

## APPLICATIONS NOTE

# FAMEs Analyses

## High-Resolution GC Analyses of Fatty Acid Methyl Esters

Fatty acid methyl esters (FAMEs) analysis is an important tool both for characterizing fats and oils and for 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 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.

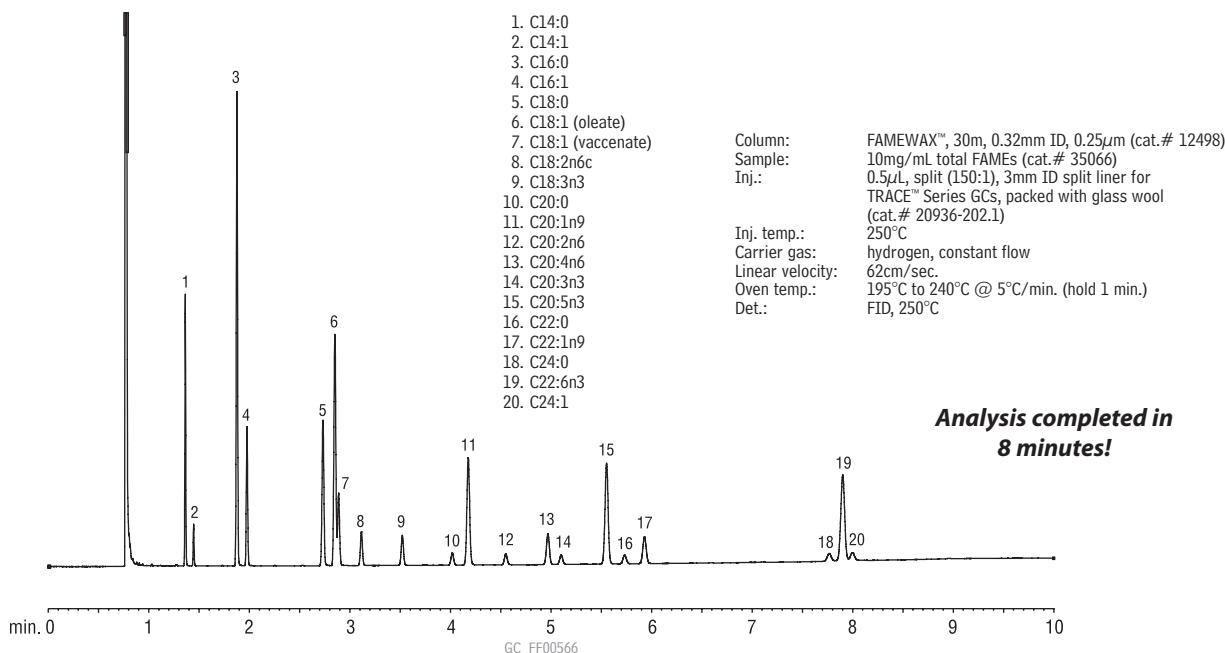
FAMEs analyses were among the first applications for gas chromatography, so many of the GC methods originally written for analysis of fats and oils described packed column technology. Capillary columns offer significant advantages, however, including more efficient separations. When analyzing fats and 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 (Figures 1 and 2), and biscyanopropyl phases are used to resolve *cis* and *trans* isomers of polyunsaturated components (Figure 3).

