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Applications note

High-Resolution Analyses of Fatty Acid Methyl Esters by Gas Chromatography

Fatty acid methyl esters (FAMEs) analysis is an important tool both in characterizing fats and oils and in determining the total fat content in foods. Fats can be extracted from a matrix, using a non-polar solvent, and saponified to produce salts of the free fatty acids. After derivatizing the free acids to form the methyl esters, the mixture readily can be analyzed by gas chromatography (GC), due to the volatility and thermal stability of the FAMEs. Gas chromatography has become an important technique in fats and oils analysis because accurate results can be obtained for complex, as well as simple, sample matrices.

FAMEs analyses were among the first applications for gas chromatography, so many of the GC methods originally written for analysis of fats and oils described packed column technology. Capillary columns offer significant advantages, however, including more efficient separations. When analyzing fats and oils with complex fatty acid profiles, such as the *cis* and *trans* forms of polyunsaturated fatty acids, higher efficiencies are needed to resolve the individual components. Capillary columns with Carbowax®-type (polyethylene glycol) stationary phases typically are used for analyses of saturated and unsaturated fatty acid methyl esters, and biscyanopropyl phases are used to resolve *cis* and *trans* isomers of polyunsaturated components.

Creating FAMEs

Lipids are normally extracted from matrices using a non-polar solvent, such as ether, and saponified to produce the free fatty acid salts. The fatty acid salts then are derivatized to form the fatty acid methyl esters, to increase volatility, improve peak symmetry, decrease sample activity, and thus provide more accurate analytical data. The official methods of AOAC International[1] and the American Oil Chemists Society (AOCS)[2] both contain procedures for the derivatization reaction, as does the European Pharmacopoeia.[3] In general, the glycerides are saponified by refluxing with methanolic sodium hydroxide. The esterification is effected with a reagent such as boron trifluoride in methanol and the FAMEs are extracted with a non-polar solvent (e.g., heptane) for analysis by GC.

Several groups of researchers have proposed simplified procedures for creating the methyl esters. For example, lipids can be transmethylated *in situ*. This option combines all of the conventional steps, except for the drying and post-reaction work-up, into one step.[4] For some samples, trimethyl-sulfonium hydroxide (TMSH), an alternative derivatization reagent, can be used for transesterification. A major advantage of this approach is that the derivatization can be performed in a single, fast reaction step.

Analyzing Polyunsaturated FAMEs

Stabilwax® and Rtx®-Wax columns provide excellent resolution of FAMEs derived from either plant or animal sources. Polyunsaturated FAMEs typically are analyzed on one of these Carbowax®-type capillary columns; analysis times of 35-50 minutes generally are required to fully resolve the C21:5 FAME from the C23:0 internal standard, and the C24:0 FAME from C22:6.

The FAMEWAX® polyethylene glycol stationary phase is specially tested with a polyunsaturated FAMEs mix to ensure resolution of the omega-3 and omega-6 fatty acids of interest, including those specified above. In addition, FAMEs such as methyl eicosapaenoate (C20:5) and methyl docosahexaenoate (C22:6), found in nutraceutical ingredients and products such as marine oils, also are resolved. FAMEWAX® columns offer excellent resolution of polyunsaturated FAMEs with significantly reduced analysis times, compared to traditional Carbowax® stationary phases. In fact, analysis times of less than 10 minutes are possible! Figure 1 shows an

Figure 1—A FAMEWAX® capillary column provides fast, efficient separations of marine oil-based FAMEs.

