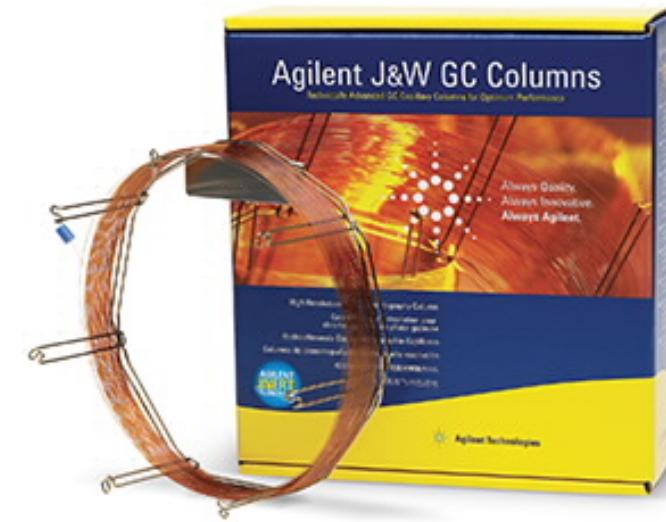


New GC Column Technology for Old Problems in Fatty Acid Analysis

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Global Product Manager – GC Columns
December 15, 2020



What Are We Testing?

Fat profiles:

- Total Fat
- Saturated Fat
- Monosaturated Fat
- Trans Fat

Free fatty acids

Triglycerides

Omega 3 and 6 fatty acids



Markets Where FAMEs in Food are Analyzed

Food/beverage testing labs

Food processing/Mfg

Fats and oil producers

- Nutritional label testing/authenticity
- QA/QC analysis, Regulatory methods
 - AOCS
 - AOAC



- Edible oils/dairy
- R&D, QA/QC, production

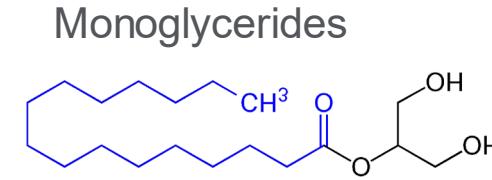
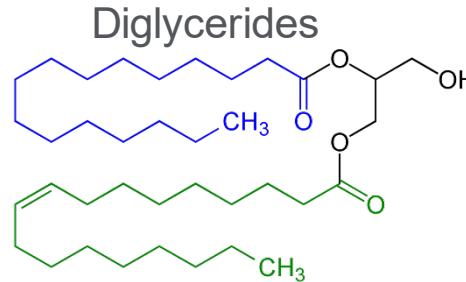
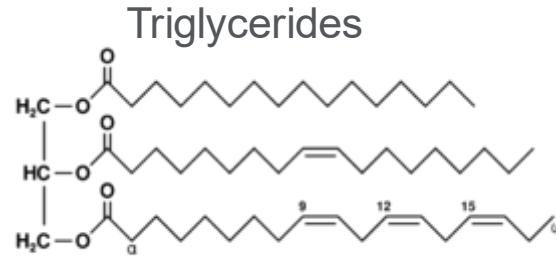
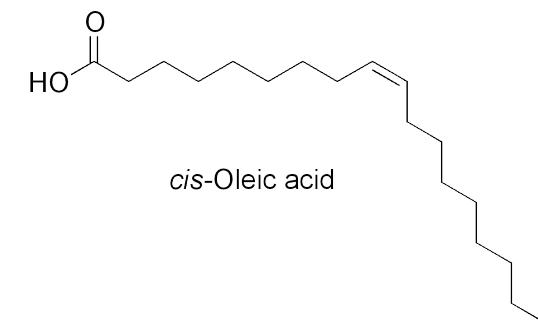
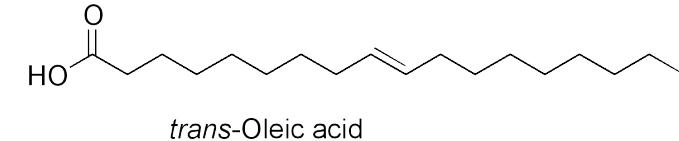
- Omega 3 and 6 supplements
- QA/QC, process control



Terminology

Fats, oils, FAMEs, triglycerides, fatty acids

- Fatty acids: carboxylic acid with long aliphatic chain (C4 – C28)
- Vegetable oils: triglycerides from plants (seeds and nuts)
mono and diglycerides in minor amounts



