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Application News

GC-MS GCMS-TQ™ Series and Smart Metabolites Database™

Quantitative Analysis of Fatty Acid Methyl Esters (FAMEs) Using Smart EI/CI Ion Source

Y. Sakamoto and Y. Kawakita

User Benefits

- PCI-MRM mode is ideal for analyzing fatty acids, as it offers higher sensitivity than EI-MRM for unsaturated fatty acids.
- Smart EI/CI ion source can switch between EI and PCI ionization methods without stopping the instrument.
- Fatty acids in foods are easily analyzed using Smart Metabolites Database and fatty acid methylation kit.

■ Introduction

Fatty acids are the components that make up lipids. They are essential to living organisms for energy metabolism and as nutrients. Fatty acid analysis has a long history, in medical applications for example to predict diseases or determine how healthy people are, and in food applications to improve the quality and functionality of food products during development.

Because of the presence of carboxyl groups, fatty acids are difficult to measure directly in a GC-MS(/MS) system, so they are measured after derivatization by methylation into fatty acid methyl esters (FAMEs).

There are many varieties of FAMEs, which differ in their carbon chain length, number or position of double bonds, and other characteristics. Although the EI ionization method is typically used for GC-MS, it tends to fragment FAMEs during ionization, resulting in the detection of large numbers of similar low-mass ions. Consequently, higher sensitivity and greater separation are needed. In contrast, positive chemical ionization (PCI) uses a reagent gas to ionize FAMEs indirectly, which makes the protonated molecules easier to detect. This is effective for separating ions with different molecular weights based on mass, but requires the separation of isomers in a chromatogram. The CI-MRM mode of GC-MS/MS involves fragmenting ionized protonated molecules via CID to improve mass separation between the contaminants and FAMEs.

This article describes the results of a comparison of the sensitivity and quantitative accuracy of EI-MRM and CI-MRM modes.

■ Smart Metabolites Database

Smart Metabolites Database contains analytical condition settings, and optimized SIM and MRM transition settings for metabolic components. Over 600 metabolic components, including organic acids, amino acids, and fatty acids, are registered in the simultaneous analysis method intended for biomarker search applications. Compound-specific quantitation methods are also included for analyzing fatty acid methyl esters (FAMEs). Fifty FAME components are registered in the method intended for DB-5MS, which can be used for either the EI or PCI ionization mode.

■ Smart EI/CI Ion Source

The hybrid Smart EI/CI ion source enables measurements by PCI while minimizing any sensitivity decrease for EI. Previously, switching between EI and CI modes required that the vacuum in the GC-MS system be compromised to allow the ion sources to be swapped. Smart EI/CI ion source enables switching between ionization modes simply by changing method files, which significantly shortens the system downtime.

■ Experiment

The sensitivity and quantitative accuracy of EI-MRM and PCI-MRM modes were compared in an analysis of fatty acids in food. PCI-MRM sensitivity was also compared when using a dedicated PCI ion source and Smart EI/CI ion source.

Standard FAME solutions were prepared by diluting a FAME reference standard containing 37 FAME components (AccuStandard Inc., cat.: FAMQ-005) in stages.

Food samples were prepared from commercial beef and mackerel. After homogenizing and freeze-drying the samples, 50 mg of each sample was weighed out, 2 mL of acetone was added and mixed in, and extract solutions were obtained by centrifugal separation. In addition, 2 mL of hexane was added to the residues and mixed in, from which extract solutions were obtained by centrifugal separation. Then the acetone and hexane extract solutions were mixed together, 2 mL of ion-exchanged water was added, the mixture was stirred and centrifuged, and the top phase was collected and dried. The dried sample was pretreated using the fatty acid methylation kit from Nacalai Tesque. The extract sample was diluted with hexane by a factor of 100 and then measured. The analytical conditions are indicated in Table 1.

