APPLICATION NOTE

Gas Chromatography

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GC-FID: Fast and Accurate Analysis of Fatty Acid Methyl Esters (FAMEs) In Edible Oils and Food Products for the Determination of Product Quality and Authenticity

Introduction

Triacylglycerols are the primary constituent of edible oils, which provide not only the bulk of the nutritive component but also serve to influence the organoleptic and chemical properties of the oil. Triacylglycerols consist of three fatty acid moieties esterified to a glycerol backbone. Due to the complex biochemical pathways involved in fatty acid synthesis, the composition of fatty acyl chains making up an oil can be complex, consisting of a mixture of saturated or unsaturated, even, and odd carbon chains. However, homogeneity within plants species results in characteristic fatty acyl profiles that can be used to determine oil authenticity. Furthermore, certain refining and industrial processing techniques, are known to alter the chemical makeup of fatty acids resulting in distinct alterations to an oil's fatty acid profile. For example, high temperature deodorization can produce unwanted *trans* fatty acids lowering oil quality, as consumption of industrially processed trans fats is associated with an increased risk of heart disease. Therefore, the gas chromatographic analysis of fatty acids isolated from edible oils is an important tool to determine both the quality of a product and to detect potential adulteration.[1]

The analysis of triacylglycerols by gas chromatography (GC) in their native state is challenging, largely due to their poor volatility and thermal decomposition in hot injector ports. To improve their properties for GC and to provide a comprehensive characterization of fatty acids, the esterified fatty acyl chains must be cleaved from the glycerol backbone and methylated to derive fatty acid methyl esters (FAMEs).

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This reaction may be carried out under acidic or basic conditions depending on the properties of the sample. For food samples, a total lipid extraction or sample digestion may be required prior to methylating. After transmethylation, the resulting higher volatility and less polar FAME products can be separated and quantitated by GC flame ionization detection. Compounds are identified based on retention time compared to injections of a known reference standard and may be quantitated based on peak area relative to that of an internal standard.

This application note reports the results from the analysis of FAMEs from edible oils performed with the PerkinElmer GC 2400™ System equipped with capillary injector, Flame Ionization Detector (FID), and Elite-2560 column showing fast analysis time and high reproducibility. PerkinElmer SimplicityChrom™ Chromatography Data System (CDS) Software manages the analytical workflow.

Experimental

Instrumentation

Analysis of FAMEs from edible oils and food samples were performed by the GC 2400 System equipped with an integrated liquid autosampler, capillary injector and FID Detector. All samples were chromatographed on a PerkinElmer Elite-2560 (100m by 0.25 mm ID by 0.20 µm df; N9311570). The column's highly polar stationary biscyanopropylpolysiloxane stationary phase provides great resolution of *cis/trans* geometric isomers of FAMEs, capable of separations of critical pairs such as methyl oleate and methyl *cis*-vaccinate.

Figure 1: PerkinElmer GC 2400 System.

Sample Preparation

Edible oil samples consisted of three commercially available oils, extra virgin olive oil, canola oil, and sunflower oil. Fatty acid methyl esters of edible oils were prepared following American Oil Chemist Society Official Method Ce 2-66.[2] To summarize, 2 mL of heptane and 0.1 mL of 2N methanolic KOH were added to aliquots of approximately 200 mg of oil. Vials were capped and vortexed for 30 seconds. Samples remained at room temperature for 30 minutes to achieve phase separation, following which 0.2 mL of supernatant was transferred to a GC vial and diluted with 1 mL of heptane.

In addition to edible oils FAMES were prepared from meat homogenate, chicken powder, and powderized dog food. Lipids were extracted by the methods of Folch, Lee, and Sloane Stanley.[3] An aliquot of total lipid extract was dissolved in hexane and methylated with 14 % methanolic boron trifluoride at 95 °C for 1 hour. The reaction was quenched with the addition of double distilled water. Fatty acid methyl esters were extracted into hexane, evaporated to dryness under a gentile nitrogen stream, then resuspended in hexane prior to transfer to a GC insert and vial for analysis.

Individual FAME were identified by comparing retention time to FAME reference standard (GLC 37, Nu-Check Prep, Inc., Elysian, MN), consisting of 37 FAMEs commonly analyzed in food and edible oils. The reference standard was prepared in hexane at 20 mg of total FAME per mL of solvent.

All FAME samples were injected at approximately 20 – 25 mg of total FAME per mL of solvent. See Table 2 for GC method parameters.

Table 1: Gas Chromatograph configuration.

Gas Chromatograph	PerkinElmer GC 2400 System
Injector	Split/Splitless Injector
Detector	Flame Ionization Detector
Software	SimplicityChrom CDS Software
Column	Elite-2560, 100 m by 0.25 mm ID by 0.20 µm; N9311570

Table 2: Gas Chromatograph method parameters.

