Rt-2560 Columns Ensure Accurate, Reliable AOAC 996.06 and AOCS Ce 1j-07 FAMEs Analysis

By Rebecca Stevens and Kristi Sellers

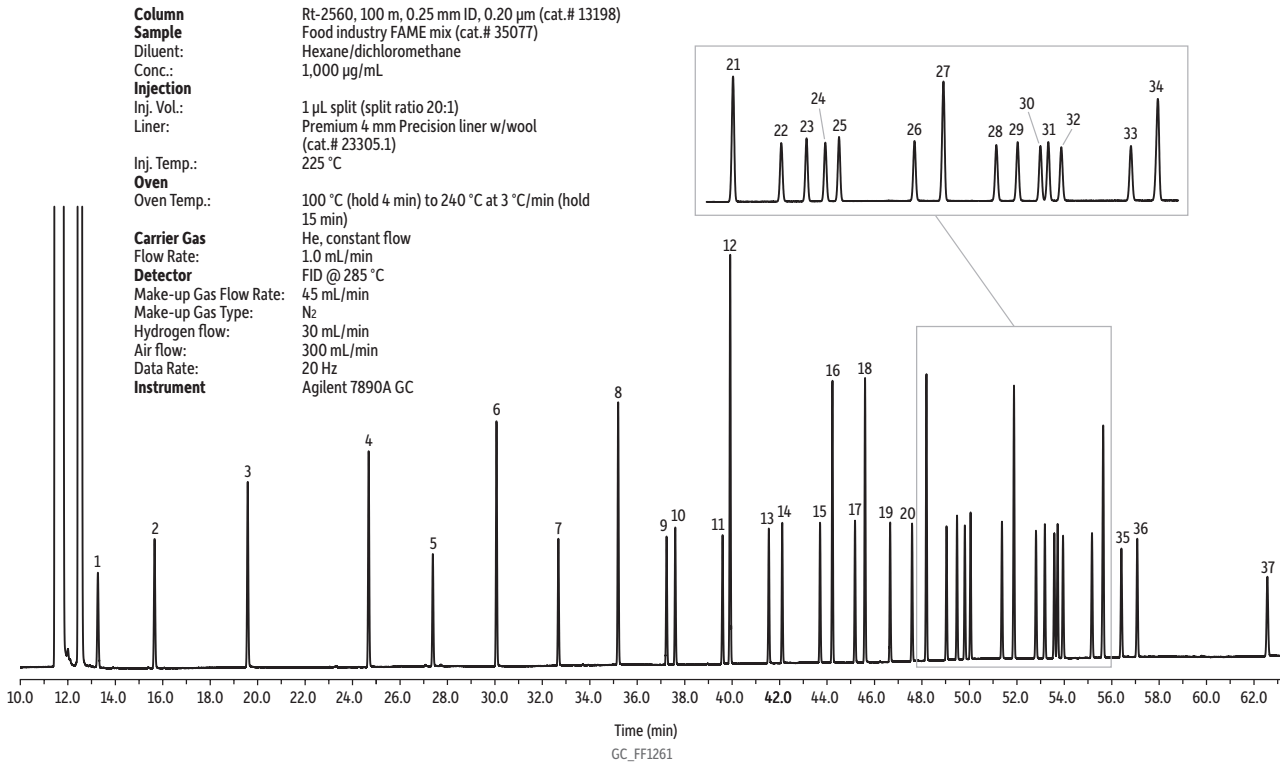
Restek's Rt-2560 GC column was originally developed for detailed analysis of FAMEs in foods and food products. Its highly polar biscyanopropyl stationary phase and long column format make it an effective tool for the difficult task of separating and accurately quantifying complex mixtures of FAMEs. Recently, Restek optimized the manufacturing process and implemented a new, application-specific, QC-testing procedure for all new Rt-2560 columns (cat.# 13198). As shown here, Rt-2560 columns produced using the optimized process exhibit excellent performance for both AOAC 996.06 and AOCS Ce 1j-07 FAMEs analysis and meet all method requirements.