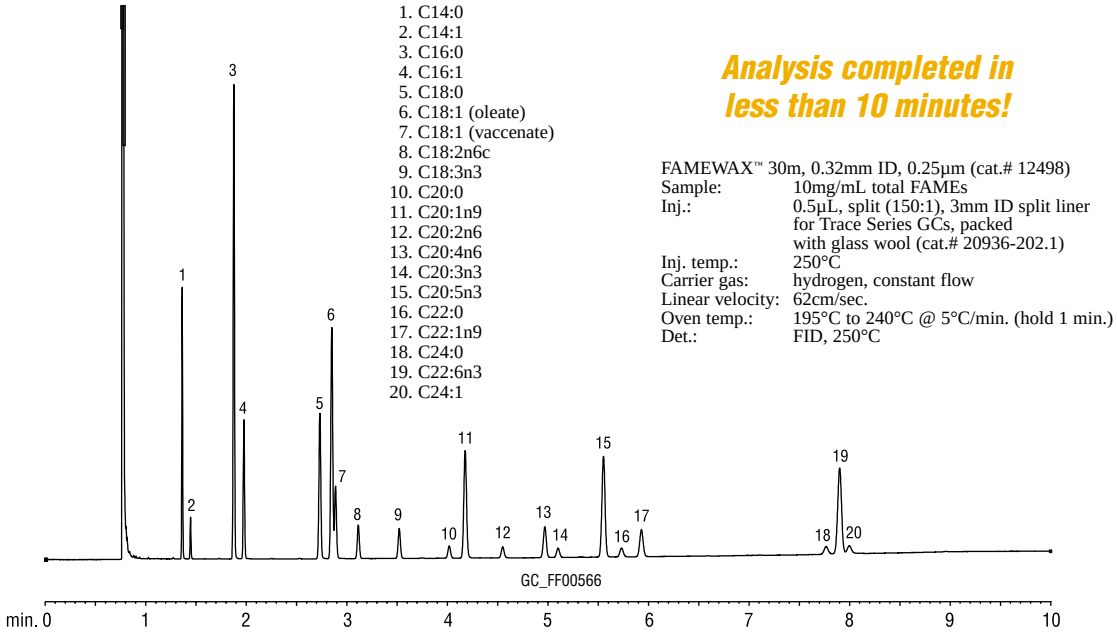

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Figure 2—Rapid, efficient analysis of FAMEs derived from a marine oil capsule, using a FAMEWAX® column.

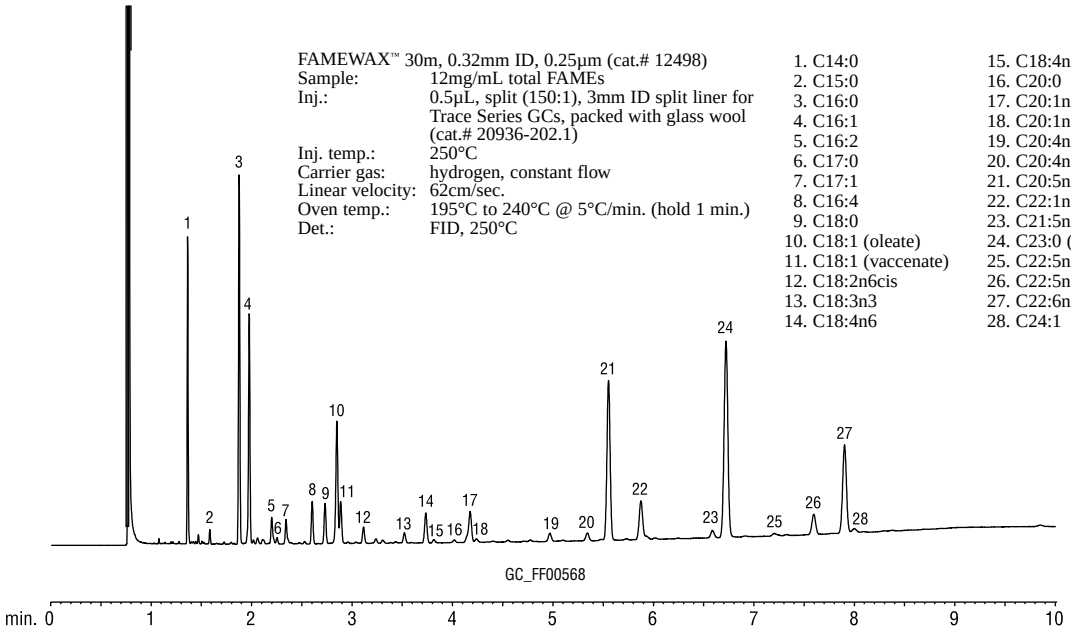
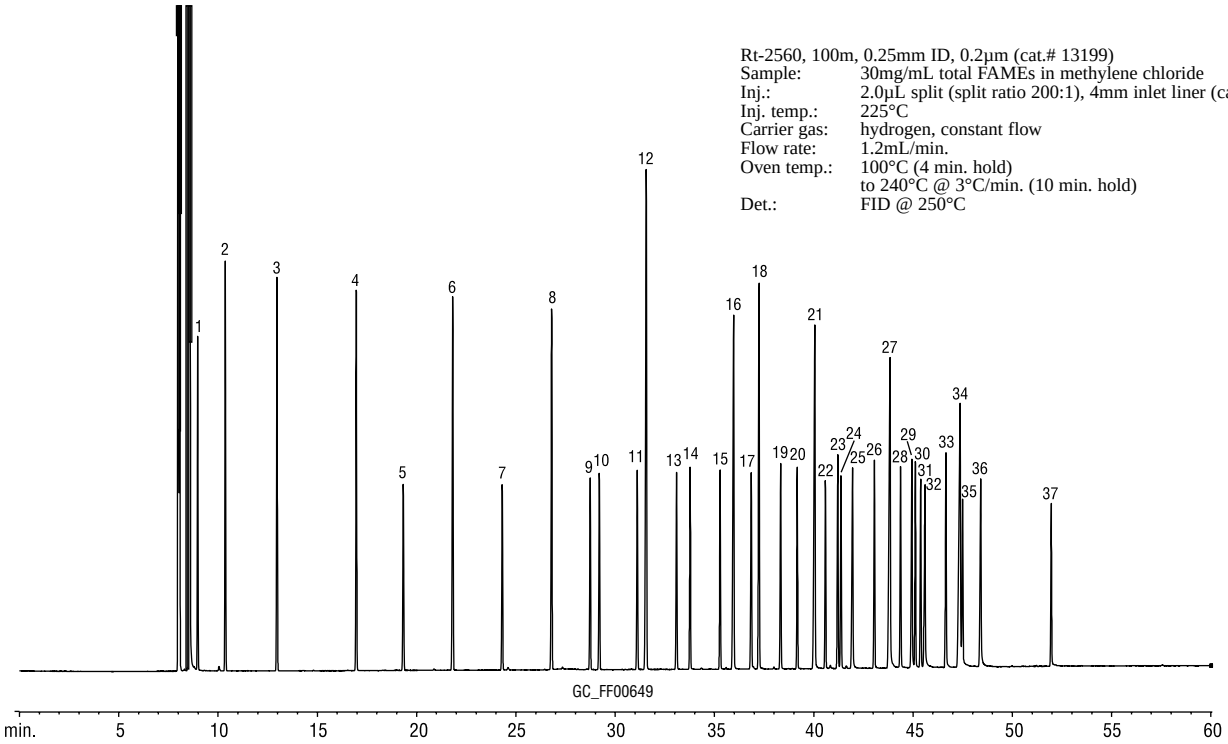