Autosampler Parameters	
Syringe	5 µL
Injection Volume	1 µL
Injection Speed	Normal
Viscosity Delay	0
Injector Parameters	
Type	Split/Splitless
Temperature	250°C
Split	100:1
Detector	
Type	FID
Temperature	300°C
Sampling Rate	10 Hz

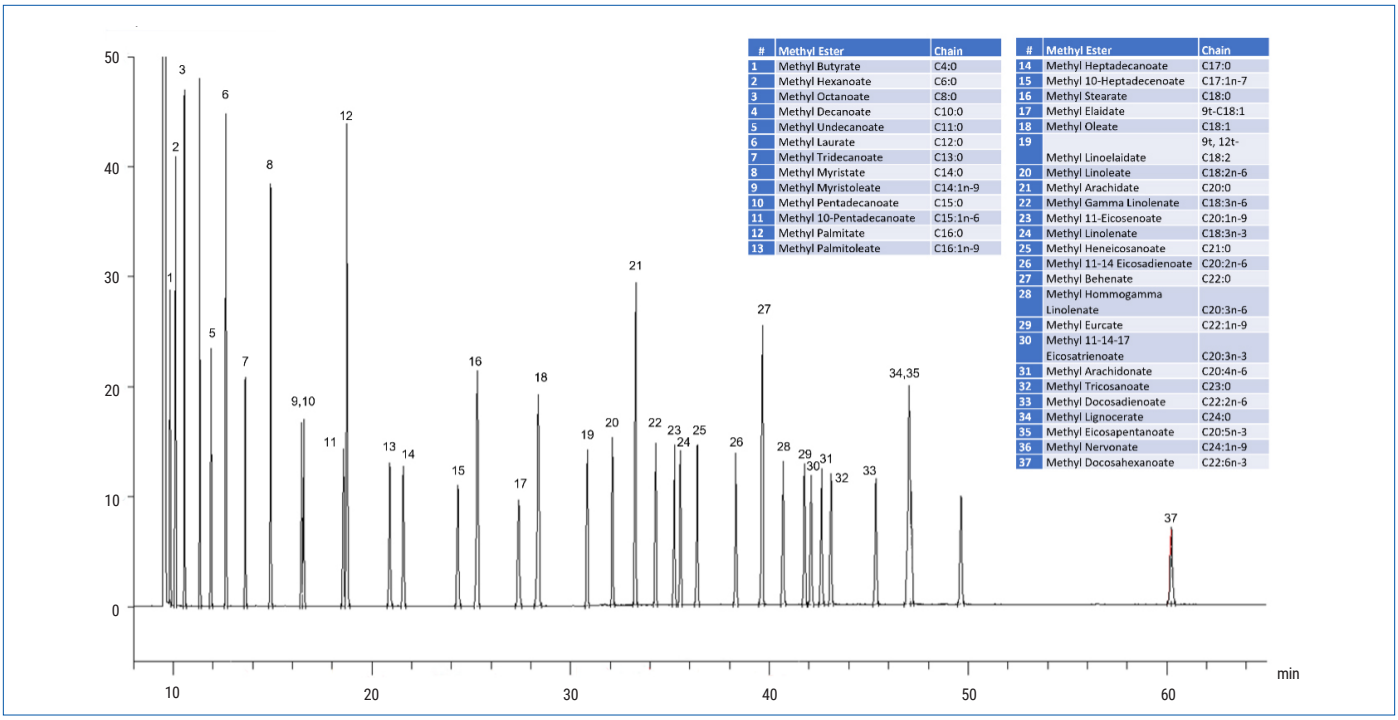
Table 2: Gas Chromatograph method parameters. Continued...

Oven Parameters: AOCS Ce 1j-07	
Initial temperature	180°C
Hold	32 min
Ramp Rate	20°C/min
Temperature	215°C
Hold	31.25 min
Total Runtime	65 min
Oven Parameters: Modified Ramp	
Initial Temperature	60°
Hold	1 min
Ramp Rate	15°C/min
Temperature	170°
Hold	12 min
Ramp Rate	6°C/min
Temperature	220°C
Ramp Rate	8°C/min
Temperature	240°C
Hold	8 min
Total Runtime	47.6

Results

Figure 2 depicts the separation of 37 FAMEs commonly analyzed in edible oil and food stuffs following the separation method AOCS Official Method Ce 1j-07.[4] The high initial oven temperature compresses the chromatography in the early portion of the GC run, while the hold at 180 °C contributes to a long gap between the last two eluting peaks, methyl nervonate and methyl docosahexanoate. By using a lower initial oven temperature and a programmed temperature ramp, the modified GC method improved the resolution of early eluting FAMEs and reduced the time required to elute methyl docosahexanoate from approximately 60 mins to 44 mins (Figure 3).

The separation of FAMEs derived from olive, canola, and sunflower oils are depicted in Figure 4. Summary results from these separations demonstrate excellent peak area repeatability across all samples (Table 3). Faster separations with similar resolution can be obtained by scheduled oven temperature ramping which contributes to reducing analytical runtimes while maintaining separation of critical pairs, such as methyl oleate and methyl cis-vaccenate. As a result, fatty acid profiling of edible oils can be delivered in less time without compromising data integrity.



