Highly Reproducible Detailed *cis/trans* FAMEs Analysis Ensured by New Optimized Rt-2560 Column Manufacturing and Application-Specific QC Test

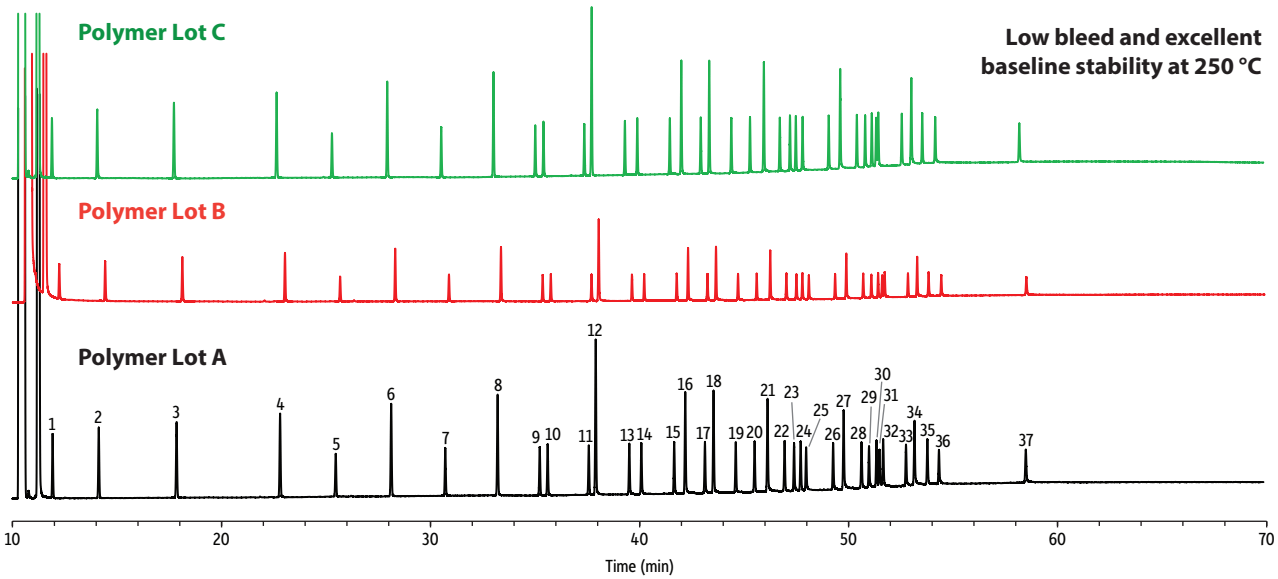
By Kristi Sellers and Rebecca Stevens

Restek's Rt-2560 GC column is commonly used for detailed analysis of fatty acid methyl esters (FAMEs) in foods; for the determination of *trans* fat content in foods and edible oils; and for profiling of fatty acids in edible oils. The column is well suited to this purpose as it contains a highly polar, nonbonded, biscyanopropyl polysiloxane stationary phase housed in a 100 m x 0.25 mm x 0.20 µm column format. The length and polarity of the column allow it to separate FAMES based on chain length and degree of unsaturation, as well as the positional and geometrical isomerism of double bonds. However, as a consequence of the column's length and high polarity, even minor variations in the manufacturing process can potentially have detrimental effects on column-to-column reproducibility. Restek has optimized the manufacturing process and implemented a new, application-specific, QC-testing procedure for all new Rt-2560 columns (cat.# 13198) in order to ensure consistent performance for detailed *cis/trans* FAMEs analysis.

Redesigned Process Produces Consistent Column Performance

The optimized manufacturing process for Rt-2560 columns results in highly consistent performance from one column to the next without affecting important features of the original column, such as selectivity, sample loading capacity, stationary phase bleed, and thermal stability. Figure 1 demonstrates the excellent column-to-column reproducibility that is typically observed across different lots of Rt-2560 polymer. In addition, low bleed levels are consistently obtained, even at the upper temperature limit of 250 °C.

Figure 1: Rt-2560 columns manufactured under Restek's optimized procedure exhibit highly reproducible column-to-column performance for detailed *cis/trans* FAMEs analysis.



