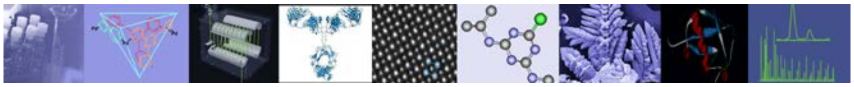


Integrated Analysis of Fatty Acid Methyl Esters using msFineAnalysis Version 2

- Molecular Ion Detection by FI -

Product: JMS-T200GC GC /MS System

Introduction

Electron ionization (EI) is a hard ionization method that is commonly used with gas chromatography mass spectrometry (GC-MS). The mass spectral fragmentation patterns produced by EI are used for library database searches to identify compounds. Conversely, soft ionization methods like field ionization (FI) tend to produce clear molecular ions with minimal fragmentation. When high-resolution MS is used with these ionization techniques, the accurate masses for the fragment ions produced by EI and the molecular ions produced by soft ionization provide an additional dimension of information for the analytes. Combining the exact mass information with the results of conventional library search can enhance the accuracy of identification compared to the use of library search alone.

Fatty acid methyl esters (FAMEs) are crucial for determining the fat content in food. Being environmentally friendly, they are also increasingly used as bio-diesel fuels. Many of the FAMEs are unsaturated with double bonds in the alkyl chains. As the number of double bonds increases (more unsaturation), the EI measurements tend to lack molecular ions. In this work, we measured a standard sample containing multiple FAMEs using EI and FI to detect their molecular ions. The resulting data was further examined by using msFineAnalysis to produce an integrated report for these compounds in which the library database search was combined with the molecular ion exact mass analysis to produce a qualitative identification of these compounds.

Experiment

A commercial 37-component FAMEs standard mixture (Restek, 200-600 ng/µL) was used as a sample. Table 1 shows the measurement conditions used for the GC/EI and GC/FI analyses.

Table 1. Measurement conditions

[GC-TOFMS Conditions]

System	JMS-T200GC (JEOL)
Ion Source	EI/FI combination ion source
Ionization mode	EI+: 70 eV, 300 µA FI+: -10 kV, 50Ma, Slope mode
Mass Range	*m/z* 35-600
GC column	DB-5MSUI, 30 m x 0.25 mm, 0.25 µm
Oven temp.	50°C (1 min) → 10°C/min → 140°C → 3°C/min → 260°C (5 min)
Inlet mode	Split 50:1

Results and discussions

Figure 1 shows the TICC for the GC/EI and GC/FI measurements. While the sample contains 37 components, there were only 36 peaks observed in each chromatogram. The *cis*-4,7,10,13,16,19-docosahexaenoic acid methyl ester ($C_{23}H_{34}O_2$) and the heneicosanoic acid methyl ester ($C_{22}H_{44}O_2$) coelute with exactly the same retention time (RT) at 38.8 min. However, the FI mass spectrum for this peak showed the molecular ions for each component (Figure 2). Because the JMS-T200GC is always measuring high-resolution mass spectra, these components, which are not quite separated in the chromatogram, can be identified by mass separation.