AOAC 996.06

AOAC official method 996.06 describes a standard procedure for the determination of total, saturated, and unsaturated fat in foods using capillary GC-FID. The procedure involves hydrolytic extraction, methylation, and capillary GC-FID analysis of the resulting fatty acid methyl esters (FAMEs). Triundecanoin, the C11:0 triglyceride, is used as an internal standard for quantitation, and response factors are included in method AOAC 996.06.

The method recommends a 100 m x 0.25 mm x 0.20 µm column with a highly polar biscyanopropyl stationary phase, such as the Rt-2560. The column must be able to separate the adjacent C18:3 and C20:1 FAME isomers as well as C22:1, C20:3, and C20:4 with a resolution of 1.0 or better. The Rt-2560 is specifically designed for FAMEs analysis and readily achieves these required critical separations as shown by peaks 22 to 24 and 29 to 31 in Figure 1.

Figure 1: Application-specific QC testing ensures that all Rt-2560 columns meet AOAC 996.06 method requirements.



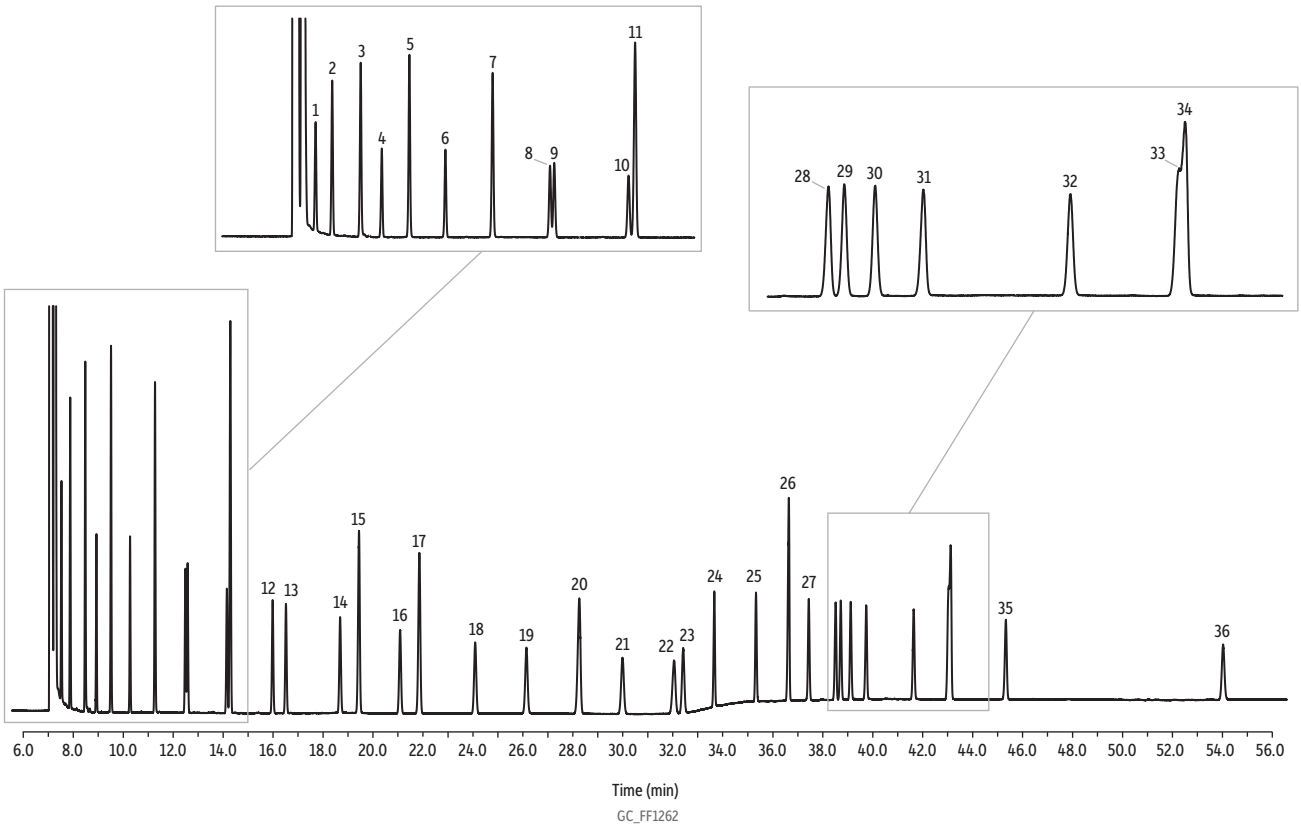
Peaks	t_R (min)	Conc. (µg/mL)	Structural Nomenclature
1. Methyl butyrate	13.16	40	C4:0
2. Methyl caproate	15.53	40	C6:0
3. Methyl octanoate	19.43	40	C8:0
4. Methyl decanoate	24.50	40	C10:0
5. Methyl undecanoate	27.20	20	C11:0
6. Methyl dodecanoate	29.87	40	C12:0
7. Methyl tridecanoate	32.47	20	C13:0