Figure 3—Effective analysis of a 37 FAME mix on an Rt-2560 capillary column.



Compound	% in Mix	Compound	% in Mix
1. C4:0 methyl butyrate	4.0	19. C18:2 methyl linoleaidate (*trans*-9,12)	2.0
2. C6:0 methyl hexanoate	4.0	20. C18:2 methyl linoleate (*cis*-9,12)	2.0
3. C8:0 methyl octanoate	4.0	21. C18:3 methyl γ-linolenate (*cis*-6,9,12)	2.0
4. C10:0 methyl decanoate	4.0	22. C20:0 methyl arachidate	4.0
5. C11:0 methyl undecanoate	2.0	23. C20:1 methyl eicosenoate (*cis*-11)	2.0
6. C12:0 methyl laurate	4.0	24. C18:3 methyl linolenate (*cis*-9,12,15)	2.0
7. C13:0 methyl tridecanoate	2.0	25. C21:0 methyl heneicosanoate	2.0
8. C14:0 methyl myristate	4.0	26. C20:2 methyl eicosadienoate (*cis*-11,14)	2.0
9. C14:1 methyl myristoleate (*cis*-9)	2.0	27. C20:3 methyl eicosatrienoate (*cis*-11,14,17)	2.0
10. C15:0 methyl pentadecanoate	2.0	28. C22:0 methyl behenate	4.0
11. C15:1 methyl pentadecenoate (*cis*-10)	2.0	29. C22:1 methyl erucate (*cis*-13)	2.0
12. C16:0 methyl palmitate	6.0	30. C20:3 methyl eicosatrienoate (*cis*-11,14,17)	2.0
13. C16:1 methyl palmitoleate (*cis*-9)	2.0	31. C20:4 methyl arachidonate (*cis*-5,8,11,14)	2.0
14. C17:0 methyl heptadecanoate	2.0	32. C23:0 methyl tricosanoate	2.0
15. C17:1 methyl heptadecenoate (*cis*-10)	2.0	33. C22:2 methyl docosadienoate (*cis*-13,16)	2.0
16. C18:0 methyl stearate	4.0	34. C20:5 methyl eicosapentaenoate (*cis*-5,8,11,14,17)	2.0
17. C18:1 methyl elaidate (*trans*-9)	2.0	35. C24:0 methyl lignocerate	4.0
18. C18:1 methyl oleate (*cis*-9)	4.0	36. C24:1 methyl nervonate (*cis*-15)	2.0
		37. C22:6 methyl docosahexaenoate (*cis*-4,7,10,13,16,19)	2.0

analysis of a marine-oil FAME standard; a marine oil sample is shown in Figure 2. Both analyses are characterized by fast, effective resolution and sharp, symmetric peaks.