Fats = Triglycerides (animal or plant origin)

FAMEs: **fatty acid methyl esters** derived by transesterification of fats with methanol (in presence base)

FAEEs: fatty acid ethyl esters, naturally occurring in alcoholic beverages

Structures of fats and oils

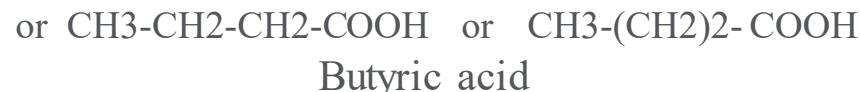
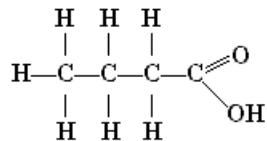
A triglyceride is called a fat if it is a solid at 25 °C; it is called an oil if it is a liquid at that temperature. These differences in melting points reflect differences in the degree of unsaturation and number of carbon atoms in the constituent fatty acids.

What Type of Fatty Acids are There?

Fatty acids are long-chain hydrocarbons that can be separated into three categories: saturated, monounsaturated (MUFA), and polyunsaturated (PUFAs).

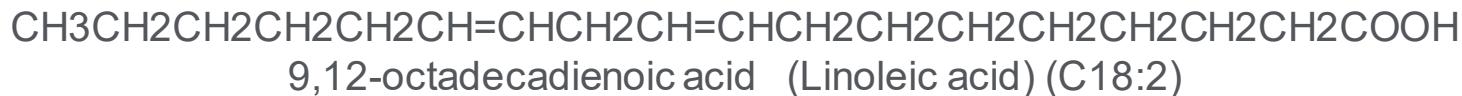
Saturated fatty acids (SFAs):

Have all the hydrogen that the carbon atoms can hold
and, therefore, have no double bonds between carbon atoms.



Monounsaturated fatty acids (MUFAs): Have only one double bond

Polyunsaturated fatty acids (PUFAs): Have more than one double bond



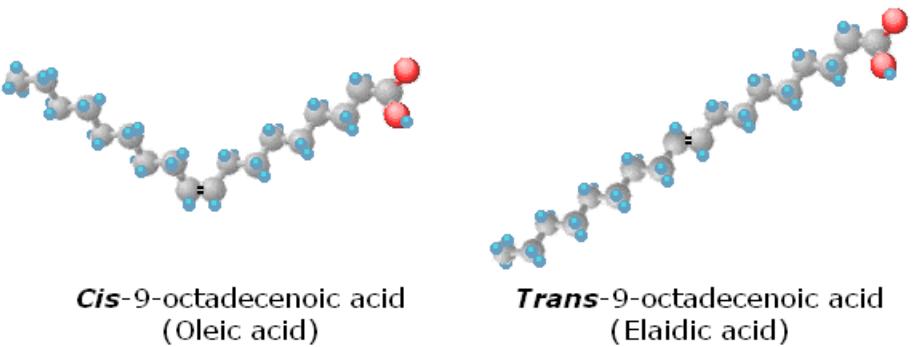
Which would be abbreviated as:
 $\text{CH}_3(\text{CH}_2)_4\text{CH=CHCH}_2\text{CH=CH}(\text{CH}_2)_7\text{COOH}$

Fatty Acid Configurations

Fatty Acid Configurations

What are *Trans* Fats?

Double bonds bind carbon atoms tightly and prevent rotation of the carbon atoms along the bond axis. This gives rise to *configurational isomers* which are arrangements of atoms that can only be changed by breaking the bonds.



These three-dimensional molecular projections show the *Cis* and *Trans* configurational isomers of 9-octadecenoic acid with the hydrogen atoms shown in blue. The Latin prefixes *Cis* and *Trans* describe the orientation of the hydrogen atoms with respect to the double bond. *Cis* means "on the same side" and *Trans* means "across" or "on the other side". Naturally occurring fatty acids generally have the *Cis* configuration. The natural form of 9-octadecenoic acid (oleic acid) found in olive oil has a "V" shape due to the *Cis* configuration at position 9. The *Trans* configuration (elaidic acid) looks more like a straight line.



Fatty acids: Positional isomers – which position in the chain are the double bonds in
Geometrical isomers – what is the configuration of the bond (cis or trans)

Trans fats

Most trans fats are manufactured through a process called hydrogenation, which is the artificial addition of hydrogen atoms to unsaturated oils.