#	Methyl Ester	Chain
1	Methyl Butyrate	C4:0
2	Methyl Hexanoate	C6:0
3	Methyl Octanoate	C8:0
4	Methyl Decanoate	C10:0
5	Methyl Undecanoate	C11:0
6	Methyl Laurate	C12:0
7	Methyl Tridecanoate	C13:0
8	Methyl Myristate	C14:0
9	Methyl Myristoleate	C14:1n-9
10	Methyl Pentadecanoate	C15:0
11	Methyl 10-Pentadecanoate	C15:1n-6
12	Methyl Palmitate	C16:0
13	Methyl Palmitoleate	C16:1n-9

#	Methyl Ester	Chain
14	Methyl Heptadecanoate	C17:0
15	Methyl 10-Heptadecenoate	C17:1n-7
16	Methyl Stearate	C18:0
17	Methyl Elaidate	9t-C18:1
18	Methyl Oleate	C18:1
19	Methyl Linoelaidate	9t, 12t-C18:2
20	Methyl Linoleate	C18:2n-6
21	Methyl Arachidate	C20:0
22	Methyl Gamma Linolenate	C18:3n-6
23	Methyl 11-Eicosenoate	C20:1n-9
24	Methyl Linolenate	C18:3n-3
25	Methyl Heneicosanoate	C21:0
26	Methyl 11-14 Eicosadienoate	C20:2n-6
27	Methyl Behenate	C22:0
28	Methyl Hommogamma Linolenate	C20:3n-6
29	Methyl Eurcate	C22:1n-9
30	Methyl 11-14-17 Eicosatrienoate	C20:3n-3
31	Methyl Arachidonate	C20:4n-6
32	Methyl Tricosanoate	C23:0
33	Methyl Docosadienoate	C22:2n-6
34	Methyl Lignocerate	C24:0
35	Methyl Eicosapentanoate	C20:5n-3
36	Methyl Nervonate	C24:1n-9
37	Methyl Docosahexanoate	C22:6n-3

Figure 2: Separation of the 37 FAME reference standard mix using AOCS Official Method Ce 1j-07.

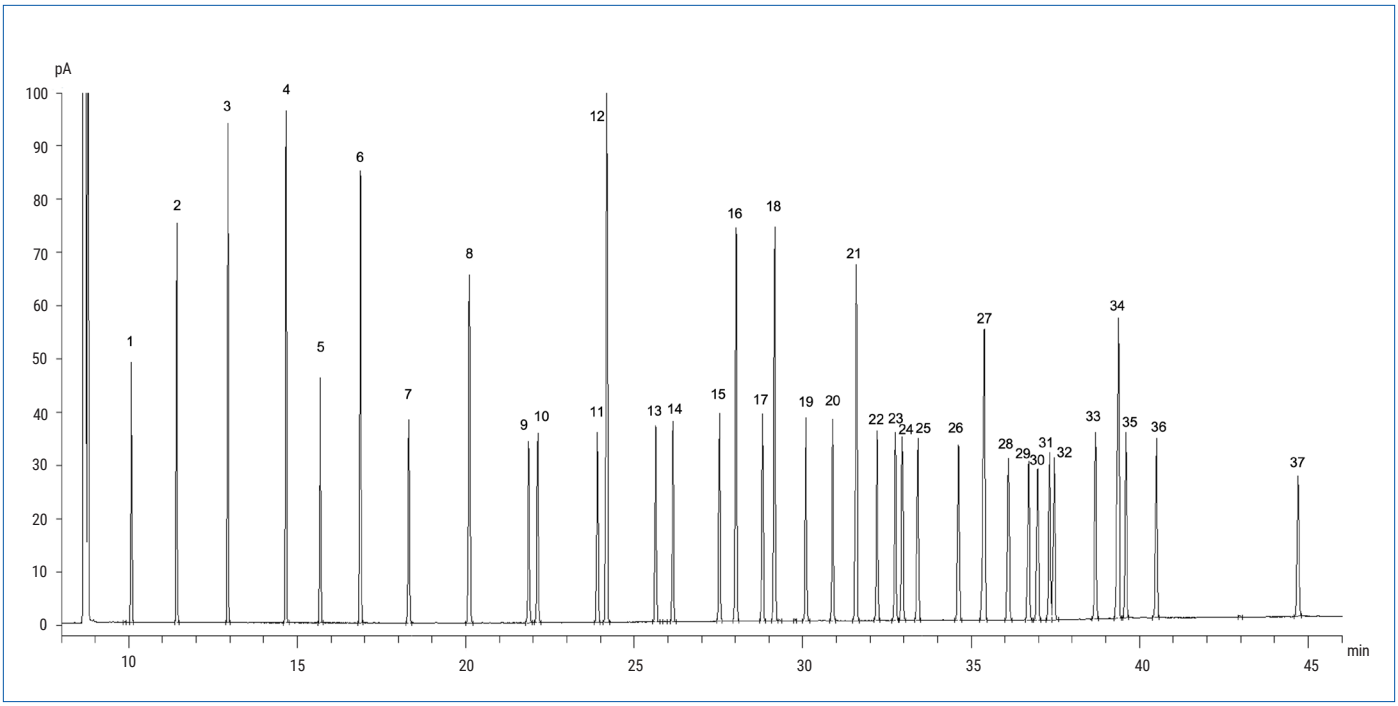


Figure 3: Separation of 37 FAME mix using the modified oven ramping temperature program.