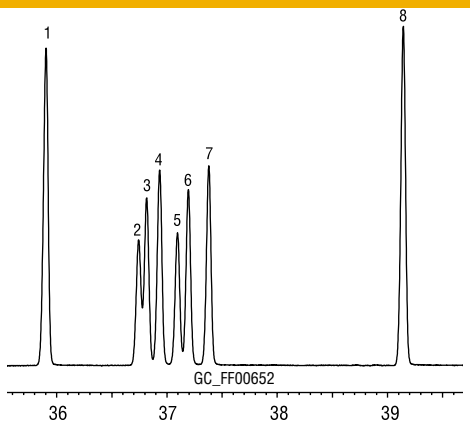
Resolving *cis* and *trans* Isomers

Individual *cis* and *trans* isomers are resolved on a 100-meter Rt-2560 column, making this the column of choice for analyzing partially hydrogenated fats. The highly polar biscyanopropyl phase gives the selectivity needed for resolving FAMEs isomers, such as the *cis* and *trans* forms of C18:1. The *trans* isomers elute before the *cis* isomers on this phase, opposite of the elution order on Carbowax®-based phases such as FAMEWAX™ or Rtx®-Wax. Figure 3 shows the chromatographic separation of 37 FAMEs typically encountered in vegetable, animal, and marine fats and oils, using an Rt-2560 column.

AOAC method 996.06[1] specifies the determination of total fat content based on the fatty acid content, after conversion to the methyl esters. This is the specified method for determining total fat content for nutritional labeling purposes. After quantifying the total FAMEs present in the derivatized sample, the amount of fat (as triglycerides) in the sample is calculated, based on initial sample weight. The 100-meter Rt-2560 column meets the requirements of this procedure. This column also allows quantification of the total *trans* content.

To calibrate the GC system for assays of this type, use a FAME mixture such as our 37-component Food Industry FAME Mix (Figure 3) or our 28-component NLEA FAME Mix (Figure 4). Both standards include a gravimetric certificate of analysis to help ensure accurate quantification. To ensure correct identifications of the individual *cis* and *trans* isomers of C18:1, use our *cis/trans* Isomer Mix, as shown in Figure 5.

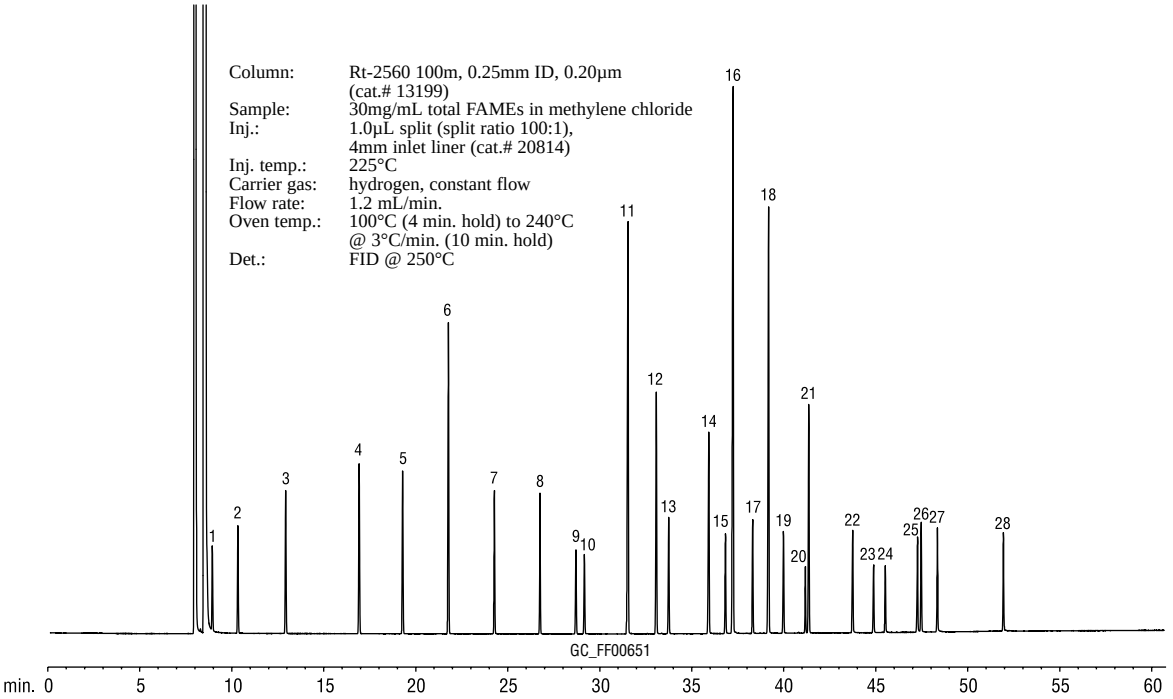
Figure 5—Resolve *cis* and *trans* isomers of unsaturated FAMEs on an Rt-2560 column.