Hydrogenation converts liquid vegetable oils to solid or semisolid fats that remain stable at room temperature.

These fats can then be incorporated into certain food products (for example, cookies, chips) to increase shelf life.

Fatty Acid Composition of Palm Oil

The major fatty acids in palm oil are oleic acid, palmitic acid, and linoleic acid.

Composition	Content (mg/g) ^a	Composition	Content (mg/g)	Composition	Content (mg/g)
C12:0	2.06 ± 0.03	cis C18:1	336.98 ± 9.34	11c C20:1	0.33 ± 0.01
C14:0	9.97 ± 0.24	9c 12t C18:2	0.92 ± 0.07	9c12c15cC18:3	1.63 ± 0.05
C15:0	0.43 ± 0.02	9t 12c C18:2	0.79 ± 0.07	trans C18:1 ^d	ND ^b
C16:0	465.49 ± 11.08	9c12c C18:2	68.85 ± 2.66	trans C18:2 ^e	1.71 ± 0.02
9cC16:1	1.12 ± 0.01	C20:0	1.65 ± 0.04	trans C18:3 ^f	ND
C17:0	0.80 ± 0.01	ctt/cct C18:3 ^c	ND	C18:2/C16:0	0.1479
C18:0	37.86 ± 0.76	9t 12c15c C18:3	ND	C18:3/C16:0	0.0035

Means of duplicate analyses ± standard deviation

^b ND: not detected

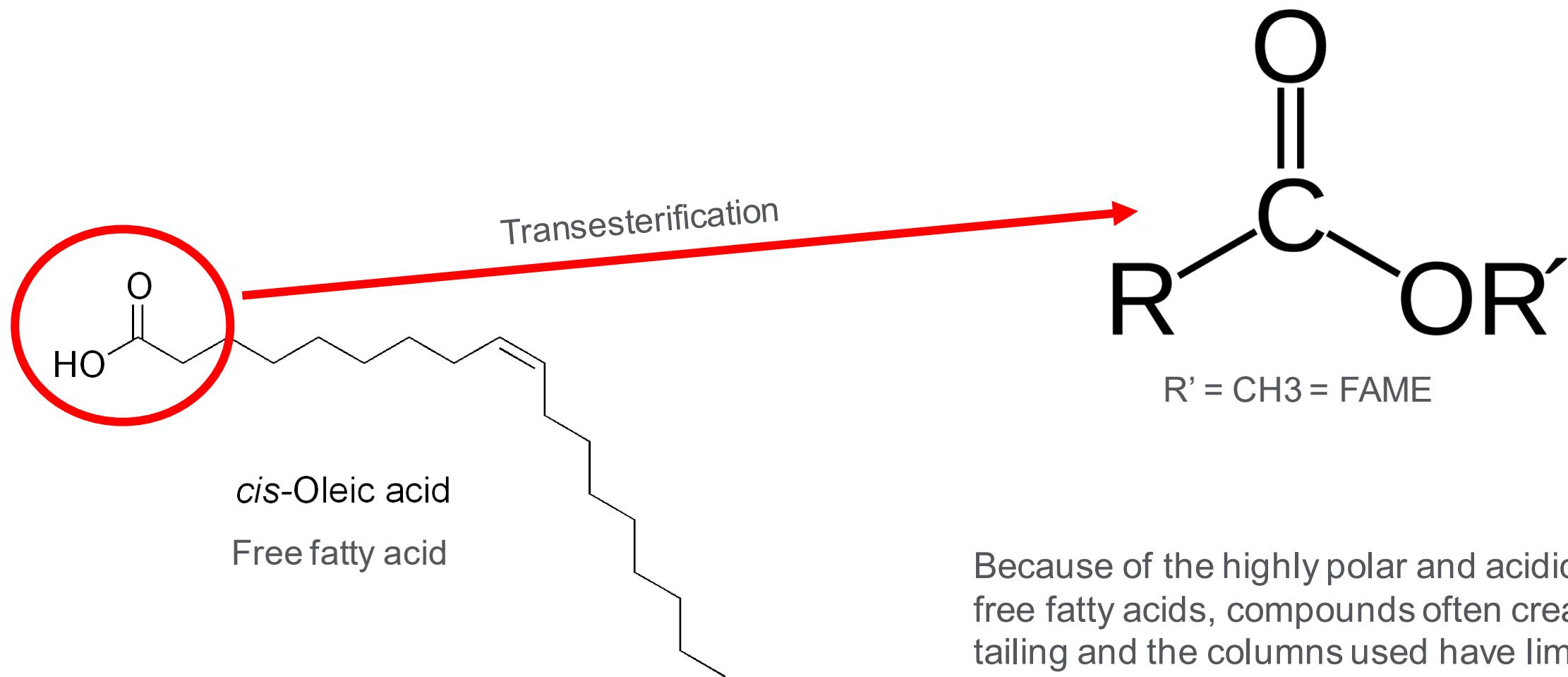
^c Total contents of 9c 12t 15t C18:3 and 9c12c 15t C18:3