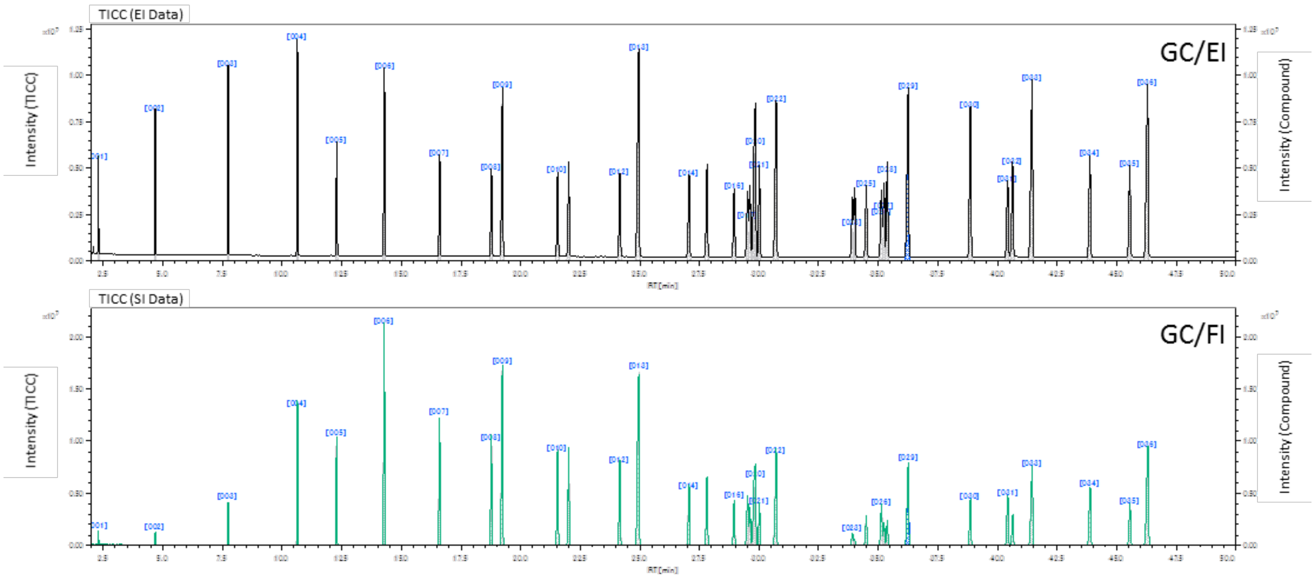
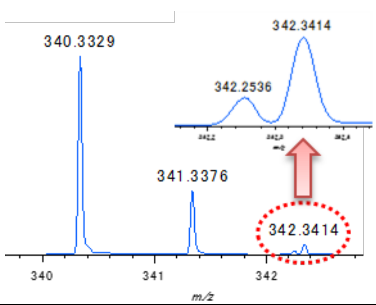


Figure 1. GC/EI and GC/FI total ion current chromatograms for the 37 FAMEs mixture



m/z	Formula	Error [mDa]	DBE
340.3329	$C_{22}H_{44}O_2$	-0.7	1.0
342.2536	$C_{23}H_{34}O_2$	-1.8	7.0

Figure 2. FI mass spectrum (enlarged) at RT 38.8 min and exact mass analysis results

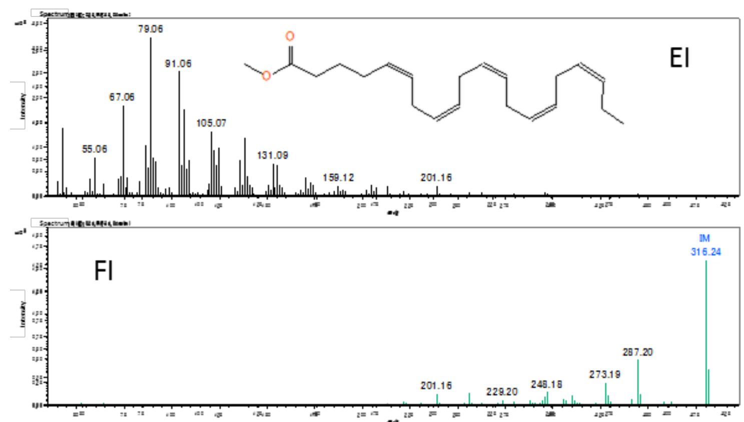


Figure 3. EI and FI mass spectra of 5, 8, 11, 14, 17-eicosapentaenoic acid methyl ester (all-Z)-

The FI mass spectra show molecular ions for all 37 FAMEs in the mixture. Additionally, these molecular ions are the base peak in each FI mass spectrum except for the 15-tetracosenoic acid methyl ester (Z)-, which is detected at a relative intensity of >80%. All of these results demonstrate that FI ionizes FAMEs softly and efficiently. As an example, Figure 3 shows the EI and FI mass spectra for 5,8,11,14,17-eicosapentaenoic acid methyl ester (all-Z)-, which has 5 double bonds and an alkyl group. In this example, the molecular ion was not observed in the EI mass spectrum, but the molecular ion is the base peak in the FI mass spectrum. Figure 4 shows the FI mass spectra and chemical formulas for 6 components that all have a carbon number of 20 (minus the ester bond) and have 0 to 5 double bonds. Lastly, Table 2 shows the integrated analysis report generated by msFineAnalysis. In each case, the FI molecular ion accurate masses were automatically used to determine the molecular formula for each component in the FAMES mixture to help identify the correct match from the EI library database search.