Compound	% in Mix
1. C18:0 methyl stearate	20.0
2. C18:1 methyl petroselaidate (*trans*-6)	8.0
3. C18:1 methyl elaidate (*trans*-9)	10.0
4. C18:1 methyl transvaccenate (*trans*-11)	12.0
5. C18:1 methyl petroselinate (*cis*-6)	8.0
6. C18:1 methyl oleate (*cis*-9)	10.0
7. C18:1 methyl vaccenate (*cis*-11)	12.0
8. C18:2 methyl linoleate (*cis*-9,12)	20.0

Rt-2560, 100m, 0.25mm ID, 0.2µm (cat.# 13199)
Sample: 10mg/mL total FAMEs in methylene chloride
Inj.: 1.0µL split (split ratio 20:1), 4mm inlet liner (cat.# 20814)
Inj. temp.: 225°C
Carrier gas: hydrogen, constant flow
Flow rate: 1.2mL/min.
Oven temp.: 100°C (4 min. hold) to 240°C @ 3°C/min. (10 min. hold)
Det.: FID @ 250°C

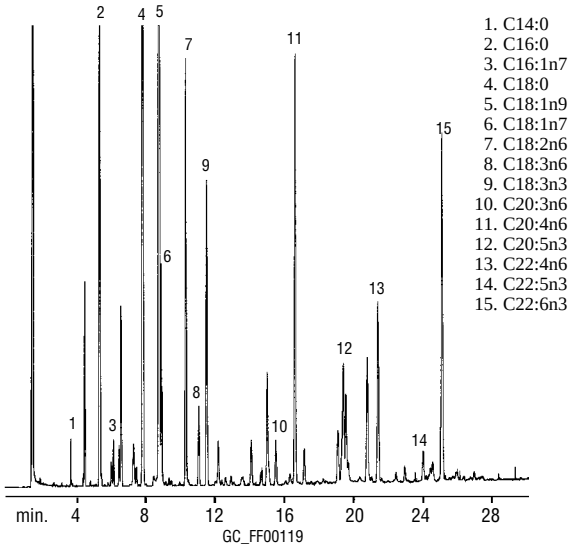
Figure 4—Use the NLEA FAME Mix to standardize Fat-by-Fatty Acid Composition methods, such as AOAC 996.06.



1. C4:0 methyl butyrate
2. C6:0 methyl heanoate
3. C8:0 methyl octanoate
4. C10:0 methyl decanoate
5. C11:0 methyl undecanoate
6. C12:0 methyl laurate
7. C13:0 methyl tridecanoate
8. C14:0 methyl myristate
9. C14:1 methyl myristoleate (*cis*-9)
10. C15:0 methyl pentadecanoate
11. C16:0 methyl palmitate
12. C16:1 methyl palmitoleate (*cis*-9)
13. C17:0 methyl heptadecanoate
14. C18:0 methyl stearate
15. C18:1 methyl elaidate (*trans*-9)
16. C18:1 methyl oleate (*cis*-9)
17. C18:2 methyl linoelaidate (*trans*-9,12)
18. C18:2 methyl linoleate (*cis*-9,12)
19. C20:0 methyl arachidate
20. C20:1 methyl eicosenoate (*cis*-11)
21. C18:3 methyl linolenate (*cis*-9,12,15)
22. C22:0 methyl behenate
23. C22:1 methyl erucate (*cis*-13)
24. C23:0 methyl tricosanoate
25. C24:0 methyl lignocerate
26. C20:5 methyl eicosapentaenoate (*cis*-5,8,11,14,17)
27. C24:1 methyl mervonate (*cis*-15)
28. C22:6 methyl docosahexaenoate (*cis*-4,7,10,13,16,19)

Rtx®-2330, a 90% biscyanopropyl phase, also resolves *cis* and *trans* FAME isomers. These columns are slightly less polar than Rt-2560 columns. Figure 6 shows the analysis of an animal-based fat, using an Rtx®-2330 column. As on Rt-2560 columns, the *trans* forms of the FAMEs elute before the *cis* forms.

Figure 6—Analysis of an animal-based fat, using an Rtx®-2330 capillary column.



30m, 0.32mm ID, 0.20µm Rtx®-2330 (cat.# 10724)
0.1µL split injection of PUFA 2 mix.

Oven temp.:	160°C to 250°C @ 2°C/min. (hold 10 min.)
Inj. & det. temp.:	260°C
Carrier gas:	hydrogen
Linear velocity:	40cm/sec.
FID sensitivity:	8 x 10^{-11} AFS
Split ratio:	20:1

Analyzing Botanical Products

Gas chromatography can be used to analyze the marker compounds in some botanical products, such as saw palmetto. In this product, the marker compounds are the fatty acids. The Institute for Nutraceutical Advancement (INA) has published a method for the analysis of the fatty acid content in saw palmetto by gas chromatography. The analysis is performed after converting the acids to the methyl esters. Both Rtx®-Wax and Stabilwax® capillary columns provide the efficiency and selectivity needed to perform this analysis, and allow accurate identification of the individual fatty acids (Figures 7 and 8).