Table 1 System Configuration and Analytical Conditions

GC-MS:	GCMS-TQ8050 NX
Autoinjector:	AOC™-30i/20s U
Column:	DB-5MS (30 m, 0.25 mm I.D., 0.25 µm)
[GC]	
Injection Temp.:	250 °C
Column Oven Temp.:	40 °C (2 min) → (6 °C /min) → 320 °C (1 min)
Injection Mode:	Splitless
Sampling Time:	1 min
Carrier Gas:	He
Carrier Gas Control:	Constant linear velocity (50.0 cm/sec)
Injection Volume:	1 µL
[MS]	
Ion Source Temp.:	200 °C
Interface Temp:	280 °C
Data Acquisition Mode:	MRM
Event Time:	0.5 sec
Ionization Method (PCI Ion Source)	
Ionization Method:	PCI
Reagent and Pressure:	Isobutane (80 kPa)
Ionization Method (Smart EI/CI Ion Source)	
Ionization Method:	PCI
Reagent and Pressure:	Isobutane (200 kPa)

■ Comparison of PCI-MRM Sensitivity

PCI-MRM mode sensitivity for a dedicated PCI ion source and Smart EI/CI ion source were compared. MRM chromatograms of methyl octanoate (C8) and methyl palmitate (C16), representative components measured from the 0.01 µg/mL standard sample, are shown in Fig. 1. S/N values were calculated by the peak-to-peak method. Although the results show minor differences for some target components, Smart EI/CI ion source generally provided near equivalent PCI sensitivity to the dedicated PCI ion source.

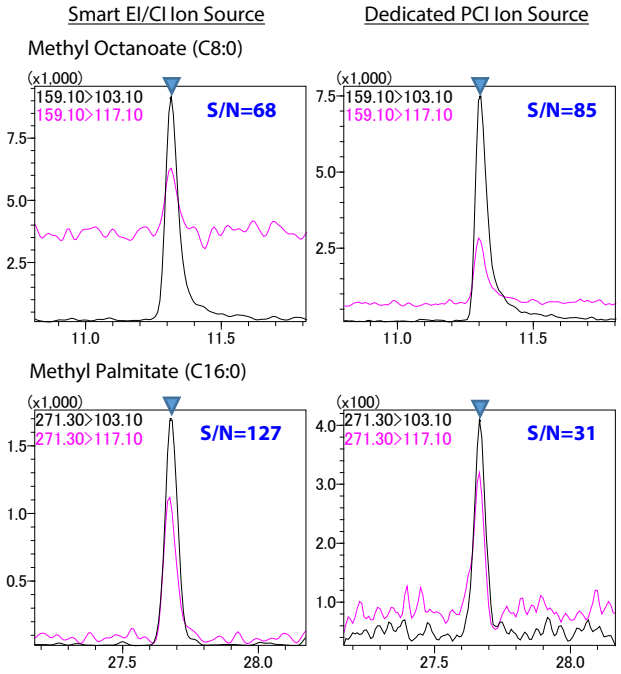

Fig. 1 Mass Chromatograms Acquired with a Dedicated PCI Ion Source or Smart EI/CI Ion Source

■ Comparison of EI-MRM and PCI-MRM Sensitivity

Next, the sensitivity of Smart EI/CI ion source PCI-MRM and EI-MRM modes were compared. The results from a comparison of representative MRM chromatograms are shown in Fig. 2. S/N values were calculated by the peak-to-peak method, with the limit of quantitation (LOQ) set to the concentration that resulted in an S/N value of 10. Table 2 lists the PCI and EI mode LOQ values for 37 FAME components. Although EI-MRM mode provided slightly better results for saturated fatty acids, PCI-MRM mode provided better results for unsaturated fatty acids. In particular, sensitivity improved more than tenfold for some FAME compounds with one double bond.