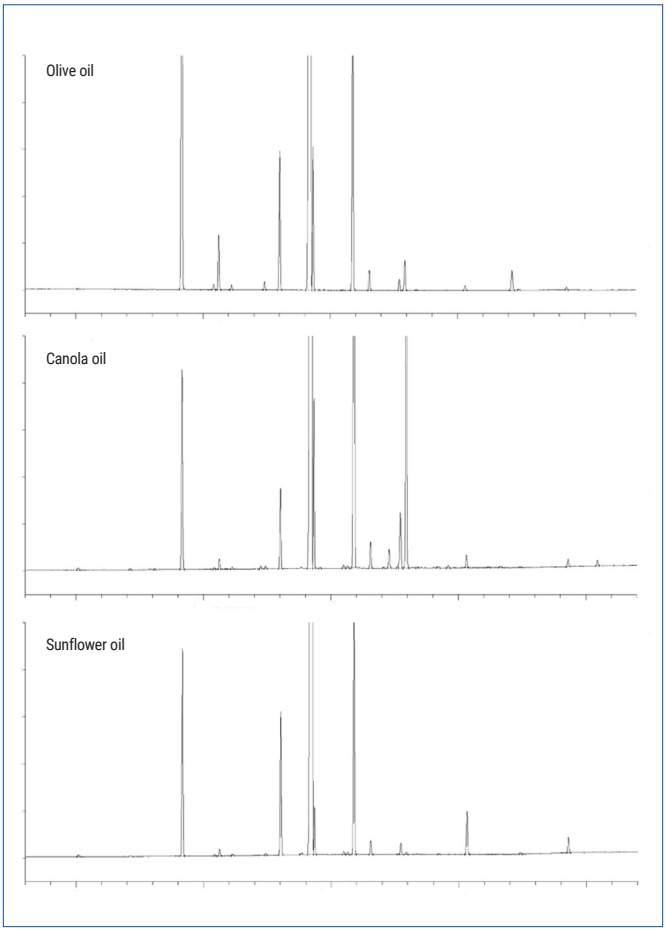


Figure 4: Olive, canola, and sunflower oil FAME separations using temperature ramping oven program.

Table 3: Fatty acid methyl ester percent composition of edible oils analyzed using the modified temperature ramping program.

Component	Olive Oil		Canola Oil		Sunflower Oil	
	Average	SD	Average	SD	Average	SD
Methyl Myristate	0.01	0.013	0.05	0.002	0.05	0.001
Methyl Palmitate	13.69	0.007	4.24	0.007	4.61	0.002
Methyl Palmitoleate	1.21	0.004	0.22	0.001	0.16	0.002
Methyl Heptadecanoate	0.10	0.006	0.05	0.003	0.03	0.001
Methyl Stearate	3.04	0.010	1.77	0.004	3.19	0.002
Methyl Oleate	69.32	0.067	60.79	0.014	83.39	0.008
Methyl cis-Vaccenate	2.58	0.003	3.03	0.002	0.83	0.003
Methyl Linoleate	8.33	0.003	19.21	0.012	5.59	0.003
Methyl Arachidate	0.46	0.013	0.58	0.003	0.32	0.001
Methyl 11-Eicosenoate	0.27	0.005	1.54	0.004	0.26	0.007
Methyl Linolenate	0.74	0.005	7.73	0.004	0.05	0.001
Methyl 11-14 Eicosadienoate	-		0.07	0.002	-	-
Methyl Behenate	0.14	0.009	0.33	0.004	1.11	0.002
Methyl Eurcate	-		0.06	0.003	-	-
Methyl Tricosanoate	0.03	0.004	0.02	0.004	0.04	0.002
Methyl Lignocerate	0.07	0.010	0.17	0.002	0.37	0.001
Methyl Nervonate	-		0.13	0.001	-	-

Abbreviations: SD; standard deviation

Notably, the fast-refreshing programmable pneumatic control modules of the GC 2400 System contribute to highly reproducible chromatographic separations demonstrated by the average retention time repeatability of 0.006% (%RSD; Table 4, Figure 5). The excellent retention time repeatability makes for consistent automated peak identification from run to run, improving data quality. This is especially true for separations of FAMEs extracted and prepared from complex matrices, such as those from dog food, meat homogenate, and chicken powder (Figure 6, Figure 7, and Figure 8, respectively).

Table 4: Retention time repeatability for 37 FAMEs mix.