### Creating FAMEs

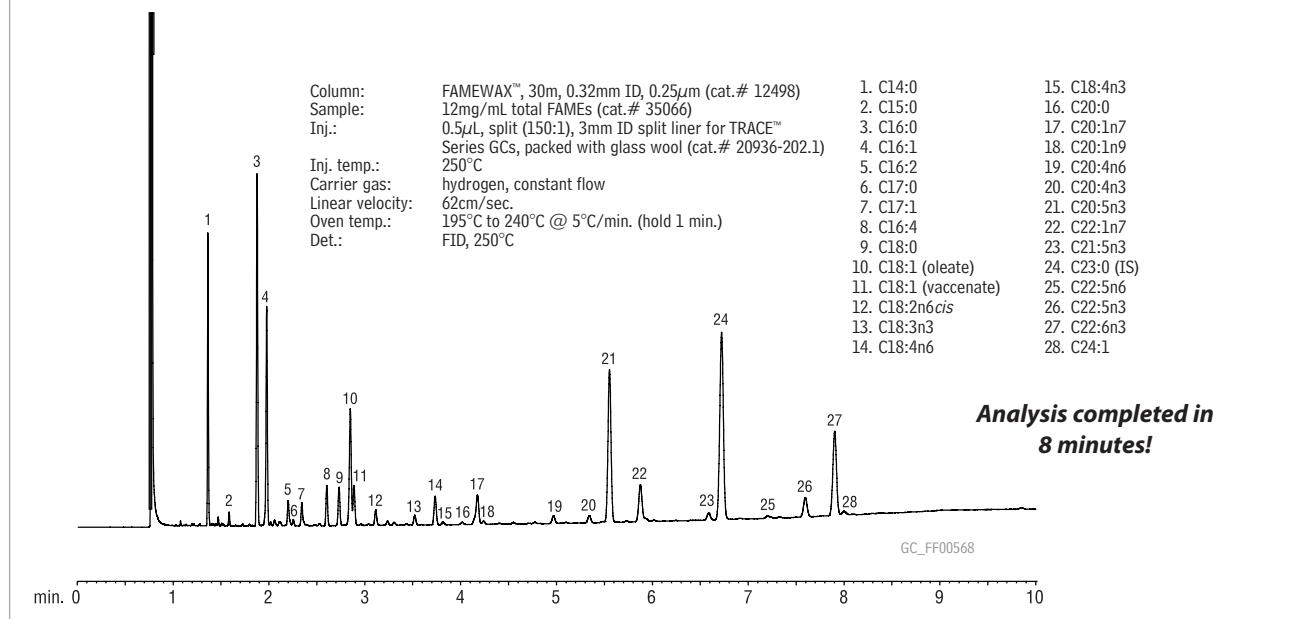
Lipids normally are extracted from matrices using a non-polar solvent, such as ether, and saponified to produce the free fatty acid salts. The fatty acid salts then are derivatized to form the fatty acid methyl esters, to increase volatility, improve peak symmetry, and decrease sample activity, and thus provide more accurate analytical data. The official methods of the Association of Official Agriculture Chemists (AOAC International)<sup>1</sup> and the American Oil Chemists Society (AOCS)<sup>2</sup> contain procedures for the derivatization reaction, as does the European Pharmacopoeia.<sup>3</sup> 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 the drying and post-reaction work-up, into one step.<sup>4</sup> 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.<sup>5</sup>