^d Total contents of 9t C18:1 and 11t C18:1

^e Total contents of 9c 12t C18:2 and 9t 12c C18:2

^f Total contents of 9c 12t 15t C18:3, 9c12c 15t C18:3 and 9t 12c15c C18:3

Why Do We Analyze FAMEs?



Because of the highly polar and acidic end of free fatty acids, compounds often create tailing and the columns used have limited lifetime. A lengthy transesterification process taking 4 to 6 hours may be required.

Free fatty acids can be an analytical challenge

Basic Application Overview

Sample preparation

Separation

Detection

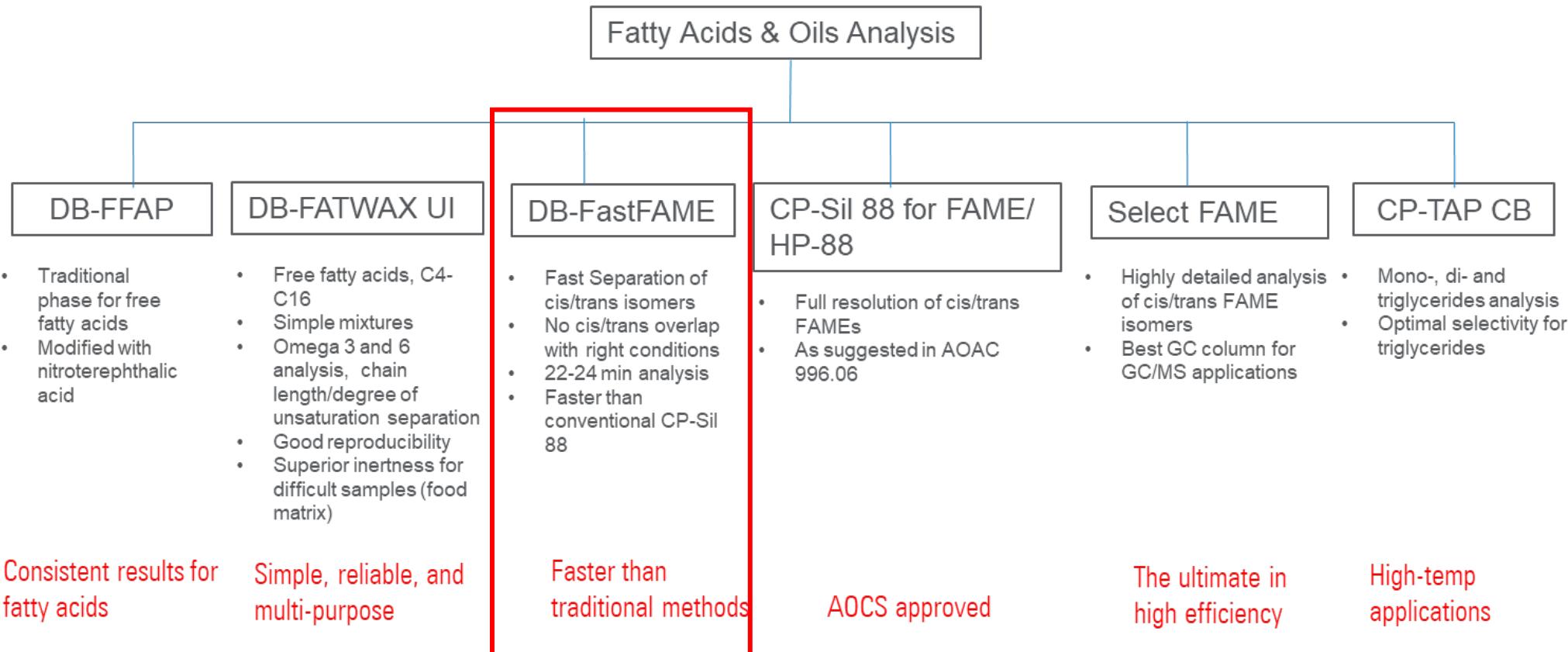
AOAC/AOCS regulatory methods using acid/basic hydrolysis and a possible methylation to form FAMEs

DB-FATWAX UI which offers superior inertness for free fatty acid analysis and the **DB-FastFAME**, designed to offer faster analysis times and improve throughput.