Table 2 LOQ of PCI-MRM and EI-MRM Modes for 37 FAME Components

	Compound Name	CI MRM LOQ (pg)	EI MRM LOQ (pg)
1	Methyl butanoate (C4:0)	**0.7**	**0.4**
2	Methyl caproate (C6:0)	**0.4**	**0.7**
3	Methyl caprylate (C8:0)	**2.9**	**1.4**
4	Methyl caprate (C10:0)	**2.5**	**2.0**
5	Methyl undecanoate (C11:0)	**2.5**	**2.0**
6	Methyl laurate (C12:0)	**6.9**	**3.9**
7	Methyl tridecanoate (C13:0)	**4.5**	**5.6**
8	Methyl myristate (C14:0)	**2.8**	**0.9**
9	Methyl myristoleate (Z) (C14:1n-5)	**0.2**	**4.8**
10	Methyl pentadecanoate (C15:0)	**2.8**	**0.9**
11	Methyl cis-10-pentadecanoate (Z) (C15:1n-5)	**0.2**	**5.9**
12	Methyl palmitate (C16:0)	**2.6**	**0.8**
13	Methyl palmitoleate (Z) (C16:1n-7)	**1.4**	**4.8**
14	Methyl margarate (C17:0)	**3.2**	**1.1**
15	Methyl cis-10-heptadecanoate (Z) (C17:1n-7)	**0.9**	**7.7**
16	Methyl stearate (C18:0)	**4.8**	**1.3**
17	Methyl elaidate (E) (C18:1n-9)	**0.6**	**9.7**
18	Methyl oleate (Z) (C18:1n-9)	**0.4**	**6.5**
19	Methyl linolelaidate (E) (C18:2n-6)	**1.5**	**8.9**
20	Methyl linoleate (Z) (C18:2n-6)	**2.6**	**16.9**
21	Methyl arachisate (C20:0)	**1.4**	**2.3**
22	Methyl ganma-linolenate (Z) (C18:3n-6)	**5.7**	**5.6**
23	Methyl cis-11-icosenoate (Z) (C20:1n-9)	**0.7**	**6.3**
24	Methyl linolenate (Z) (C18:3n-3)	**13.2**	**47.6**
25	Methyl heneicosanoate (C21:0)	**0.8**	**2.1**
26	Methyl cis-11,14-Icosadienoate (Z) (C20:2n-6)	**6.3**	**22.2**
27	Methyl behenate (C22:0)	**2.2**	**4.4**
28	Methyl eicosa-8,11,14-trienoate (C20:3n-6)	**14.7**	**16.9**
29	Methyl erucate (C22:1n-9)	**0.5**	**35.7**
30	Methyl cis-11,14,17-Icosatrienoate (Z) (C20:3n-3)	**24.4**	**31.3**
31	Methyl tricosanoate (C23:0)	**5.0**	**6.3**
32	Methyl arachidonate (Z) (C20:4n-6)	**20.4**	**34.5**
33	Methyl cis-13,16-Docosadienate (Z) (C22:2n-6)	**4.6**	**41.7**
34	Methyl lignocerate (C24:0)	**6.5**	**9.1**
35	Methyl cis-5,8,11,14,17-Eicosapentaenoate (Z) (C20:5n-3)	**42.0**	**138.9**
36	Methyl nervonate (Z) (C24:1n-9)	**2.1**	**50.0**
37	Methyl cis-4,7,10,13,16,19-Docosahexaenoate (Z) (C22:6n-3)	**59.5**	**68.5**