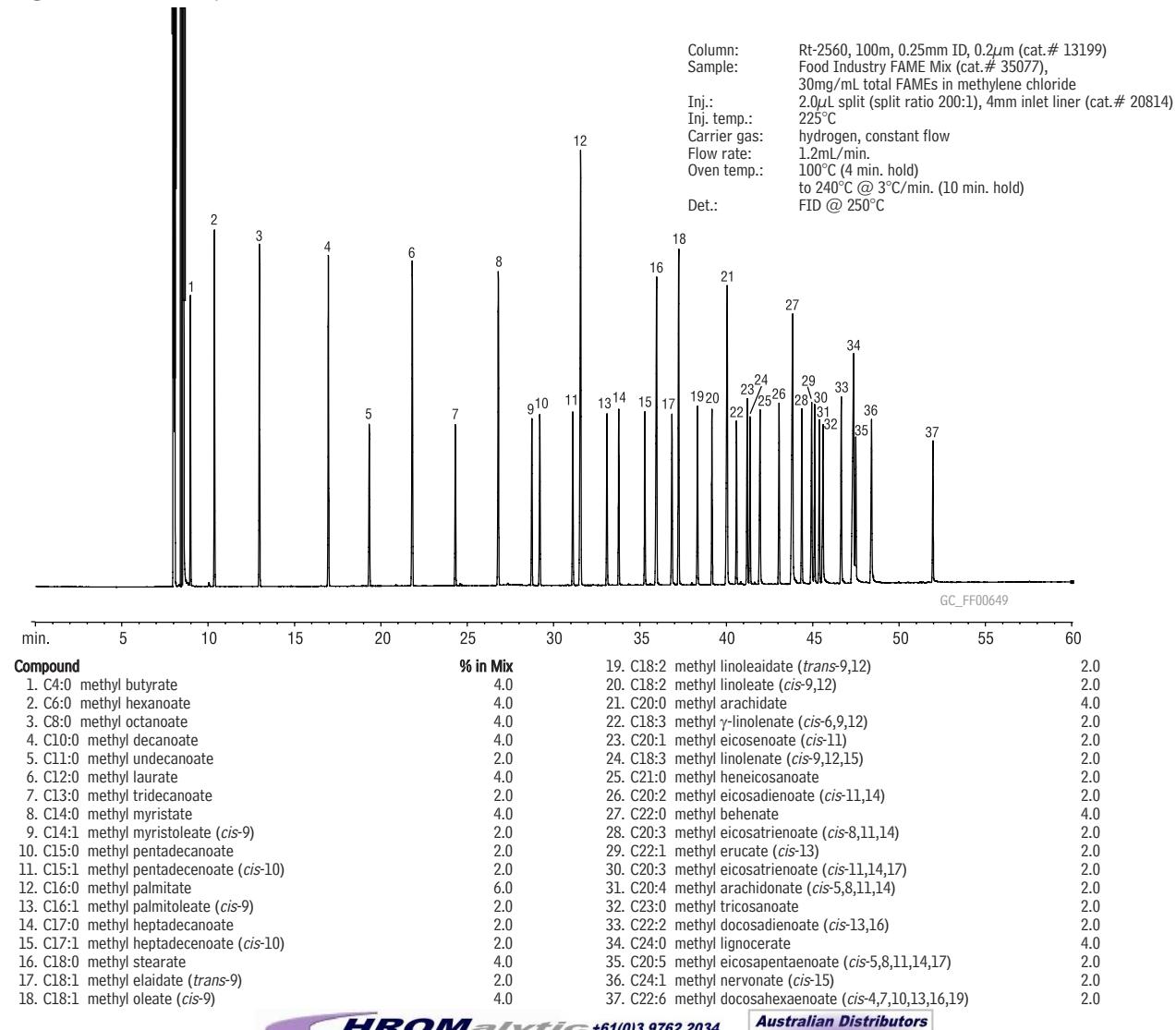
**Figure 1** A FAMEWAX™ column provides fast, efficient separations of marine oil-based FAMEs.



**Figure 2** Rapid, excellent resolution of marine oil FAMEs, using a FAMEWAX™ column.



**Figure 3** Near complete resolution of a 37 FAME mix on an Rt-2560 column.



## Analyzing Polyunsaturated FAMEs

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. FAMEs such as methyl eicosapentaenoate (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! Figures 1 and 2 show analyses of marine-oil FAME standards. Both analyses are characterized by fast, effective resolution and sharp, symmetric peaks.