These fatty acid/FAME molecules can have the same mass but have different structures. MS cannot tell them apart.
FID > MS

The critical step in the analysis of FAMEs that will ensure consistent results is the GC column

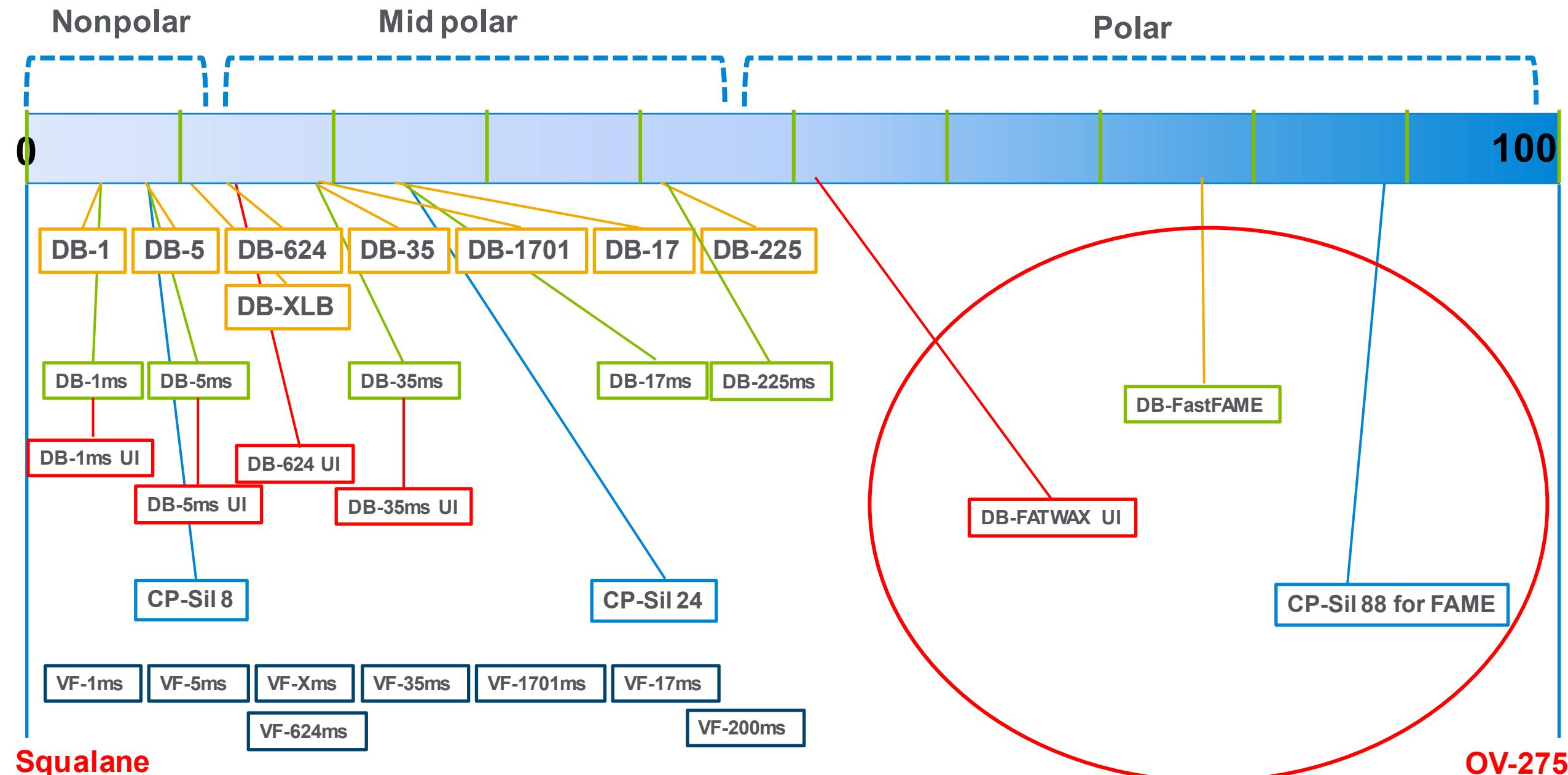
Comprehensive Portfolio for Fatty Acids, FAME, and Oils Analysis