8. Methyl myristate	34.97	40	C14:0
9. Methyl myristoleate	37.01	20	C14:1 (c9)
10. Methyl pentadecanoate	37.37	20	C15:0
11. Methyl pentadecenoate	39.36	20	C15:1 (c10)
12. Methyl palmitate	39.68	60	C16:0
13. Methyl palmitoleate	41.30	20	C16:1 (c9)
14. Methyl heptadecanoate	41.86	20	C17:0
15. Methyl heptadecenoate	43.45	20	C17:1 (c10)
16. Methyl stearate	43.97	40	C18:0
17. Methyl octadecenoate	44.92	20	C18:1 (t9)
18. Methyl oleate	45.34	40	C18:1 (c9)
19. Methyl linoleaidate	46.39	20	C18:2 (t9,t12)
20. Methyl linoleate	47.32	20	C18:2 (c9,c12)
21. Methyl arachidate	47.92	40	C20:0
22. Methyl linolenate	48.77	20	C18:3 (c6,c9,c12)
23. Methyl eicosenoate	49.20	20	C20:1 (c11)
24. Methyl linolenate	49.54	20	C18:3 (c9,c12,c15)
25. Methyl heneicosanoate	49.78	20	C21:0
26. Methyl eicosadienoate	51.09	20	C20:2 (c11,c14)
27. Methyl behenate	51.60	40	C22:0
28. Methyl eicosatrienoate	52.52	20	C20:3 (c8,c11,c14)
29. Methyl erucate	52.88	20	C22:1 (c13)
30. Methyl eicosatrienoate	53.28	20	C20:3 (c11,c14,c17)
31. Methyl tricosanoate	53.42	20	C23:0
32. Methyl arachidonate	53.65	20	C20:4 (c5,c8,c11,c14)
33. Methyl docosadienoate	54.85	20	C22:2 (c13,c16)
34. Methyl lignocerate	55.32	40	C24:0
35. Methyl eicosapentaenoate	56.09	20	C20:5 (c5,c8,c11,c14,c17)
36. Methyl nervonate	56.74	20	C24:1 (C15)
37. Methyl docosahexaenoate	62.17	20	C22:6 (c4,c7,c10,c13,c16,c19)