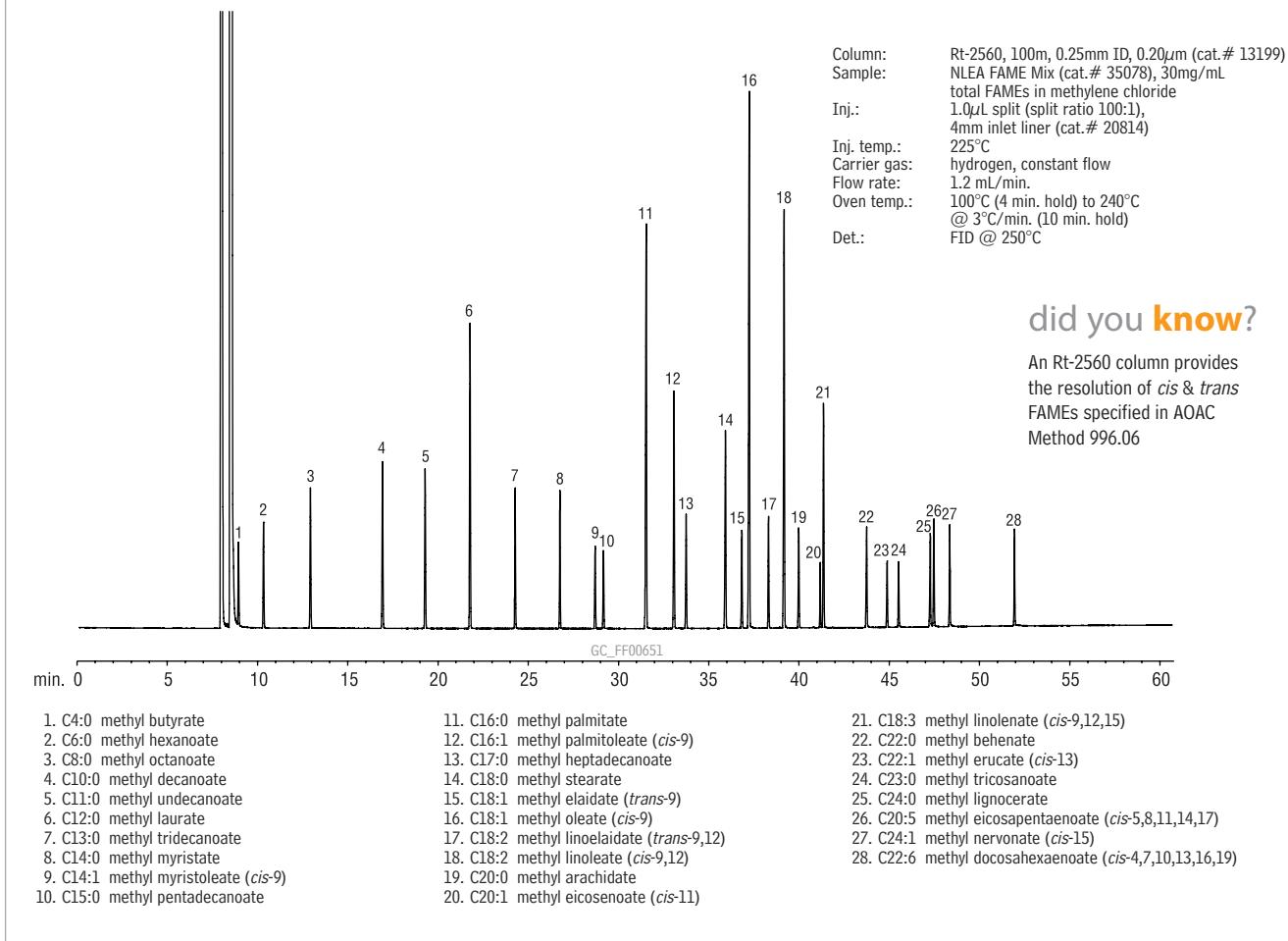
Like FAMEWAX™ columns, Stabilwax® columns and Rtx®-Wax columns provide excellent resolution of FAMEs derived from either plant or animal sources. When polyunsaturated FAMEs are analyzed on one of these Carbowax®-type capillary columns, analysis times of 35-50 minutes generally are required to fully resolve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

## 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 FAME 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, or marine fats and oils, using an Rt-2560 column.

AOAC method 996.06<sup>1</sup> describes the determination of total fat content based on the fatty acid content, after conversion to 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 (Figure 4). This column also allows quantification of the total *trans* content.