#	Methyl Ester	Chain	Average RT (min; n=5)	%RSD
1	Methyl Butyrate	C4:0	10.077	0.005
2	Methyl Hexanoate	C6:0	11.420	0.005
3	Methyl Octanoate	C8:0	12.941	0.005
4	Methyl Decanoate	C10:0	14.667	0.005
5	Methyl Undecanoate	C11:0	15.681	0.004
6	Methyl Laurate	C12:0	16.875	0.005
7	Methyl Tridecanoate	C13:0	18.311	0.004
8	Methyl Myristate	C14:0	20.110	0.009
9	Methyl Myristoleate	C14:1n-9	21.870	0.006
10	Methyl Pentadecanoate	C15:0	22.136	0.005
11	Methyl 10-Pentadecanoate	C15:1n-6	23.911	0.006
12	Methyl Palmitate	C16:0	24.192	0.007
13	Methyl Palmitoleate	C16:1n-9	25.641	0.005
14	Methyl Heptadecanoate	C17:0	26.152	0.005
15	Methyl 10-Heptadecenoate	C17:1n-7	27.533	0.004
16	Methyl Stearate	C18:0	28.030	0.006
17	Methyl Elaidate	9t-C18:1	28.815	0.004
18	Methyl Oleate	C18:1	29.172	0.004
19	Methyl Linoelaidate	9t, 12t-C18:2	30.099	0.006
20	Methyl Linoleate	C18:2n-6	30.895	0.006
21	Methyl Arachidate	C20:0	31.600	0.007
22	Methyl Gamma Linolenate	C18:3n-6	32.215	0.006
23	Methyl 11-Eicosenoate	C20:1n-9	32.753	0.006
24	Methyl Linolenate	C18:3n-3	32.960	0.006
25	Methyl Heneicosanoate	C21:0	33.424	0.004
26	Methyl 11-14 Eicosadienoate	C20:2n-6	34.627	0.007
27	Methyl Behenate	C22:0	35.395	0.006
28	Methyl Hommogamma Linolenate	C20:3n-6	36.110	0.006
29	Methyl Eurcate	C22:1n-9	36.712	0.006
30	Methyl 11-14-17 Eicosatrienoate	C20:3n-3	36.979	0.010
31	Methyl Arachidonate	C20:4n-6	37.332	0.006
32	Methyl Tricosanoate	C23:0	37.476	0.005
33	Methyl Docosadienoate	C22:2n-6	38.695	0.005
34	Methyl Lignocerate	C24:0	39.379	0.003
35	Methyl Eicosapentanoate	C20:5n-3	39.600	0.006
36	Methyl Nervonate	C24:1n-9	40.503	0.005
37	Methyl Docosahexanoate	C22:6n-3	44.710	0.007
			Average	**0.006**