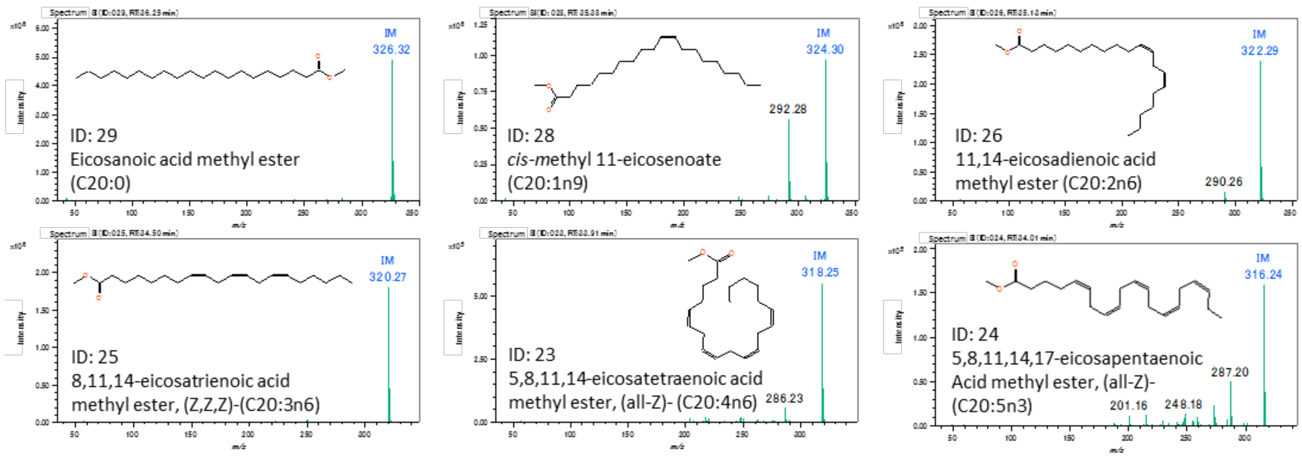


Figure 4. FI mass spectra for the C20 FAMEs

Table 2. Integrated qualitative analysis results report using msFineAnalysis

General					Total Result					
ID	RT [min]	Area	Area [%]	IM m/z	Library Name	CAS#	Similarity	Formula	Calculated m/z	Mass Error [mDa]
001	2.29	7,802,029	14.13	102.06850	Butanoic acid, methyl ester	623-42-7	902	C5 H10 O2	102.06753	0.97
002	4.68	12,509,365	22.65	130.09965	Hexanoic acid, methyl ester	106-70-7	954	C7 H14 O2	130.09883	0.82
003	7.74	17,836,754	32.30	158.13091	Octanoic acid, methyl ester	111-11-5	921	C9 H18 O2	158.13013	0.78
004	10.64	22,872,875	41.42	186.16241	Decanoic acid, methyl ester	110-42-9	951	C11 H22 O2	186.16143	0.98
005	12.28	13,873,023	25.12	200.17785	Undecanoic acid, methyl ester	1731-86-8	947	C12 H24 O2	200.17708	0.77
006	14.29	28,637,881	51.86	214.19378	Dodecanoic acid, methyl ester	111-82-0	924	C13 H26 O2	214.19273	1.05
007	16.61	16,639,305	30.13	228.20907	Tridecanoic acid, methyl ester	1731-88-0	962	C14 H28 O2	228.20838	0.68
008	18.78	15,928,365	28.84	240.20818	Methyl myristoleate	56219-06-8	951	C15 H28 O2	240.20838	-0.20
009	19.24	33,711,996	61.05	242.22503	Methyl tetradecanoate	124-10-7	956	C15 H30 O2	242.22403	1.00
010	21.55	16,820,354	30.46	254.22386	Methyl (Z)-10-pentadecenoate	-	928	C16 H30 O2	254.22403	-0.18
011	22.02	18,711,161	33.88	256.24022	Pentadecanoic acid, methyl ester	7132-64-1	949	C16 H32 O2	256.23968	0.54
012	24.16	17,805,851	32.24	268.23913	9-Hexadecenoic acid, methyl ester, (Z)-	1120-25-8	942	C17 H32 O2	268.23968	-0.55
013	24.95	55,221,475	100.00	270.25537	Hexadecanoic acid, methyl ester	112-39-0	946	C17 H34 O2	270.25533	0.04
014	27.07	18,586,681	33.66	282.25402	cis-10-Heptadecenoic acid, methyl ester	-	943	C18 H34 O2	282.25533	-1.32
015	27.82	20,566,515	37.24	284.27051	Heptadecanoic acid, methyl ester	1731-92-6	933	C18 H36 O2	284.27098	-0.47
016	28.96	15,344,971	27.79	292.23830	Methyl γ-linolenate	16326-32-2	945	C19 H32 O2	292.23968	-1.38
017	29.52	8,858,244	16.04	294.25444	9,12-Octadecadienoic acid, methyl ester, (E,E)-	2566-97-4	835	C19 H34 O2	294.25533	-0.89