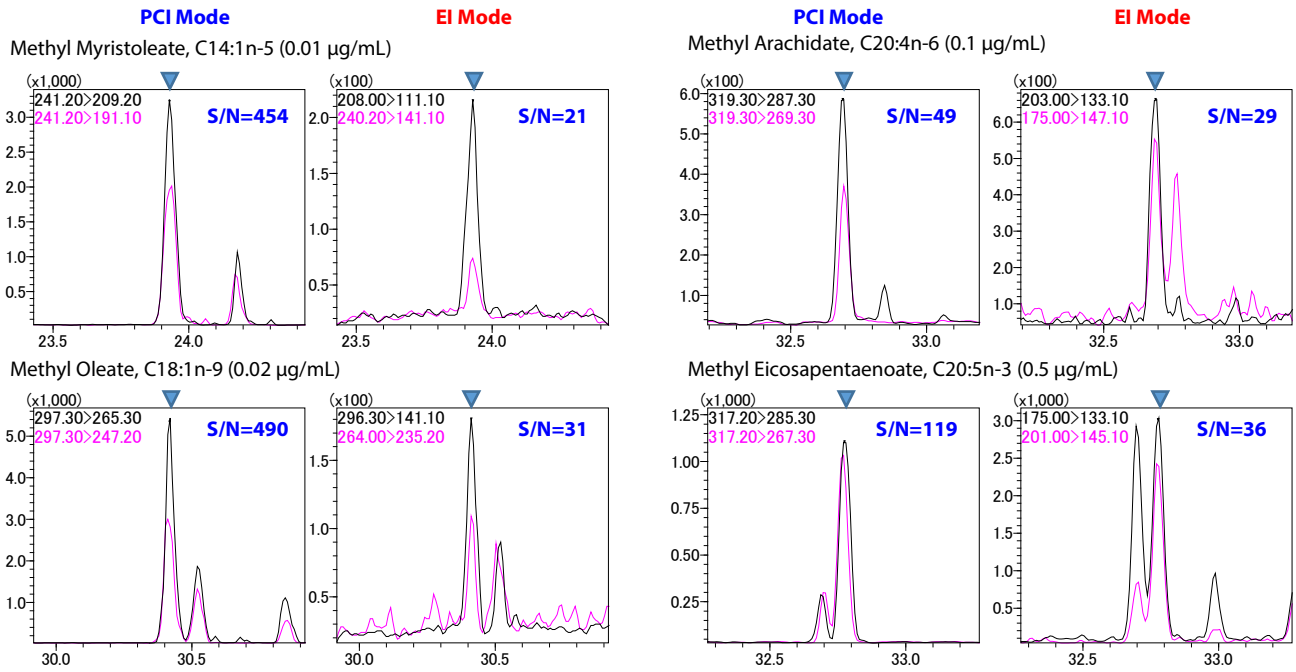

Fig. 2 Mass Chromatograms Acquired with PCI and EI Modes

■ EI-MRM and PCI-MRM Sensitivity

The calibration curve linearity for Smart EI/CI ion source PCI-MRM and EI-MRM modes were compared. Calibration curves for methyl palmitate (C16:0) and methyl docosahexaenoate (C22:6n-3) are shown in Fig. 3 as representative of the calibration curves acquire in PCI-MRM mode. The results of a comparison of the contribution rate (R^2) and the calibration curve range for a PCI-MRM and EI-MRM analysis of 37 FAME components are listed in Table 3.

The contribution rate for the calibration curve acquired by PCI-MRM was 0.995 or higher, which is about equivalent to the calibration curve acquired by EI-MRM.

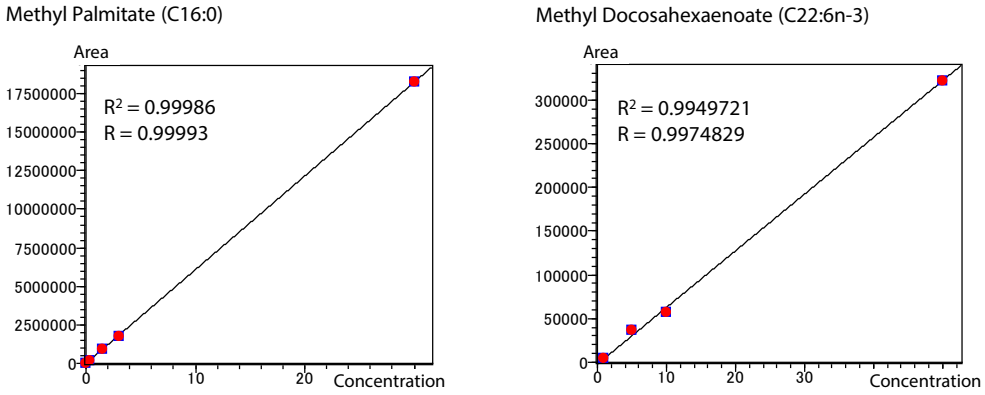

Fig. 3 Calibration Curves for Methyl Palmitate and Methyl Docosahexaenoate Acquired with the PCI Mode

Table 3 Contribution Rate (R^2) and Calibration Curve Rang in CI-MRM and EI-MRM Modes for 37 FAME Components