**Figure 4** Use the NLEA FAME Mix to standardize fat-by-fatty acid composition methods, such as AOAC 996.06.



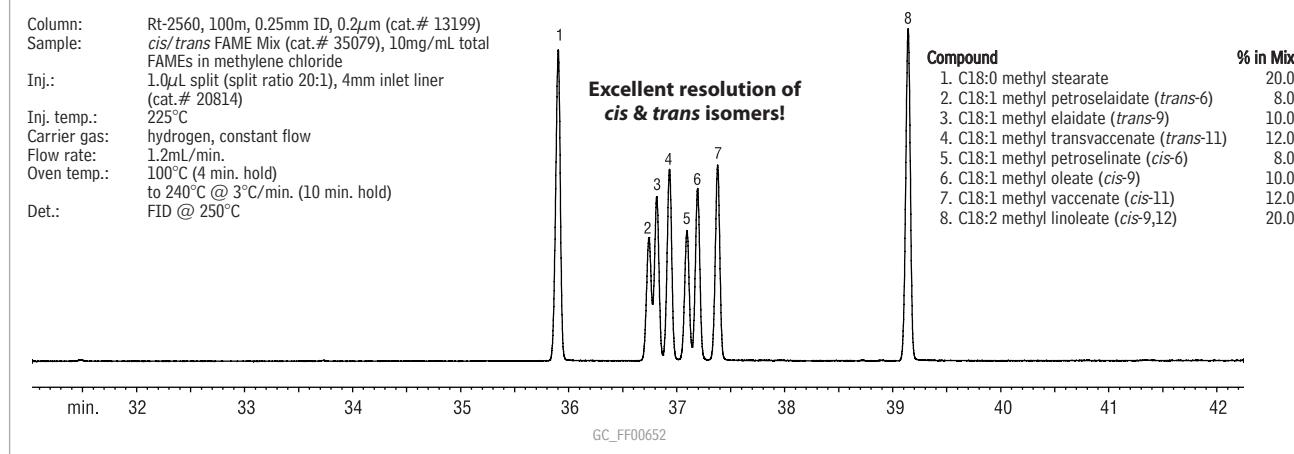
To calibrate the GC system for assays of this type, use a FAME mixture such as our 37-component Food Industry FAME Mix (Figure 3) or our 28-component NLEA FAME Mix (Figure 4). Both standards include a gravimetric certificate of analysis to help ensure accurate quantification. To ensure correct identifications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

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.

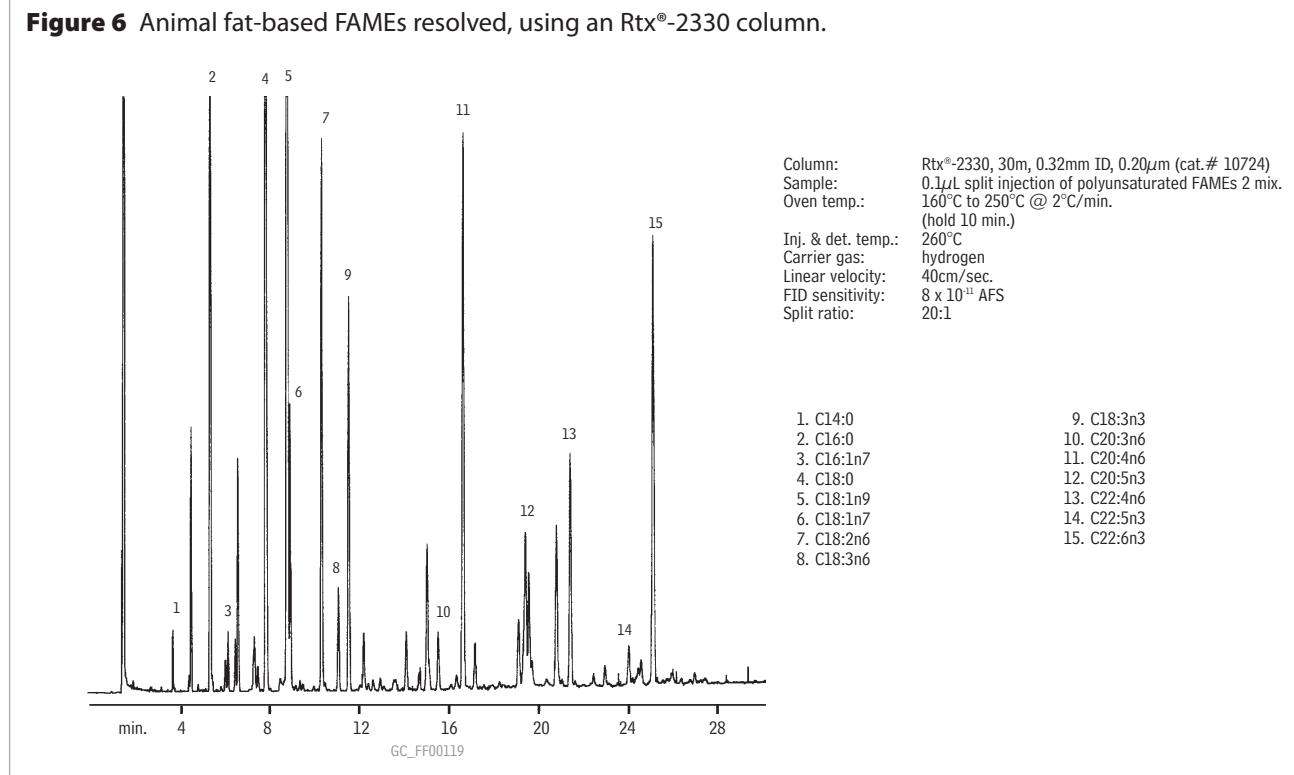
### Analyzing Botanical Products

Gas chromatography can be used to analyze fatty acid marker compounds in some botanical products. The Institute for Nutraceutical Advancement (INA) has published a method for analyzing the fatty acid content in saw palmetto by gas chromatography, after converting the acids to 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).