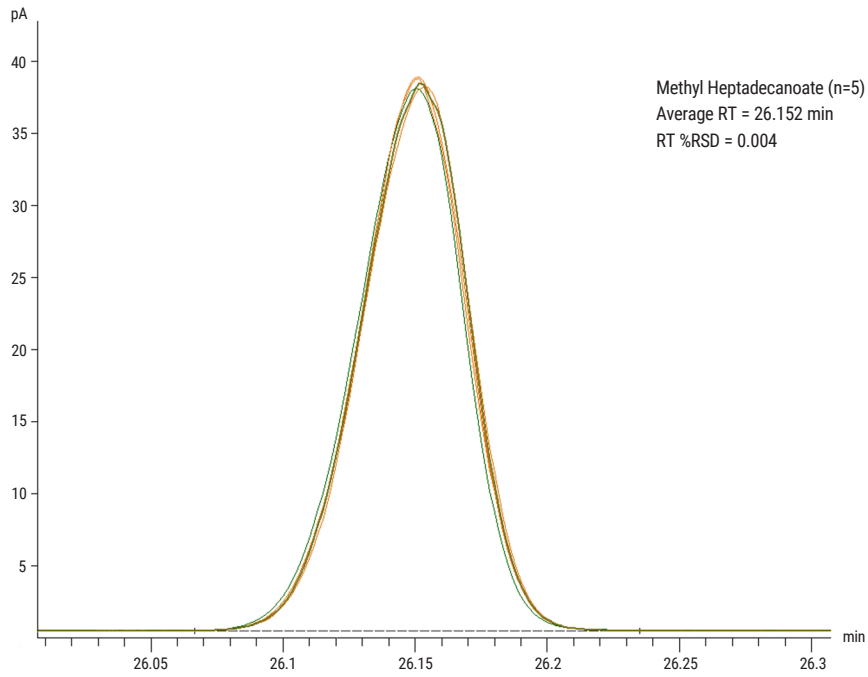
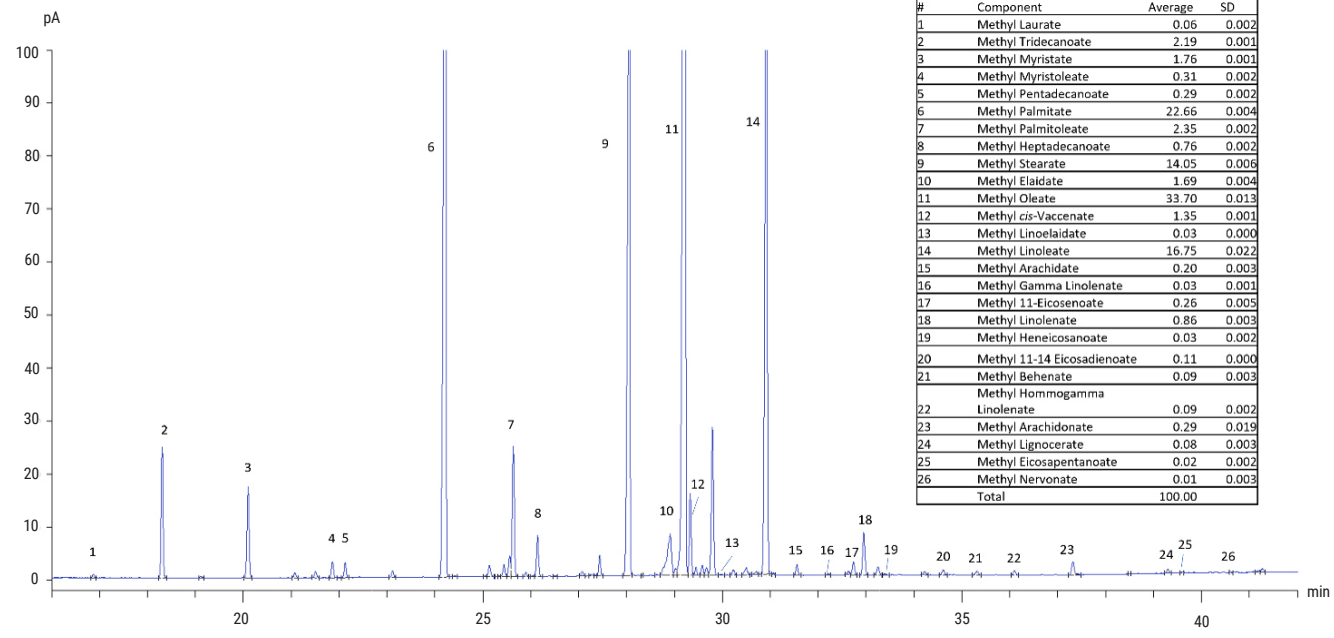
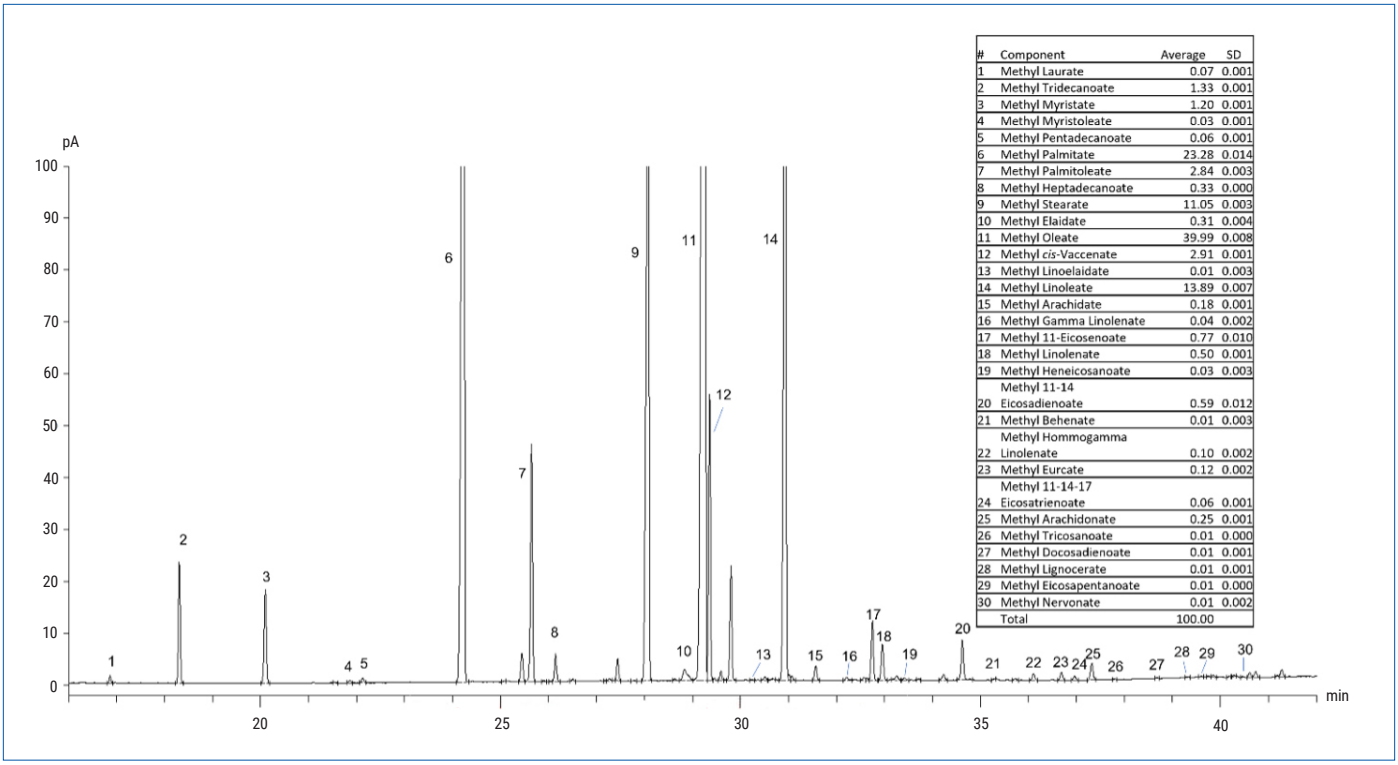


Figure 5: Overlay chromatogram showing excellent retention time repeatability.