Summary

Capillary gas chromatography is an essential tool in analyses of fats, oils, and fat-containing products. GC is especially useful for determining total fat content, *trans* fat content, and total omega-3 polyunsaturated fatty acid content in foods. The choice of capillary column depends on the information required. For polyunsaturated FAMEs analysis, a FAMEWAX® column allows fast, accurate quantification. A more polar Rt-2560 column is the column of choice when determining the total fat content, or the amount of *trans* fat, in an ingredient or end product.

Whatever your fatty acid analysis requirements, Restek can provide the consistent-performance analytical columns and reference materials that will help you to accurately characterize your materials.

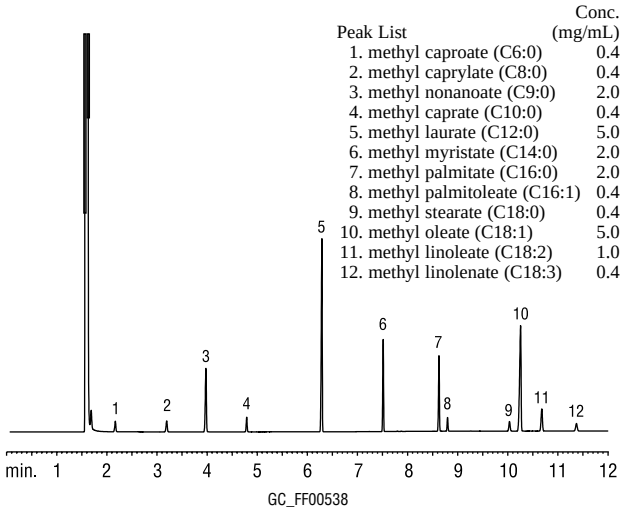
References

1. *Official Methods of Analysis*, 17th edition, AOAC International, 2000.
2. *Official Methods and Recommended Practices of the AOCS*, 5th edition, American Oil Chemists Society.
3. *European Pharmacopoeia*, 4th edition, method 2001:1352.
4. Liu, K.-S., *JAOCS* 71 (11): 1179 (1994).
5. Miller, K.D. *et. Al.*, *JHRC* 16: 161 (1993).

Also Available from Restek

Selection Guide for Polar WAX GC Column Phases (free on request, lit. cat.# 59890).

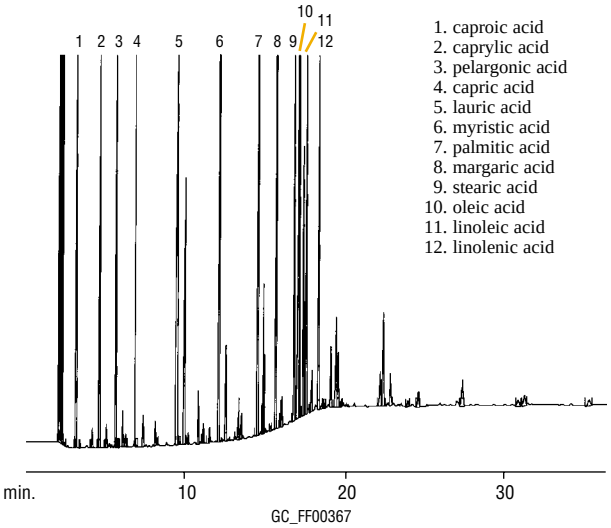
Figure 7—Saw palmetto FAME standard resolved on an Rtx®-Wax column.



30m, 0.25mm, 0.25µm Rtx®-Wax (cat.# 12423)
1µL injection of saw palmetto standard
Conc.: see peak list

Oven temp.:	120°C (hold 3 min.) to 220°C at 20°C/min. (hold 12 min.)
Inj./det. temp.:	250°C/300°C
Carrier gas:	helium
Linear velocity:	1mL/min. 34 cm/sec.
Split ratio:	100:1

Figure 8—Fatty acids in saw palmetto, a Stabilwax® column after conversion to methyl esters.



50m, 0.25mm ID, 0.25µm Stabilwax® (cat.# 10626-105).
1µL split injection.

Oven temp.:	110°C (hold 1 min.) to 240°C @ 8°C/min. (hold 25 min.)
Inj./FID temp.:	230°C/250°C
Carrier gas:	hydrogen @ 2.5mL/min.
Split flow:	37.5mL/min.
Septum purge:	3mL/min.