018	29.62	8,877,607	16.08	292.23851	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	301-00-8	918	C19 H32 O2	292.23968	-1.17
019	29.79	18,365,132	33.26	294.25465	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	112-63-0	866	C19 H34 O2	294.25533	-0.68
020	29.84	32,367,534	58.61	296.26984	9-Octadecenoic acid (Z)-, methyl ester	112-62-9	894	C19 H36 O2	296.27098	-1.14
021	30.02	19,095,805	34.58	296.27036	9-Octadecenoic acid, methyl ester, (E)-	1937-62-8	927	C19 H36 O2	296.27098	-0.62
022	30.72	41,648,466	75.42	298.28652	Methyl stearate	112-61-8	939	C19 H38 O2	298.28663	-0.11
023	33.91	6,037,723	10.93	318.25265	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	2566-89-4	909	C21 H34 O2	318.25533	-2.68
024	34.01	6,574,800	11.91	316.23636	5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-	2734-47-6	945	C21 H32 O2	316.23968	-3.32
025	34.50	16,559,406	29.99	320.26864	8,11,14-Eicosatrienoic acid, methyl ester, (Z,Z,Z)-	21061-10-9	937	C21 H36 O2	320.27098	-2.34
026	35.13	10,403,767	18.84	322.28535	11,14-Eicosadienoic acid, methyl ester	2463-02-7	820	C21 H38 O2	322.28663	-1.28
027	35.25	9,642,111	17.46	320.26997	11,14,17-Eicosatrienoic acid, methyl ester	55682-88-7	861	C21 H36 O2	320.27098	-1.01
028	35.38	18,118,358	32.81	324.30086	cis-Methyl 11-eicosenoate	2390-09-2	948	C21 H40 O2	324.30228	-1.42
029	36.25	45,036,437	81.56	326.31784	Eicosanoic acid, methyl ester	1120-28-1	895	C21 H42 O2	326.31793	-0.10
030	38.86	36,312,501	65.76	340.33291	Heneicosanoic acid, methyl ester	6064-90-0	781	C22 H44 O2	340.33358	-0.67
031	40.42	18,929,601	34.28	350.31571	cis-13,16-Docasadienoic acid, methyl ester	-	947	C23 H42 O2	350.31793	-2.22
032	40.63	22,206,388	40.21	352.33120	13-Docosenoic acid, methyl ester, (Z)-	1120-34-9	881	C23 H44 O2	352.33358	-2.38
033	41.44	48,576,846	87.97	354.34851	Docosanoic acid, methyl ester	929-77-1	940	C23 H46 O2	354.34923	-0.73
034	43.88	25,785,457	46.69	368.36379	Tricosanoic acid, methyl ester	2433-97-8	947	C24 H48 O2	368.36488	-1.09
035	45.55	23,274,202	42.15	380.36207	15-Tetracosenoic acid, methyl ester, (Z)-	2733-88-2	871	C25 H48 O2	380.36488	-2.81
036	46.29	51,225,970	92.76	382.37968	Tetracosanoic acid, methyl ester	2442-49-1	922	C25 H50 O2	382.38053	-0.85