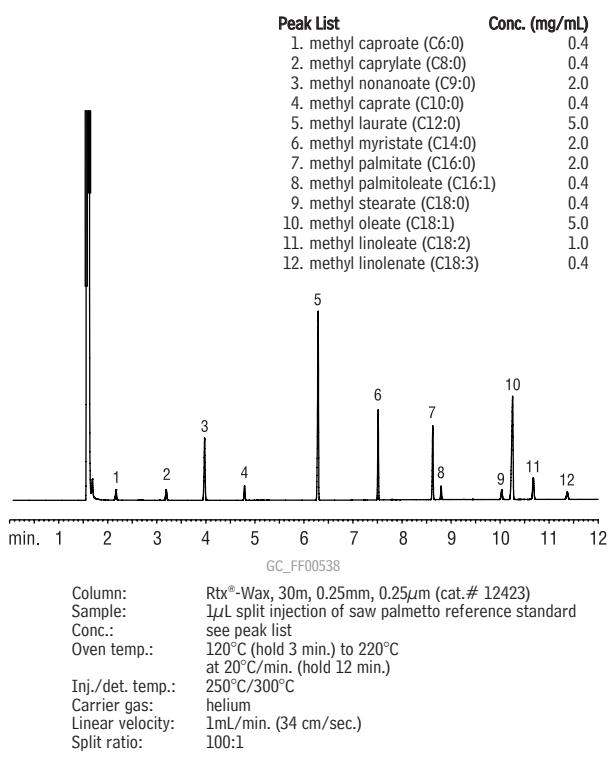
**Figure 5** Resolve *cis* and *trans* isomers of unsaturated FAMEs on an Rt-2560 column.



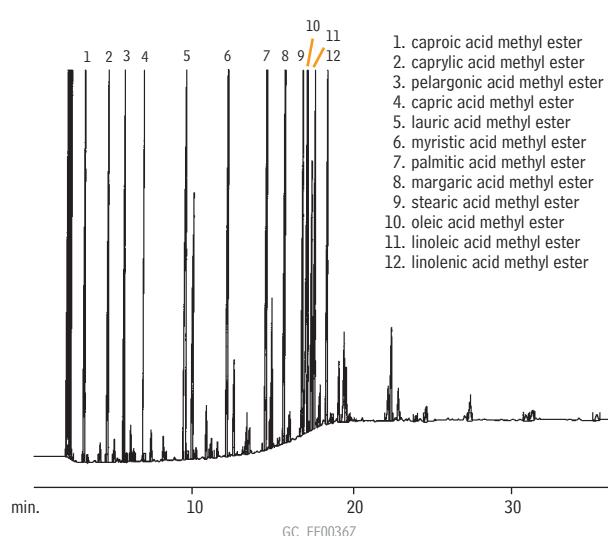
**Figure 6** Animal fat-based FAMEs resolved, using an Rtx®-2330 column.



**Figure 7** Saw palmetto FAMEs resolved on an Rtx®-WAX column.



**Figure 8** Saw palmetto FAMEs on a Stabilwax® column.



Chromatogram provided by The Institute for Nutraceutical Advancement (INA)

## Summary

Capillary 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 mixes that will help you to accurately characterize your materials.