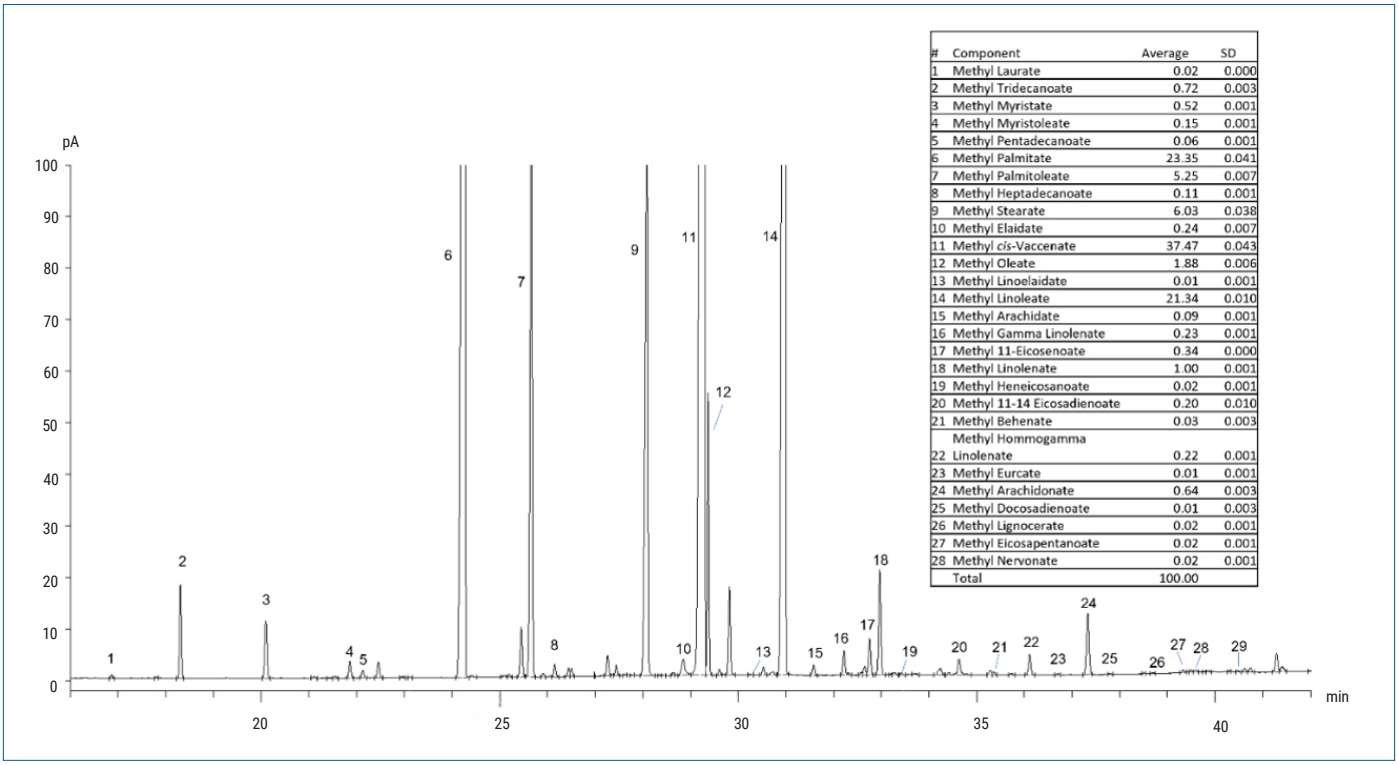
#	Component	Average	SD
1	Methyl Laurate	0.06	0.002
2	Methyl Tridecanoate	2.19	0.001
3	Methyl Myristate	1.76	0.001
4	Methyl Myristoleate	0.31	0.002
5	Methyl Pentadecanoate	0.29	0.002
6	Methyl Palmitate	22.66	0.004
7	Methyl Palmitoleate	2.35	0.002
8	Methyl Heptadecanoate	0.76	0.002
9	Methyl Stearate	14.05	0.006
10	Methyl Elaidate	1.69	0.004
11	Methyl Oleate	33.70	0.013
12	Methyl *cis*-Vaccenate	1.35	0.001
13	Methyl Linoelaidate	0.03	0.000
14	Methyl Linoleate	16.75	0.022
15	Methyl Arachidate	0.20	0.003
16	Methyl Gamma Linolenate	0.03	0.001
17	Methyl 11-Eicosenoate	0.26	0.005
18	Methyl Linolenate	0.86	0.003
19	Methyl Heneicosanoate	0.03	0.002
20	Methyl 11-14 Eicosadienoate	0.11	0.000
21	Methyl Behenate	0.09	0.003
22	Methyl Hommogamma Linolenate	0.09	0.002
23	Methyl Arachidonate	0.29	0.019
24	Methyl Lignocerate	0.08	0.003
25	Methyl Eicosapentanoate	0.02	0.002
26	Methyl Nervonate	0.01	0.003
	Total	100.00	

Figure 6: Separation of FAME prepared from powderized dog food.