AOCS Ce 1j-07

AOCS official method Ce 1j-07 is very similar in scope and principle to AOAC 996.06 and other standard methods for GC-based determination of fat in foods. This method recommends triundecanoin, the C13:0 triglyceride, as an internal standard. Some suitable methods for extraction and methylation are referenced in the method, but no single approach is specified.

The same type of 100 m biscyanopropyl capillary GC column is recommended for AOCS Ce 1j-07, which requires baseline separation of C18:1 (*cis*-9) and C18:1 (*cis*-11). The column must also separate adjacent C18:3 and C20:1 isomers with a resolution of 1.0 or more. Figure 2 demonstrates that the Rt-2560 column meets these requirements and is suitable for AOCS Ce 1j-07, as well as other similar methods. In the AOCS method, a high initial GC oven temperature and long isothermal hold (180 °C for 32 minutes) decrease resolution for the more volatile saturated FAMEs, but may result in enhanced resolution of the C18:1 and C18:2 geometric and positional isomer clusters.

Figure 2: Application-specific QC testing ensures that all Rt-2560 columns meet AOCS Ce 1j-07 method requirements.



Peaks	t_R (min)	Conc. (µg/mL)	Structural Nomenclature
1. Methyl caproate	7.52	40	C6:0
2. Methyl octanoate	7.88	40	C8:0
3. Methyl decanoate	8.48	40	C10:0
4. Methyl undecanoate	8.93	20	C11:0
5. Methyl dodecanoate	9.51	40	C12:0
6. Methyl tridecanoate	10.27	20	C13:0
7. Methyl myristate	11.27	40	C14:0
8. Methyl myristoleate	12.48	20	C14:1 (c9)
9. Methyl pentadecanoate	12.57	20	C15:0
10. Methyl pentadecenoate	14.15	20	C15:1 (C10)
11. Methyl palmitate	14.28	60	C16:0
12. Methyl palmitoleate	15.98	20	C16:1 (c9)
13. Methyl heptadecanoate	16.51	20	C17:0
14. Methyl heptadecenoate	18.68	20	C17:1 (c10)
15. Methyl stearate	19.43	40	C18:0
16. Methyl octadecenoate	21.08	20	C18:1 (t9)
17. Methyl oleate	21.85	40	C18:1 (c9)
18. Methyl linoleaidate	24.09	20	C18:2 (t9,t12)
19. Methyl linoleate	26.14	20	C18:2 (c9,c12)
20. Methyl arachidate	28.25	40	C20:0
21. Methyl linolenate	29.98	20	C18:3 (c6,c9,c12)
22. Methyl eicosenoate	32.05	20	C20:1 (c11)
23. Methyl linolenate	32.41	20	C18:3 (c9,c12,c15)
24. Methyl heneicosanoate	33.66	20	C21:0
25. Methyl eicosadienoate	35.33	20	C20:2 (c11,c14)
26. Methyl behenate	36.64	40	C22:0
27. Methyl eicosatrienoate	37.44	20	C20:3 (c8,c11,c14)
28. Methyl erucate	38.51	20	C22:1 (c13)
29. Methyl eicosatrienoate	38.72	20	C20:3 (c11,c14,c17)
30. Methyl arachidonate	39.12	20	C20:4 (c5,c8,c11,c14,c17)
31. Methyl tricosanoate	39.74	20	C23:0
32. Methyl docosadienoate	41.64	20	C22:2 (c13,c16)
33. Methyl eicosapentaenoate	43.07	20	C20:5 (c5,c8,c11,c14,c17)
34. Methyl lignocerate	43.11	40	C24:0
35. Methyl nervonate	45.33	20	C24:1 (c15)
36. Methyl docosahexaenoate	54.02	20	C22:6 (c4,c7,c10,c13,c16,c19)

Column	Rt-2560, 100 m, 0.25 mm ID, 0.20 µm (cat.# 13198)
Sample	Food industry FAME mix (cat.# 35077)
Diluent:	Hexane/dichloromethane
Conc.:	1,000 µg/mL
Injection	
Inj. Vol.:	1 µL split (split ratio 20:1)
Liner:	Premium 4 mm Precision liner w/wool (cat.# 23305.1)
Inj. Temp.:	235 °C
Oven	
Oven Temp.:	180 °C (hold 32 min) to 215 °C at 20 °C/min (hold 31.25 min)
Carrier Gas	He, constant flow
Flow Rate:	2.0 mL/min
Detector	FID @ 325 °C
Make-up Gas Flow Rate:	45 mL/min
Make-up Gas Type:	N_2
Hydrogen flow:	30 mL/min
Air flow:	300 mL/min
Data Rate:	20 Hz
Instrument	Agilent 7890A GC
Notes	C4:0 Methyl butyrate (623-42-7) elutes in the solvent front.

Conclusion

The improved manufacturing process and application-specific quality control test both ensure that Rt-2560 columns provide a consistently high level of performance in the detailed analysis of FAMEs. The Rt-2560 column meets method requirements for standard methods of fat speciation in food, including AOAC 996.06 and AOCS Ce-1j-07. In addition, Rt-2560 columns can be used for other methods that call for a 100 m high-percentage biscyanopropyl capillary GC column.

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