		CI		EI	
ID	Compound Name	R^2	Calibration Range	R^2	Calibration Range
1	Methyl butanoate (C4:0)	0.9989	0.02 to 10	0.9998	0.02 to 10
2	Methyl caproate (C6:0)	0.9992	0.02 to 10	0.9995	0.02 to 2
3	Methyl caprylate (C8:0)	0.9998	0.02 to 10	0.9997	0.02 to 20
4	Methyl caprate (C10:0)	0.9999	0.02 to 10	0.9991	0.02 to 100
5	Methyl undecanoate (C11:0)	0.9995	0.01 to 10	0.9996	0.01 to 50
6	Methyl laurate (C12:0)	0.9982	0.02 to 10	0.9999	0.02 to 100
7	Methyl tridecanoate (C13:0)	0.9997	0.01 to 10	0.9997	0.01 to 50
8	Methyl myristate (C14:0)	0.9997	0.02 to 20	0.9999	0.02 to 20
9	Methyl myristoleate (Z) (C14:1n-5)	0.9990	0.01 to 10	0.9996	0.01 to 50
10	Methyl pentadecanoate (C15:0)	0.9998	0.01 to 10	0.9995	0.01 to 50
11	Methyl cis-10-pentadecanoate (Z) (C15:1n-5)	0.9995	0.01 to 10	0.9995	0.01 to 50
12	Methyl palmitate (C16:0)	0.9999	0.03 to 30	0.9999	0.03 to 30
13	Methyl palmitoleate (Z) (C16:1n-7)	0.9997	0.01 to 10	0.9993	0.01 to 50
14	Methyl margarate (C17:0)	0.9992	0.01 to 10	0.9992	0.01 to 50
15	Methyl cis-10-heptadecanoate (Z) (C17:1n-7)	0.9999	0.01 to 10	0.9990	0.01 to 50
16	Methyl stearate (C18:0)	0.9993	0.02 to 20	0.9990	0.02 to 100
17	Methyl elaidate (E) (C18:1n-9)	0.9999	0.01 to 10	0.9997	0.01 to 50
18	Methyl oleate (Z) (C18:1n-9)	0.9999	0.02 to 20	0.9989	0.02 to 20
19	Methyl linolelaidate (E) (C18:2n-6)	0.9991	0.1 to 50	0.9989	0.1 to 50
20	Methyl linoleate (Z) (C18:2n-6)	0.9988	0.01 to 50	0.9994	0.1 to 50
21	Methyl arachisate (C20:0)	0.9984	0.02 to 20	0.9992	0.02 to 100
22	Methyl ganma-linolenate (Z) (C18:3n-6)	0.9995	0.1 to 50	0.9973	0.01 to 50
23	Methyl cis-11-icosenoate (Z) (C20:1n-9)	0.9982	0.01 to 10	0.9996	0.1 to 50
24	Methyl linolenate (Z) (C18:3n-3)	0.9996	0.1 to 50	0.9996	0.1 to 50
25	Methyl heneicosanoate (C21:0)	0.9981	0.01 to 50	0.9976	0.01 to 50
26	Methyl cis-11,14-Icosadienoate (Z) (C20:2n-6)	0.9984	0.1 to 50	0.9990	0.1 to 50
27	Methyl behenate (C22:0)	0.9989	0.02 to 100	0.9986	0.02 to 100
28	Methyl eicosa-8,11,14-trienoate (C20:3n-6)	0.9995	0.1 to 50	0.9996	0.1 to 50
29	Methyl erucate (C22:1n-9)	0.9988	0.01 to 50	0.9970	0.1 to 50
30	Methyl cis-11,14,17-Icosatrienoate (Z) (C20:3n-3)	0.9990	0.1 to 50	0.9995	0.1 to 50
31	Methyl tricosanoate (C23:0)	0.9979	0.1 to 50	0.9980	0.1 to 50
32	Methyl arachidonate (Z) (C20:4n-6)	0.9989	0.1 to 50	0.9992	0.1 to 50
33	Methyl cis-13,16-Docosadienate (Z) (C22:2n-6)	0.9983	0.1 to 50	0.9989	0.1 to 50
34	Methyl lignocerate (C24:0)	0.9995	0.2 to 100	0.9977	0.2 to 100
35	Methyl cis-5,8,11,14,17-Eicosapentaenoate (Z) (C20:5n-3)	0.9971	0.5 to 50	0.9988	0.1 to 50
36	Methyl nervonate (Z) (C24:1n-9)	0.9993	0.1 to 50	0.9998	0.5 to 50
37	Methyl cis-4,7,10,13,16,19-Docosahexaenoate (Z) (C22:6n-3)	0.9950	0.5 to 50	0.9986	0.5 to 50

■ Examples of Food Sample Applications

Beef and mackerel samples were pretreated using the fatty acid methylation kit, and measured in EI-MRM and PCI-MRM modes. The concentrations in the samples were calculated based on the calibration curves created using the EI-MRM and CI-MRM results. The quantitative results and the PCI/EI concentration ratios for the beef sample are listed in Table 4.

The quantitative results and the PCI/EI concentration ratios for the mackerel sample are listed in Table 5. Quantitative results for PCI mode were within ±30 % of the reference EI-MRM quantitative results. Quantitative accuracy remained basically unchanged. The PCI/EI concentration ratio for methyl linolenate in the beef sample was 146.9 %, but this was presumably because the concentration in the EI extract solution was close to the limit of quantitation.