#	Component	Average	SD
1	Methyl Laurate	0.07	0.001
2	Methyl Tridecanoate	1.33	0.001
3	Methyl Myristate	1.20	0.001
4	Methyl Myristoleate	0.03	0.001
5	Methyl Pentadecanoate	0.06	0.001
6	Methyl Palmitate	23.28	0.014
7	Methyl Palmitoleate	2.84	0.003
8	Methyl Heptadecanoate	0.33	0.000
9	Methyl Stearate	11.05	0.003
10	Methyl Elaidate	0.31	0.004
11	Methyl Oleate	39.99	0.008
12	Methyl *cis*-Vaccenate	2.91	0.001
13	Methyl Linoelaidate	0.01	0.003
14	Methyl Linoleate	13.89	0.007
15	Methyl Arachidate	0.18	0.001
16	Methyl Gamma Linolenate	0.04	0.002
17	Methyl 11-Eicosenoate	0.77	0.010
18	Methyl Linolenate	0.50	0.001
19	Methyl Heneicosanoate	0.03	0.003
20	Methyl 11-14 Eicosadienoate	0.59	0.012
21	Methyl Behenate	0.01	0.003
22	Methyl Hommogamma Linolenate	0.10	0.002
23	Methyl Eurcate	0.12	0.002
24	Methyl 11-14-17 Eicosatrienoate	0.06	0.001
25	Methyl Arachidonate	0.25	0.001
26	Methyl Tricosanoate	0.01	0.000
27	Methyl Docosadienoate	0.01	0.001
28	Methyl Lignocerate	0.01	0.001
29	Methyl Eicosapentanoate	0.01	0.000
30	Methyl Nervonate	0.01	0.002
	Total	100.00	

Figure 7: Separation of FAME prepared from meat homogenate.



#	Component	Average	SD
1	Methyl Laurate	0.02	0.000
2	Methyl Tridecanoate	0.72	0.003
3	Methyl Myristate	0.52	0.001
4	Methyl Myristoleate	0.15	0.001
5	Methyl Pentadecanoate	0.06	0.001
6	Methyl Palmitate	23.35	0.041
7	Methyl Palmitoleate	5.25	0.007
8	Methyl Heptadecanoate	0.11	0.001
9	Methyl Stearate	6.03	0.038
10	Methyl Elaidate	0.24	0.007
11	Methyl *cis*-Vaccenate	37.47	0.043
12	Methyl Oleate	1.88	0.006
13	Methyl Linoelaidate	0.01	0.001
14	Methyl Linoleate	21.34	0.010
15	Methyl Arachidate	0.09	0.001
16	Methyl Gamma Linolenate	0.23	0.001
17	Methyl 11-Eicosenoate	0.34	0.000
18	Methyl Linolenate	1.00	0.001
19	Methyl Heneicosanoate	0.02	0.001
20	Methyl 11-14 Eicosadienoate	0.20	0.010
21	Methyl Behenate	0.03	0.003
22	Methyl Hommogamma Linolenate	0.22	0.001
23	Methyl Eurcate	0.01	0.001
24	Methyl Arachidonate	0.64	0.003
25	Methyl Docosadienoate	0.01	0.003
26	Methyl Lignocerate	0.02	0.001
27	Methyl Eicosapentanoate	0.02	0.001
28	Methyl Nervonate	0.02	0.001
	Total	100.00	

Figure 8: Separation of FAME prepared from chicken powder.

Conclusion

The analysis of FAMEs from edible oils is a critical tool to determine oil authenticity and quality, and essential for the profiling of fatty acids in food products for labeling purposes. The GC 2400 System provides excellent resolution and highly repeatable separations of complex FAME mixtures from food and oils contributing to improve data quality. Fast analysis time contribute to increased lab productivity. The intuitive SimplicityChrom CDS Software provides a practical, customizable user experience with multifunctionality and accessibility options. In addition, the detachable touchscreen provides versatility and portability which ultimately offers time optimization for busy lab environments.

References

1. Sirgley, C.T., and Mossoba M.M (2017), Current Analytical Techniques for Food Lipids. Food and Drug Administration Papers 7.

2. American Oil Chemists' Society (2017), AOCS Official Method Ce 2-66: Preparation of Methyl Esters of Fatty Acids. In Firestone D (ed) Official Methods and Recommended Practices of the AOCS, 7th ed. AOAC International: Urbana, Il.

3. Folch, J., Lees, M., and Sloane Stanley, G.H. (1957), A simplified method for the isolation and purification of total lipids from animal tissues. J Biol Chem; 226(1):497-509.

4. American Oil Chemists' Society (2017), AOCS Official Method Ce 1j-07: cis-, trans-, Saturated, Monounsaturated and Polyunsaturated Fatty Acids in Extracted Fats by Capillary GLC. In Firestone D (ed) Official Methods and Recommended Practices of the AOCS, 7th ed. AOAC International.

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