Chromatogram provided by The Institute for Nutraceutical Advancement (INA)

GC Columns for FAMEs Analyses

Ordering Information | FAMEWAX™ (Fused Silica)

(Crossbond® polyethylene glycol)

ID	df (µm)	temp. limits	30-Meter
0.25mm	0.25	20 to 250°C	12497
0.32mm	0.25	20 to 250°C	12498
0.53mm	0.50	20 to 250°C	12499

Ordering Information | Stabilwax® (Fused Silica)

(Crossbond® Carbowax®—provides oxidation resistance)

ID	df (µm)	temp. limits	15-Meter	30-Meter	30-Meter 6/pk.	60-Meter
0.25mm	0.10	40 to 250°C	10605	10608		10611
	0.25	40 to 250°C	10620	10623		10626
	0.50	40 to 250°C	10635	10638		10641
0.32mm	0.10	40 to 250°C	10606	10609		10612
	0.25	40 to 250°C	10621	10624		10627
	0.50	40 to 250°C	10636	10639		10642
0.53mm	0.10	40 to 250°C	10607	10610		10613
	0.25	40 to 250°C	10622	10625		10628
	0.50	40 to 250°C	10637	10640		10643
	1.00	40 to 240/250°C	10652	10655	10655-600	10658

Ordering Information | Rtx®-Wax (Fused Silica)

(Crossbond® polyethelene glycol)

ID	df (µm)	temp. limits**	15-Meter	30-Meter	60-Meter
0.25mm	0.10	20 to 250°C	12405	12408	
	0.25	20 to 250°C	12420	12423	12426
	0.50	20 to 250°C	12435	12438	12441
0.32mm	0.10	20 to 250°C	12406	12409	
	0.25	20 to 250°C	12421	12424	12427
	0.50	20 to 250°C	12436	12439	12442
	1.00	20 to 240/250°C	12451	12454	12457
0.53mm	0.25	20 to 250°C	12422	12425	
	0.50	20 to 250°C	12437	12440	12443
	1.00	20 to 240/250°C	12452	12455	12458
ID	**df (µm)**	**temp. limits**	**10-Meter**	**20-Meter**	
0.10mm	0.10	20 to 250°C	41601	41602	
	0.20	20 to 240/250°C	41603	41604	

Ordering Information | Rt-2560 (Fused Silica)

ID	df (µm)	temp. limits	100-Meter
0.25mm	0.20	20 to 250°C	13199

Ordering Information | Rtx®-2330* (Fused Silica)

(90% biscyanopropyl/10% phenylcyanopropyl)

ID	df (µm)	temp. limits**	15-Meter	30-Meter	60-Meter	105-Meter
0.25mm	0.10	0 to 260/275°C	10705	10708	10711	10714
	0.20	0 to 260/275°C	10720	10723	10726	10729
0.32mm	0.10	0 to 260/275°C	10706	10709	10712	10715
	0.20	0 to 260/275°C	10721	10724	10727	10730
0.53mm	0.10	0 to 260/275°C	10707	10710	10713	
	0.20	0 to 260/275°C	10722	10725	10728	
ID	**df (µm)**	**temp. limits**	**10-Meter**	**20-Meter**	**40-Meter**	
0.18mm	0.10	0 to 260/275°C	40701	40702	40703	

**Not solvent rinsable.*
***The maximum temperatures listed are for 15- and 30-meter lengths. Longer lengths may have a slightly reduced maximum temperature.*

Vials and Limited Volume Inserts

Convenience Kits: Includes Vials*, Caps, & Septa

Description	100-pk.	1000-pk.
2mL Clear Vial, deactivated**, PTFE/Natural Rubber Seal	24671	24672
2mL Amber Vial, deactivated**, PTFE/Natural Rubber Seal	24673	24674
2.0mL Clear Vial, untreated, PTFE/Natural Rubber Seal	21196	21197
2.0mL Amber Vial, untreated, PTFE/Natural Rubber Seal	21198	21199
2.0mL Clear Vial, untreated, PTFE/Silicone Seal	24646	24647
2.0mL Amber Vial, untreated, PTFE/Silicone Seal	24648	24649

**Crimp top vials, 2.0mL, 12 x 32mm, 11mm crimp finish.*
***Silcote™ CL7 deactivation.*

Limited Volume Inserts for 2mL, Crimp-Top & Short-Cap, Screw-Thread Vials

Description	100-pk.	1000-pk.
50µL Glass, Polypropylene, Bottom Spring	24513	21782
250µL Glass, BM Insert w/ Bottom Spring	21776	21777
250µL Glass, BM Insert w/ Glass Flange (Step™ Design)	24516	21779
350µL Glass, Flat Bottom Insert	21780	24517
250µL Polypropylene, Bottom Spring	24518	—
250µL Polypropylene, Top Flange	24519	—
250µL Polypropylene, No Spring	24520	—