GC_FF1263

Peaks	t_R (min)	Conc. (µg/mL)	Structural Nomenclature
1. Methyl butyrate	11.92	40	C4:0
2. Methyl caproate	14.13	40	C6:0
3. Methyl octanoate	17.85	40	C8:0
4. Methyl decanoate	22.80	40	C10:0
5. Methyl undecanoate	25.46	20	C11:0
6. Methyl dodecanoate	28.12	40	C12:0
7. Methyl tridecanoate	30.71	20	C13:0
8. Methyl myristate	33.21	40	C14:0
9. Methyl myristoleate	35.22	20	C14:1 (c9)
10. Methyl pentadecanoate	35.60	20	C15:0
11. Methyl pentadecenoate	37.57	20	C15:1 (c10)
12. Methyl palmitate	37.90	60	C16:0
13. Methyl palmitoleate	39.51	20	C16:1 (c9)
14. Methyl heptadecanoate	40.09	20	C17:0
15. Methyl heptadecenoate	41.66	20	C17:1 (c10)
16. Methyl stearate	42.19	40	C18:0
17. Methyl octadecenoate	43.13	20	C18:1 (t9)
18. Methyl oleate	43.54	40	C18:1 (c9)
19. Methyl linoleaidate	44.60	20	C18:2 (t9,t12)
20. Methyl linoleate	45.50	20	C18:2 (c9,c12)
21. Methyl arachidate	46.12	40	C20:0
22. Methyl linolenate	46.94	20	C18:3 (c6,c9,c12)
23. Methyl eicosenoate	47.39	20	C20:1 (c11)
24. Methyl linolenate	47.71	20	C18:3 (c9,c12,c15)
25. Methyl heneicosanoate	47.97	20	C21:0
26. Methyl eicosadienoate	49.26	20	C20:2 (c11,c14)
27. Methyl behenate	49.77	40	C22:0
28. Methyl eicosatrienoate	50.62	20	C20:3 (c8,c11,c14)
29. Methyl erucate	50.98	20	C22:1 (c13)
30. Methyl eicosatrienoate	51.34	20	C20:3 (c11,c14,c17)
31. Methyl tricosanoate	51.48	20	C23:0
32. Methyl arachidonate	51.66	20	C20:4 (c5,c8,c11,c14)
33. Methyl docosadienoate	52.75	20	C22:2 (c13,c16)
34. Methyl lignocerate	53.16	40	C24:0
35. Methyl eicosapentaenoate	53.77	20	C20:5 (c5,c8,c11,c14,c17)
36. Methyl nervonate	54.33	20	C24:1 (C15)
37. Methyl docosahexaenoate	58.48	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.:	225 °C
Oven	
Oven Temp.:	100 °C (hold 4 min) to 250 °C at 3 °C/min (hold 30 min)
Carrier Gas	He, constant flow
Flow Rate:	1.3 mL/min
Detector	FID @ 285 °C
Make-up Gas Flow Rate:	30 mL/min
Make-up Gas Type:	N_2
Hydrogen flow:	30 mL/min
Air flow:	300 mL/min
Data Rate:	50 Hz
Instrument	Agilent 7890B GC

New QC Test Ensures High-Quality, Detailed *cis/trans* FAMEs Analysis

In order to verify that the optimized process and original process both produce Rt-2560 columns of equivalent performance, a new application-specific QC test was designed and stringent specifications were set. The test mix used in the new QC test contains both saturated and unsaturated FAMEs that are commonly encountered in fats and oils analysis. Because every column manufactured under the improved process is tested, only high-performance columns suitable for detailed *cis/trans* FAMEs analysis are certified for sale. Table I lists the components of the test mix and some of the chromatographic performance metrics that are calculated for each. Stationary phase bleed is measured at 250 °C following elution of the FAMEs.

Table I: Application-specific components in the new QC test are used to verify the performance of Rt-2560 columns (cat.# 13198) by measuring critical chromatographic performance metrics. Components are listed in elution order.

Test Mix Component	Structural Nomenclature	Performance Metric
Methyl octadecanoate	C18:0	
Methyl elaidate	C18:1-t9	Retention index
Methyl oleate	C18:1-c9	
Methyl nonadecanoate	C19:0	
Methyl eicosanoate	C20:0	Retention factor
Methyl eicosenoate	C20:1-c11	Resolution with methyl linolenate
Methyl linolenate	C18:3-c9,c12,c15	Retention index
Methyl heneicosanoate	C21:0	
Methyl docosanoate	C22:0	Retention factor and efficiency
Methyl eicosatrienoate	C20:3-c11,c14,c17	Retention index
Methyl tricosanoate	C23:0	

The retention index values measured for the series of unsaturated FAMES are used to characterize stationary phase selectivity and confirm that each column exhibits the same elution pattern and separation capabilities. These indices are measured using the bracketing provided by homologous saturated FAMEs, rather than by *n*-alkanes, in order to avoid potential interfacial adsorption effects on the highly polar stationary phase.