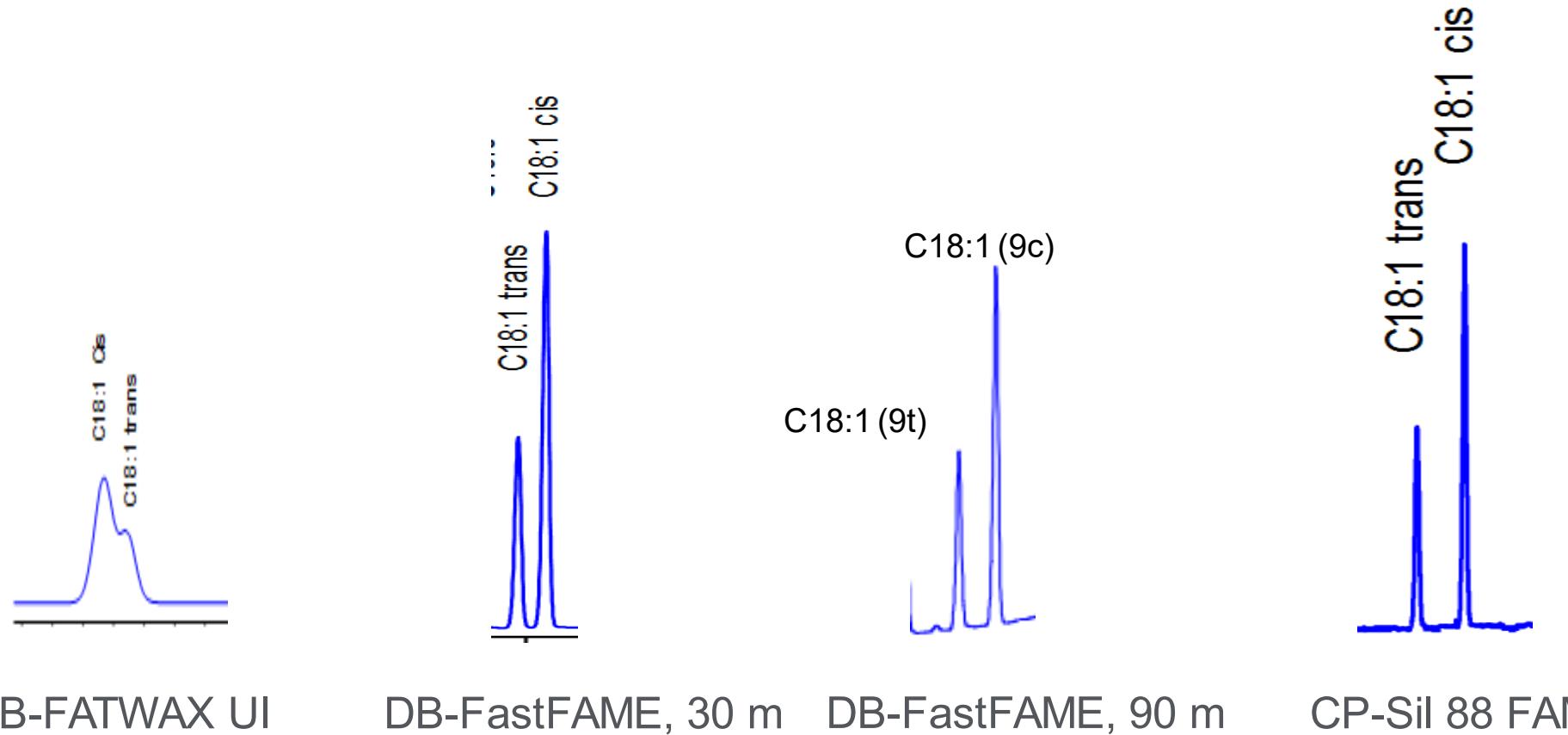
FFA

FAME

TG



Column Polarity/Interaction Effects – FAMEs



Traditional GC Columns

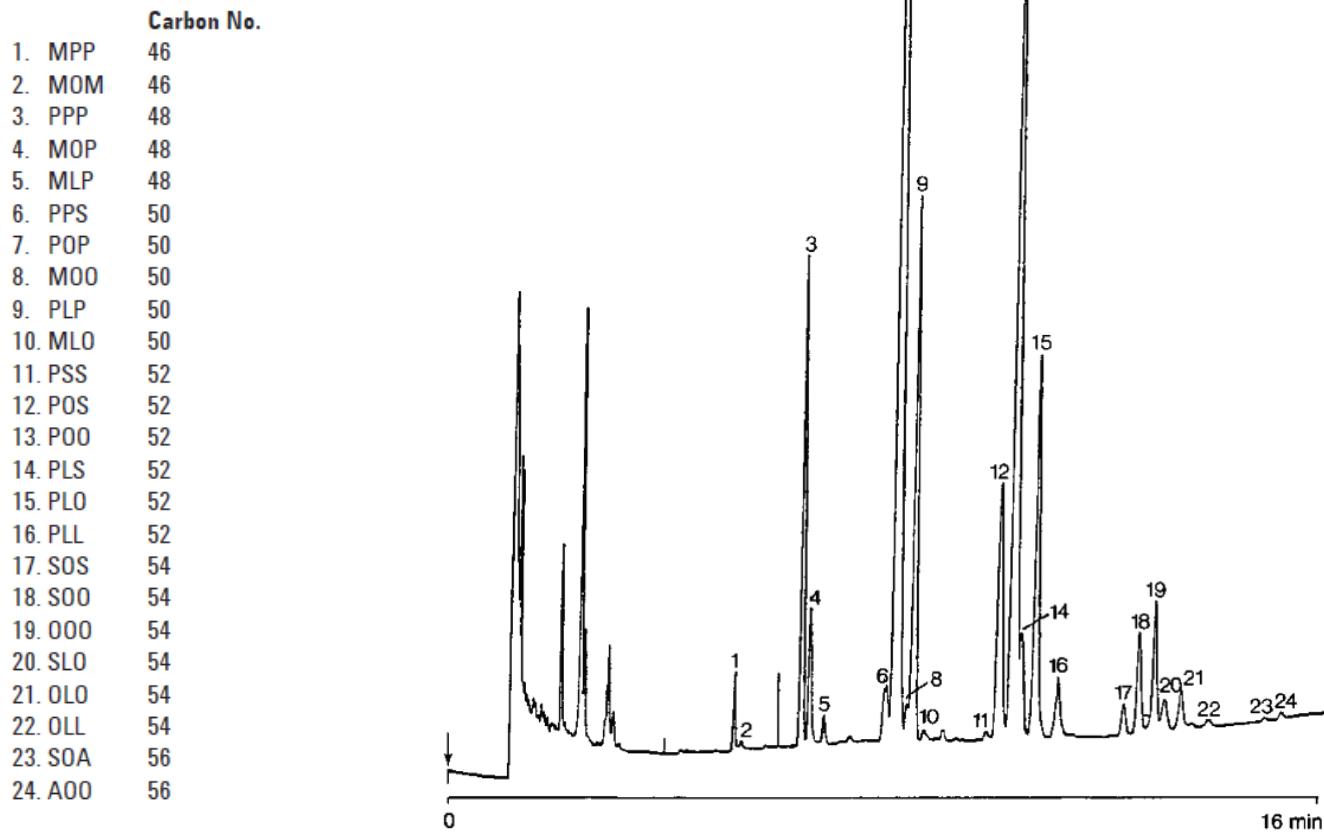
For fatty acids, FAMEs and triglycerides

Triglycerides Analysis of Palm Oil

CP-TAP CB for triglycerides

Column CP-TAP CB, 25 m, 0.25 mm, 0.10 µm (p/n CP7483)
Inlet Oncolumn
Carrier Hydrogen, 100 kPa (1 bar, 15 psi)
Oven 340 °C (1 min) to 355 °C at 1 °C/min
Detector FID
Injection 0.2 µL
Sample 0.05% palm oil in hexane

M: Myristic acid (tetradecanoic acid) C14:0
P: Palmitic acid, (hexadecanoic acid) C16:0
S: Stearic acid (octadecanoic acid) C18:0
O: Oleic acid (cis-9-octadecenoic acid) C18:1
L: Linoleic acid (cis,cis-9,12,octadecadienoic acid) C18:2
A: Arachidic acid (eicosanoic acid) C20:0