BM = Big Mouth

Analytical Reference Materials for FAMEs Analyses

Marine Oil FAME Mix (20 components)

Chain	Description	% by Weight
C14:0	methyl myristate	6.0
C14:1	methyl myristoleate	1.0
C16:0	methyl palmitate	16.0
C16:1	methyl palmitoleate	5.0
C18:0	methyl stearate	8.0
C18:1	methyl oleate	13.0
C18:1	methyl vaccenate	4.0
C18:2	methyl linoleate	2.0
C18:3	methyl linolenate	2.0
C20:0	methyl arachidate	1.0
C20:1	methyl 11-eicosenoate	9.0
C20:2	methyl 11-14-eicosadienoate	1.0
C20:4	methyl arachidonate	3.0
C20:3	methyl 11-14-17-eicosatrienoate	1.0
C20:5	methyl eicosapentaenoate	10.0
C22:0	methyl behenate	1.0
C22:1	methyl erucate	3.0
C22:6	methyl docosahexaenoate	12.0
C24:0	methyl lingnocerate	1.0
C24:1	methyl nervonate	1.0

100mg
35066

Food Industry FAME Mix (37 components)

Chain	% by Weight	Chain	% by Weight
C4:0	4.0	C18:2(all-*cis*-9,12)	2.0
C6:0	4.0	C18:3(all-*cis*-6,9,12)	2.0
C8:0	4.0	C18:3(all-*cis*-9,12,15)	2.0
C10:0	4.0	C20:0	4.0
C11:0	2.0	C20:1(*cis*-11)	2.0
C12:0	4.0	C20:2(all-*cis*-11,14,)	2.0
C13:	2.0	C20:3 (all-*cis*-8,11,14)	2.0
C14:0	4.0	C20:3(all-*cis*-11,14,17)	2.0
C14:1(*cis*-9)	2.0	C20:4(all-*cis*-5,8,11,14)	2.0
C15:0	2.0	C20:5(all-*cis*-5,8,11,14,17)	2.0
C15:1(*cis*-10)	2.0	C21:0	2.0
C16:0	6.0	C22:0	4.0
C16;1(*cis*-9)	2.0	C22:1(*cis*-13)	2.0
C17:0	2.0	C22:2(all-*cis*-13,16)	2.0
C17:1(*cis*-10)	2.0	22:6(all-*cis*-4,7,10,13,16,19)	2.0
C18:0	4.0	C23:0	2.0
C18:1(*trans*-9)	2.0	C24:0	4.0
C18:1(*cis*-9)	4.0	C24:1(*cis*-15)	2.0
C18:2(all-*trans*-9,12)	2.0		

30mg/mL total in methylene chloride, 1mL/ampul

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35077

NLEA FAME Mix (28 components)

Chain	% by Weight	Chain	% by Weight
C4:0	1.5	C18:1(*trans*-9)	2.5
C6:0	1.5	C18:1(*cis*-9)	15.0
C8:0	2.0	C18:2(all-*trans*-9,12)	2.5
C10:0	2.5	C18:2(all-*cis*-9,12)	10.0
C11:0	2.5	C18:3(all-*cis*-9,12,15)	5.0
C12:0	5.0	C20:0	2.5
C13:	2.5	C20:1(*cis*-11)	1.5
C14:0	2.5	C20:5(all-*cis*-5,8,11,14,17)	2.5
C14:1(*cis*-9)	1.5	C22:0	2.5
C15:0	1.5	C22:1(*cis*-13)	1.5
C16:0	10.0	C22:6(all-*cis*-4,7,10,13,16,19)	2.5
C16;1(*cis*-9)	5.0	C23:0	1.5
C17:0	2.5	C24:0	2.5
C18:0	5.0	C24:1(*cis*-15)	2.5

30mg/mL total in methylene chloride, 1mL/ampul

ea.
35078

cis/trans FAME Mix (8 components)

Description	% by Weight
methyl elaidate (C18:1 *trans*-9)	10.0
methyl linoleate (C18:2 *cis*-9,12)	20.0
methyl oleate (C18:1 *cis*-9)	10.0
methyl petroselinate (C18:1*cis*-6)	8.0
methyl petroselaidate (C18:1*trans*-6)	8.0
methyl stearate (C18:0)	20.0
methyl transvaccenate (C18:1 *trans*-11)	12.0
methyl vaccenate (C18:1 *cis*-11)	12.0

10mg/mL total in methylene chloride, 1mL/ampul

ea.
35079

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