## References

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

## Rtx®-Wax Columns (fused silica)

(Crossbond® Carbowax® polyethylene glycol)

ID	df ( $\mu$ m)	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
0.53mm	1.00	20 to 240/250°C	12451	12454	12457
	0.25	20 to 250°C	12422	12425	
	0.50	20 to 250°C	12437	12440	12443
ID	df ( $\mu$ m)	temp. limits	10-Meter	20-Meter	
	0.10mm	20 to 250°C	41601	41602	
	0.20	20 to 240/250°C	41603	41604	

## free literature

Selection Guide for Polar WAX GC Column Phases (technical guide, lit. cat.# 59890)

Fast, Selective Triglyceride Analysis (application note, lit. cat.# 59580A)

Foods Flavors Fragrances (technical guide, lit. cat.# 59260A)

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### Stabilwax® Columns (fused silica)

(Crossbond® Carbowax® polyethylene glycol—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
	1.00	40 to 240/250°C	10651	10654	10654-600	10657
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
	1.50	40 to 230/240°C	10666	10669		10672
	2.00	40 to 220/230°C	10667	10670		

### FAMEWAX™ Columns (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

### Rt-2560 Column (fused silica)

(biscyanopropyl polysiloxane)

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

### Rtx®-2330 Columns (fused silica)

(90% biscyanopropyl/10% phenylcyanopropyl polysiloxane)\*\*

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	

\*Maximum temperatures are for 15- and 30-meter lengths. Longer lengths may have a slightly reduced maximum temperature.

\*\*Not solvent rinsable.

### Convenience Kits: Vials, Caps, & Septa

Vials packaged in a clear-lid tray. Caps with septa packaged in a plastic bag.



12 x 32mm vials, 11mm crimp finish

Description	100-pk.	1000-pk.
2.0mL Clear Vial, deactivated, PTFE/Natural Rubber Seal†	24671	24672
2.0mL 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



Glass, with Bottom Spring

Glass, with Top Flange

### Limited Volume Inserts for 2mL Crimp-Top Vials

Description	100-pk.	1000-pk.
50μL Glass, Big Mouth, Bottom Spring	24513	21782
250μL Glass, Big Mouth Insert w/ Bottom Spring	21776	21777
250μL Glass, Big Mouth Insert w/ Glass Flange (Step™ Design)	24516	21779
350μL Glass, Flat Bottom Insert	21780	24517
350μL Glass, Flat Bottom Insert w/ ID Ring	24692	24693
250μL Polypropylene, Big Mouth, Bottom Spring	24518	—
250μL Polypropylene, Big Mouth, Top Flange	24519	—
250μL Polypropylene, Big Mouth, No Spring	24520	—

†Silcote™ CL7 deactivation.

**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 lignocerate	1.0
C24:1	methyl nervonate	1.0

Neat, 100mg/1mL ampul

cat. # 35066 (ea.)

For chromatography, see Figure 1.

**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	
C16:1(cis-9)	5.0	(all-cis-4,7,10,13,16,19)	2.5
C17:0	2.5	C23:0	1.5
C18:0	5.0	C24:0	2.5
		C24:1(cis-15)	2.5

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

cat. # 35078 (ea.)

No data pack available.

For chromatography, see Figure 4.

**Neat Fatty Acid Methyl Esters**

Use these materials to prepare specific mixtures not commercially available. These products are of the highest purity available, typically 99% by GC/FID analysis. Each compound is packaged under a nitrogen blanket to ensure product stability. A Certificate of Analysis is provided with each ampul.

