:0	74.4	56.5	6.6	10.9
7	Methyl tridecanoate;13:0	42.2	48.7	5.2	13.2
8	Methyl myristate;14:0	69.7	5.6	5.5	10.1
9	Methyl myristoleate;(Z)14:1n-5	134.0	178.3	4.7	2.7
10	Methyl pentadecanoate;15:0	32.1	29.3	4.8	36.6
11	Methyl cis-10-pentadecenoate;(Z)15:1n-5	33.7	225.3	4.0	4.6
12	Methyl palmitate;16:0	74.5	12.6	7.3	15.2
13	Methyl palmitoleate;(Z)16:1n-7	249.5	36.0	19.0	16.2
14	Methyl margarate;17:0	22.0	5.6	20.0	29.2
15	Methyl cis-10-heptadecenoate;(Z)17:1n-7	245.9	215.7	22.1	14.5
16	Methyl stearate;18:0	11.6	10.2	8.9	35.3
17	Methyl elaidate;(E)18:1n-9	173.9	180.7	5.5	19.8
18	Methyl oleate;(Z)18:1n-9	58.1	353.2	6.4	9.8
19	Methyl linolelaidate;(E)18:2n-6	52.0	253.9	28.8	23.2
20	Methyl linoleate;(Z)18:2n-6	160.7	297.9	23.2	16.5
21	Methyl arachisate;20:0	11.6	9.1	11.5	58.5
22	Methyl ganma-linolenate;(Z)18:3n-6	349.8	167.8	17.6	81.2
23	Methyl cis-11-icosenoate;(Z)20:1n-9	145.1	45.1	22.0	36.0
24	Methyl linolenate;(Z)18:3n-3	213.1	414.6	23.9	135.7
25	Methyl heneicosanoate;21:0	41.0	33.7	25.5	108.5
26	Methyl cis-11,14-Icosadienoate;(Z)20:2n-6	238.4	282.1	13.4	36.5
27	Methyl behenate;22:0	7.0	29.6	23.3	279.0
28	Methyl eicosa-8,11,14-trienoate;20:3n-6	140.5	405.2	31.7	220.3
29	Methyl erucate;22:1n-9	143.3	387.8	31.2	96.1
30	Methyl cis-11,14,17-Icosatrienoate;(Z)20:3n-3	446.0	-	24.3	284.5
31	Methyl tricosanoate;23:0	24.8	54.2	19.3	357.2
32	Methyl arachidonate;(Z)20:4n-6	292.2	181.2	45.5	151.7
33	Methyl cis-13,16-Docosadienate;(Z)22:2n-6	283.2	335.3	315.7	128.1
34	Methyl lignocerate;24:0	10.3	52.6	41.8	503.8
35	Methyl cis-5,8,11,14,17-Eicosapentaenoate;(Z)20:5n-3	437.4	286.5	54.9	184.9
36	Methyl nervonate;(Z)24:1n-9	230.7	445.2	56.5	99.4
37	Methyl cis-4,7,10,13,16,19-Docosahexaenoate;(Z)22:6n-3	281.7	161.5	304.2	255.7

Notes:
- Compound names in blue indicate unsaturated fatty acids.
- [ ] cells indicate that the LOQ is within twice the value for the analysis mode with the highest sensitivity.

Table 3: Sensitivity Predominance for Each Analysis Mode

Measurement Mode	Total Fatty Acids	Saturated Fatty Acids	Unsaturated Fatty Acids
EI SIM	7	6	1
EI MRM	10	9	1
PCI SIM	32	13	19
PCI MRM	18	7	11

Notes:
- The analysis methods having a sensitivity difference within twice the lower limit of quantitation for the analysis method offering the highest sensitivity are taken as advantageous.

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