ID	Library Search Result				
ID	BEST Similarity	Library Name	CAS#	Formula	MW
001	902	Butanoic acid, methyl ester	623-42-7	C5 H10 O2	102
002	954	Hexanoic acid, methyl ester	106-70-7	C7 H14 O2	130
003	921	Octanoic acid, methyl ester	111-11-5	C9 H18 O2	158
004	951	Decanoic acid, methyl ester	110-42-9	C11 H22 O2	186
005	947	Undecanoic acid, methyl ester	1731-86-8	C12 H24 O2	200
006	924	Dodecanoic acid, methyl ester	111-82-0	C13 H26 O2	214
007	962	Tridecanoic acid, methyl ester	1731-88-0	C14 H28 O2	228
008	951	Methyl myristoleate	56219-06-8	C15 H28 O2	240
009	956	Methyl tetradecanoate	124-10-7	C15 H30 O2	242
010	928	Methyl (Z)-10-pentadecenoate	-	C16 H30 O2	254
011	949	Pentadecanoic acid, methyl ester	7132-64-1	C16 H32 O2	256
012	942	9-Hexadecenoic acid, methyl ester, (Z)-	1120-25-8	C17 H32 O2	268
013	946	Hexadecanoic acid, methyl ester	112-39-0	C17 H34 O2	270
014	943	cis-10-Heptadecenoic acid, methyl ester	-	C18 H34 O2	282
015	933	Heptadecanoic acid, methyl ester	1731-92-6	C18 H36 O2	284
016	945	Methyl γ-linolenate	16326-32-2	C19 H32 O2	292
017	877	11,14-Octadecadienoic acid, methyl ester	56554-61-1	C19 H34 O2	294
018	918	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	301-00-8	C19 H32 O2	292
019	866	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	112-63-0	C19 H34 O2	294
020	894	9-Octadecenoic acid (Z)-, methyl ester	112-62-9	C19 H36 O2	296
021	941	trans-13-Octadecenoic acid, methyl ester	-	C19 H36 O2	296
022	939	Methyl stearate	112-61-8	C19 H38 O2	298
023	909	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	2566-89-4	C21 H34 O2	318
024	945	5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-	2734-47-6	C21 H32 O2	316
025	937	8,11,14-Eicosatrienoic acid, methyl ester, (Z,Z,Z)-	21061-10-9	C21 H36 O2	320
026	902	cis-11,14-Eicosadienoic acid, methyl ester	-	C21 H38 O2	322
027	861	11,14,17-Eicosatrienoic acid, methyl ester	55682-88-7	C21 H36 O2	320
028	948	cis-Methyl 11-eicosenoate	2390-09-2	C21 H40 O2	324
029	936	Methyl 18-methylnonadecanoate	-	C21 H42 O2	326
030	781	Heneicosanoic acid, methyl ester	6064-90-0	C22 H44 O2	340
031	947	cis-13,16-Docasadienoic acid, methyl ester	-	C23 H42 O2	350
032	912	Methyl 11-docosenoate	-	C23 H44 O2	352
033	940	Docosanoic acid, methyl ester	929-77-1	C23 H46 O2	354
034	947	Tricosanoic acid, methyl ester	2433-97-8	C24 H48 O2	368
035	876	15-Tetracosenoic acid, methyl ester	56554-33-7	C25 H48 O2	380
036	922	Tetracosanoic acid, methyl ester	2442-49-1	C25 H50 O2	382

Conclusions

The msFineAnalysis integrated analysis method produces highly accurate qualitative analysis results for the FAMEs by combining the library search results and molecular formula estimation. This combination of using GC/EI and GC/FI measurements together for qualitative analysis is particularly important for FAMEs as these types of compounds do not produce molecular ions for EI, making it difficult to use database searches alone for identification.

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