Chain	Description	CAS #	qty.	cat.#
C6:0	methyl caproate	106-70-7	100mg	35037
C7:0	methyl heptanoate	106-73-0	100mg	35038
C8:0	methyl caprylate	111-11-5	100mg	35039
C9:0	methyl nonanoate	1731-84-6	100mg	35040
C10:0	methyl caprate	110-42-9	100mg	35041
C11:0	methyl undecanoate	1731-86-8	100mg	35042
C12:0	methyl laurate	111-82-0	100mg	35043
C13:0	methyl tridecanoate	1731-88-0	100mg	35044
C14:0	methyl myristate	124-10-7	100mg	35045
C14:1 Δ 9 cis	methyl myristoleate	56219-06-8	100mg	35046
C15:0	methyl pentadecanoate	7162-64-1	100mg	35047
C16:0	methyl palmitate	112-39-0	100mg	35048
C16:1 Δ 9 cis	methyl palmitoleate	1120-25-8	100mg	35049
C17:0	methyl heptadecanoate	1731-92-6	100mg	35050
C18:0	methyl stearate	112-61-8	100mg	35051
C18:1 Δ 9 cis	methyl oleate	112-62-9	100mg	35052
C18:2 Δ 9,12 cis	methyl linoleate	112-63-0	100mg	35053
C18:3 Δ 9,12,15 cis	methyl linolenate	301-00-8	100mg	35054
C19:0	methyl nonadecanoate	1731-94-8	100mg	35055
C20:0	methyl arachidate	1120-28-1	100mg	35056
C20:1 Δ 11 cis	methyl eicosenoate	2390-09-2	100mg	35057
C20:2 Δ 11,14 cis	methyl eicosadienoate	2463-02-7	100mg	35058
C20:3 Δ 11,14,17 cis	methyl eicosatrienoate	55682-88-7	100mg	35059
C20:4 Δ 5,8,11,14 cis	methyl arachidonate	2566-89-4	100mg	35060
C21:0	methyl heneicosanoate	6064-90-0	100mg	35061
C22:0	methyl behenate	929-77-1	100mg	35062
C22:1 Δ 13 cis	methyl erucate	1120-34-9	100mg	35063
C24:0	methyl lignocerate	2442-49-1	100mg	35064
C24:1 Δ 15 cis	methyl nervonate	2733-88-2	100mg	35065

**free data**

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## Quantitative Fatty Acid Methyl Ester (FAME) Mixtures

These mixtures can be used for quantification (AOCS Method CE 1-62) and approximate the compositions of the following types of oils:

AOCS #1: corn, poppy seed, cotton seed, soybean, walnut, safflower, sunflower, rice, bran, and sesame oil  
 AOCS #2: linseed, perilla, hempseed, and rubberseed oil  
 AOCS #3: peanut, rapeseed, and mustard seed oil  
 AOCS #4: olive, teaseed, and neatsfoot oil  
 AOCS #5: coconut, palm kernel, babassu, and ouri-curi oil  
 AOCS #6: lard, beef or mutton tallow, and palm oil  
 FAME #1: oils of mid-range chain lengths (C16 - C18)  
 FAME #2: oils of short to mid-range chain lengths (C6 - C14)  
 FAME #3: oils of short to mid-range chain lengths (C8 - C16)

FAME #4: oils of mid-range to long chain lengths (C16 - C24)  
 FAME #5: oils of mid-range to long chain lengths (C16 - C24)  
 FAME #6: oils of long chain lengths (C20 - C21)  
 FAME #7: oils of short chain lengths (C6 - C10)  
 FAME #8: oils of short to mid-range chain lengths (C11 - C15)  
 FAME #9: oils of mid-range to long chain lengths (C16 - C20)  
 FAME #12: oils of mid-range to long chain lengths (C13 - C21)  
 FAME #13: mustard seed oil  
 FAME #14: cocoa butter  
 FAME #15: peanut oil

Mix	Cat. #	Composition of each mixture listed as a weight/weight % basis (minimum 50mg/ampul)													
AOCS #1	35022														
AOCS #2	35023														
AOCS #3	35024					1.0									
AOCS #4	35025														
AOCS #5	35026		7.0	5.0	48.0	15.0	7.0								
AOCS #6	35027					2.0	30.0	3.0	14.0	41.0	7.0	3.0			
FAME #1	35010						20.0		20.0	20.0	20.0				
FAME #2	35011	20.0	20.0	20.0	20.0	20.0									
FAME #3	35012		20.0	20.0	20.0	20.0	20.0								
FAME #4	35013						20.0	20.0					20.0	20.0	
FAME #5	35014							20.0	20.0				20.0	20.0	
FAME #6	35015												20.0	20.0	20.0
FAME #7	35016	20.0	20.0	20.0	20.0	20.0									
FAME #8	35017						20.0	20.0	20.0	20.0	20.0	20.0			
FAME #9	35018							20.0	20.0	20.0		20.0	20.0		
FAME #12	35021						20.0	20.0				20.0		20.0	
FAME #13	35034							3.0	1.0	2.0	20.0	15.0	10.0	1.0	10.0
FAME #14	35035							0.1	26.3	0.4	0.3	33.7	34.3	3.1	0.2
FAME #15	35036								10.0	3.0	50.0	30.0		1.5	1.5

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