Table 4 Quantitative Results for FAMEs in Beef Sample

Compound	PCI Conc. (μg/mg)	EI Conc. (μg/mg)	PCI/EI Conc. Ratio (%)
Methyl caprate (C10:0)	0.06	0.06	100.0
Methyl laurate (C12:0)	0.14	0.13	107.7
Methyl tridecanoate (C13:0)	0.02	0.02	100.0
Methyl myristoleate (Z) (C14:1n-5)	4.00	3.65	109.6
Methyl myristate (C14:0)	5.91	6.05	97.7
Methyl cis-10-pentadecanoate (Z) (C15:1n-5)	0.07	0.07	100.0
Methyl pentadecanoate (C15:0)	0.69	0.58	119.0
Methyl palmitoleate (Z) (C16:1n-7)	12.67	12.77	99.2
Methyl palmitate (C16:0)	51.48	52.49	98.1
Methyl cis-10-heptadecanoate (Z) (C17:1n-7)	2.49	2.22	112.2
Methyl margarate (C17:0)	1.55	1.36	114.0
Methyl linoleate (Z) (C18:2n-6)	7.22	6.43	112.3
Methyl linolenate (Z) (C18:3n-3)	0.47	0.32	146.9
Methyl oleate (Z) (C18:1n-9)	77.76	84.57	91.9
Methyl elaidate (E) (C18:1n-9)	7.40	7.19	102.9
Methyl stearate (C18:0)	17.16	17.18	99.9
Methyl eicosa-8,11,14-trienoate (C20:3n-6)	0.30	0.29	103.4
Methyl cis-11,14-Icosadienoate (Z) (C20:2n-6)	0.13	0.16	81.3
Methyl cis-11-icosenoate (Z) (C20:1n-9)	0.89	0.87	102.3
Methyl arachisate (C20:0)	0.11	0.11	100.0

Table 5 Quantitative Results for FAMEs in Mackerel Sample

	PCI Conc. (μg/mg)	EI Conc. (μg/mg)	PCI/ EI Conc. Ratio (%)
Methyl caprate (C10:0)	0.01	0.01	100.0
Methyl laurate (C12:0)	0.07	0.07	100.0
Methyl tridecanoate (C13:0)	0.05	0.05	100.0
Methyl myristoleate (Z) (C14:1n-5)	0.04	0.05	80.0
Methyl myristate (C14:0)	6.02	6.32	95.3
Methyl cis-10-pentadecanoate (Z) (C15:1n-5)	0.05	0.04	125.0
Methyl pentadecanoate (C15:0)	0.74	0.65	113.8
Methyl palmitoleate (Z) (C16:1n-7)	4.98	4.88	102.0
Methyl palmitate (C16:0)	36.77	38.16	96.4
Methyl cis-10-heptadecanoate (Z) (C17:1n-7)	0.63	0.56	112.5
Methyl margarate (C17:0)	1.11	0.95	116.8
Methyl ganma-linolenate (Z) (C18:3n-6)	0.27	0.21	128.6
Methyl linoleate (Z) (C18:2n-6)	2.43	2.50	97.2
Methyl linolenate (Z) (C18:3n-3)	1.15	1.21	95.0
Methyl oleate (Z) (C18:1n-9)	42.43	41.62	101.9
Methyl elaidate (E) (C18:1n-9)	5.98	5.59	107.0
Methyl stearate (C18:0)	10.26	10.54	97.3
Methyl arachidonate (Z) (C20:4n-6)	1.19	1.31	90.8
Methyl cis-5,8,11,14,17-Eicosapentaenoate (Z) (C20:5n-3)	11.46	13.45	85.2
Methyl eicosa-8,11,14-trienoate (C20:3n-6)	0.30	0.30	100.0
Methyl cis-11,14-Icosadienoate (Z) (C20:2n-6)	0.46	0.46	100.0
Methyl cis-11,14,17-Icosatrienoate (Z) (C20:3n-3)	0.30	0.35	85.7
Methyl cis-11-icosenoate (Z) (C20:1n-9)	4.74	4.96	95.6
Methyl arachisate (C20:0)	0.38	0.37	102.7
Methyl heneicosanoate (C21:0)	0.07	0.07	100.0
Methyl cis-4,7,10,13,16,19-Docosahexaenoate (Z) (C22:6n-3)	32.85	31.96	102.8
Methyl erucate (C22:1n-9)	0.80	0.92	87.0
Methyl behenate (C22:0)	0.17	0.18	94.4
Methyl nervonate (Z) (C24:1n-9)	1.38	1.42	97.2
Methyl lignocerate (C24:0)	0.38	0.45	84.4

■ Conclusion

In a quantitative analysis of fatty acids in foods, PCI-MRM mode can detect fatty acid methyl esters with higher sensitivity than EI-MRM and with comparable quantitative accuracy. A major disadvantage of the PCI method has been the need to switch to a dedicated ion source, but Smart EI/CI ion source can switch between EI and PCI without stopping the system.

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