Efficiency and retention factor measurements are used to verify proper stationary phase deposition and consistent separation performance. Columns with low efficiency will show significantly reduced performance in the speciation of *trans* fat, particularly in the separation of the complex C18:1 region, and in the detailed profiling of unsaturated fatty acids.

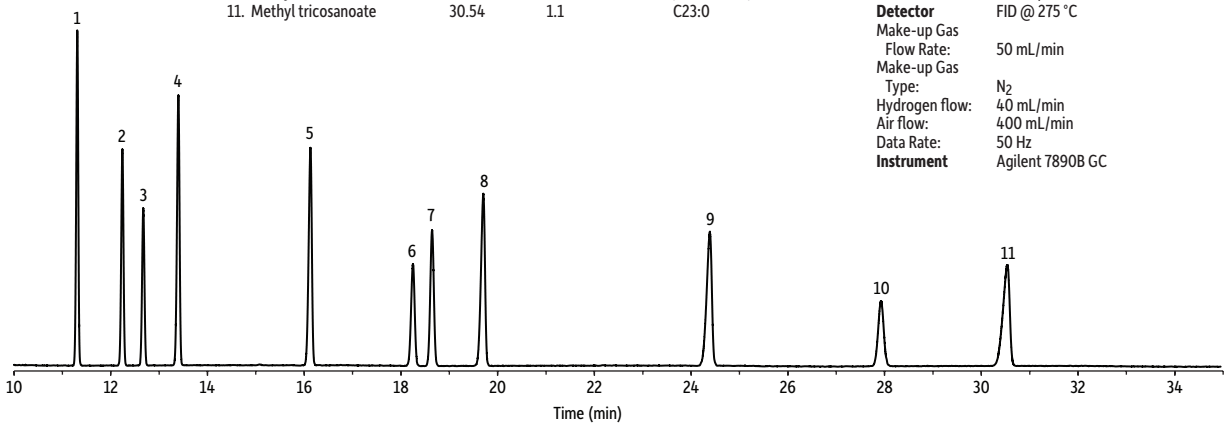
The baseline resolution of methyl eicosenoate (C20:1-c11) and methyl linolenate (C18:3-c9,c12,c15) is a critical separation required by standard methods, such as AOAC 996.06 and AOCS Ce 1j-07, in order to demonstrate system suitability. Using an optimal column and standard conditions, C18:3-c9, c12, c15 elutes after C20:1-c11 with baseline resolution. If these conditions are met, peaks eluting between C20:0 and C20:1-c11 can be tentatively identified as C18:3 isomers containing at least one *trans* double bond. In Figure 2, the new QC test is analyzed on columns manufactured using the original and the optimized procedures. This comparison demonstrates that equivalent separation characteristics are obtained both before and after process improvement. In addition, Figure 2 shows baseline resolution of C20:1-c11 and C18:3-c9, c12, c15.

Figure 2: Analysis of the FAMES QC test mix confirms that Rt-2560 columns from before and after the manufacturing process redesign show equivalent separation characteristics. Baseline resolution of C20:1-c11 and C18:3-c9, c12, c15 as well as *cis* and *trans* C18:1-9 isomers is obtained on both columns.

Before Redesign

Peaks	t_R (min)	Conc. (mg/mL)	Structural Nomenclature
1. Methyl octadecanoate	11.31	1.1	C18:0
2. Methyl elaidate	12.24	0.8	C18:1 (t9)
3. Methyl oleate	12.67	0.6	C18:1 (c9)
4. Methyl nonadecanoate	13.40	1.1	C19:0
5. Methyl eicosanoate	16.13	1.1	C20:0
6. Methyl eicosenoate	18.25	0.6	C20:1 (c11)
7. Methyl linolenate	18.64	0.8	C18:3 (c9,c12,c15)
8. Methyl heneicosanoate	19.70	1.1	C21:0
9. Methyl docosanoate	24.39	1.1	C22:0
10. Methyl eicosatrienoate	27.93	0.6	C20:3 (c11,c14,c17)
11. Methyl tricosanoate	30.54	1.1	C23:0

Column	Rt-2560, 100 m, 0.25 mm ID, 0.20 µm (cat.# 13199)
Sample	Unsaturated FAMES QC test mix
Diluent:	Hexane
Injection	
Inj. Vol.:	1 µL split (split ratio 200:1)
Liner:	Premium 4 mm Precision liner w/wool (cat.# 23305.1)
Inj. Temp.:	250 °C
Oven	
Oven Temp.:	180 °C (hold 40 min)
Carrier Gas	H_2, constant flow
Flow Rate:	2.5 mL/min
Detector	FID @ 275 °C
Make-up Gas Flow Rate:	50 mL/min
Make-up Gas Type:	N_2
Hydrogen flow:	40 mL/min
Air flow:	400 mL/min
Data Rate:	50 Hz
Instrument	Agilent 7890B GC

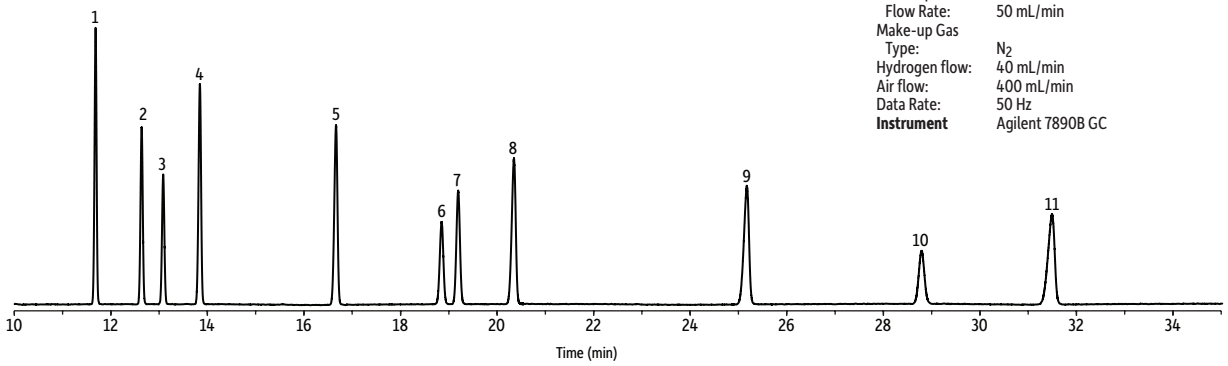


GC_EX1134

After Redesign

Peaks	t_R (min)	Conc. (mg/mL)	Structural Nomenclature
1. Methyl octadecanoate	11.68	1.1	C18:0
2. Methyl elaidate	12.64	0.8	C18:1 (t9)
3. Methyl oleate	13.09	0.6	C18:1 (c9)
4. Methyl nonadecanoate	13.84	1.1	C19:0
5. Methyl eicosanoate	16.66	1.1	C20:0
6. Methyl eicosenoate	18.85	0.6	C20:1 (c11)
7. Methyl linolenate	19.20	0.8	C18:3 (c9,c12,c15)
8. Methyl heneicosanoate	20.35	1.1	C21:0
9. Methyl docosanoate	25.17	1.1	C22:0
10. Methyl eicosatrienoate	28.79	0.6	C20:3 (c11,c14,c17)
11. Methyl tricosanoate	31.49	1.1	C23:0

Column	Rt-2560, 100 m, 0.25 mm ID, 0.20 µm (cat.# 13198)
Sample	Unsaturated FAMES QC test mix
Diluent:	Hexane
Injection	
Inj. Vol.:	1 µL split (split ratio 200:1)
Liner:	Premium 4 mm Precision liner w/wool (cat.# 23305.1)
Inj. Temp.:	250 °C
Oven	
Oven Temp.:	180 °C (hold 40 min)
Carrier Gas	H_2, constant flow
Flow Rate:	2.5 mL/min
Detector	FID @ 275 °C
Make-up Gas Flow Rate:	50 mL/min
Make-up Gas Type:	N_2
Hydrogen flow:	40 mL/min
Air flow:	400 mL/min
Data Rate:	50 Hz
Instrument	Agilent 7890B GC



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Conclusion

The improved manufacturing process and application-specific QC test both ensure that Rt-2560 columns provide a consistently high level of performance for detailed *cis/trans* FAMEs analysis and are suitable for standard methods of fat speciation in food, such as AOAC 996.06 and AOCS Ce 1j-07. Rt-2560 columns produced using the optimized process exhibit the same selectivity expected from a high cyanopropyl column with little column-to-column variability, while the new QC test ensures a high-quality product that meets method performance requirements.

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Lit. Cat.# FFAR2589-UNV