Application note: A00195

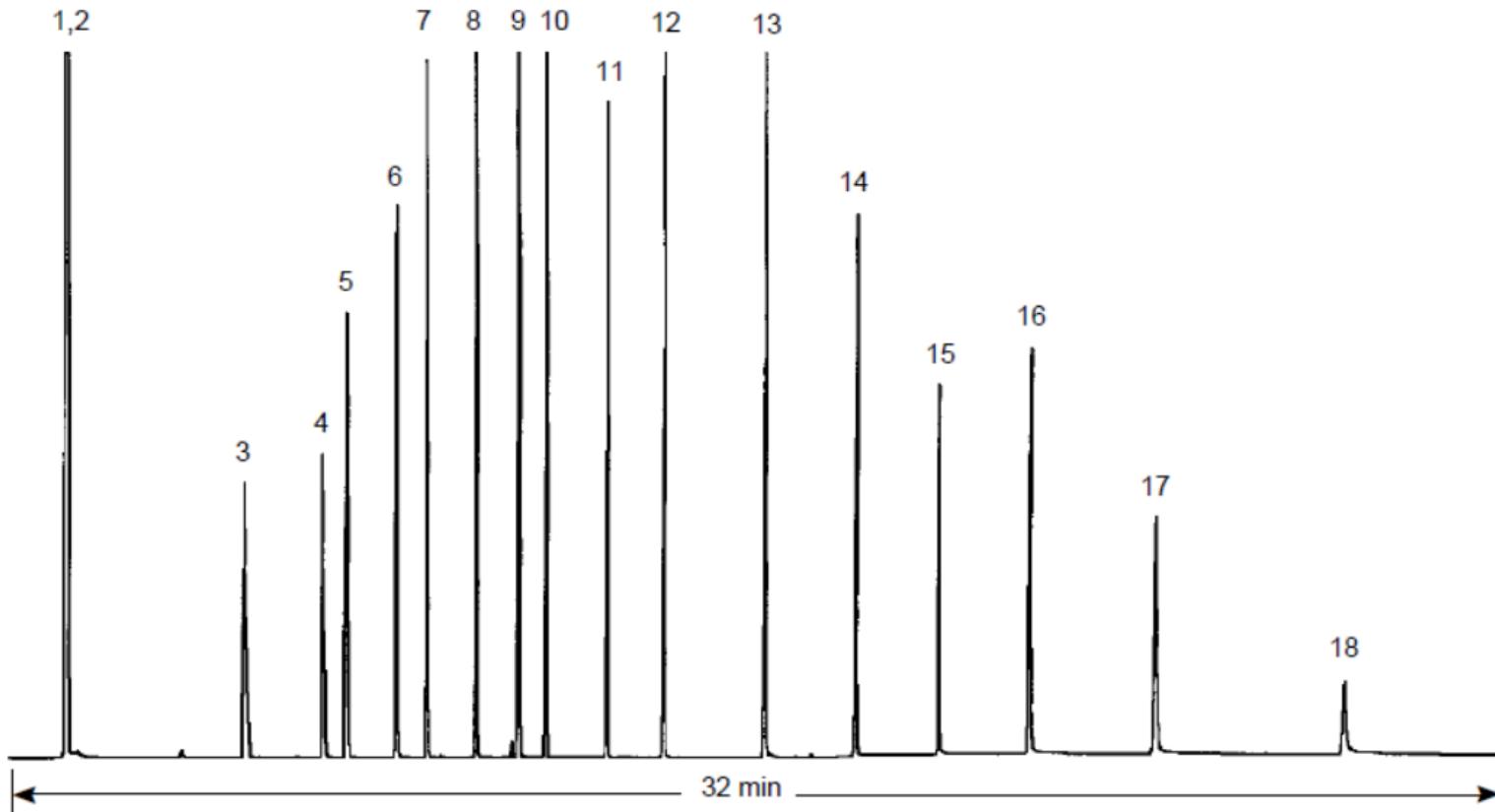
Analysis of Free Fatty Acids

Column DB-FFAP, 30 m, 0.25 mm, 0.25 µm (p/n 122-3232)
Carrier Helium at 40 cm/s, measured at 100 °C
Oven 100 °C (5 min) to 250 °C (12 min) at 10 °C/min
Detector FID, 300 °C
Injector Nitrogen makeup gas at 30 mL/min
Split 1:50, 250 °C

Acid-based WAX: DB-FFAP

Chromatogram C077

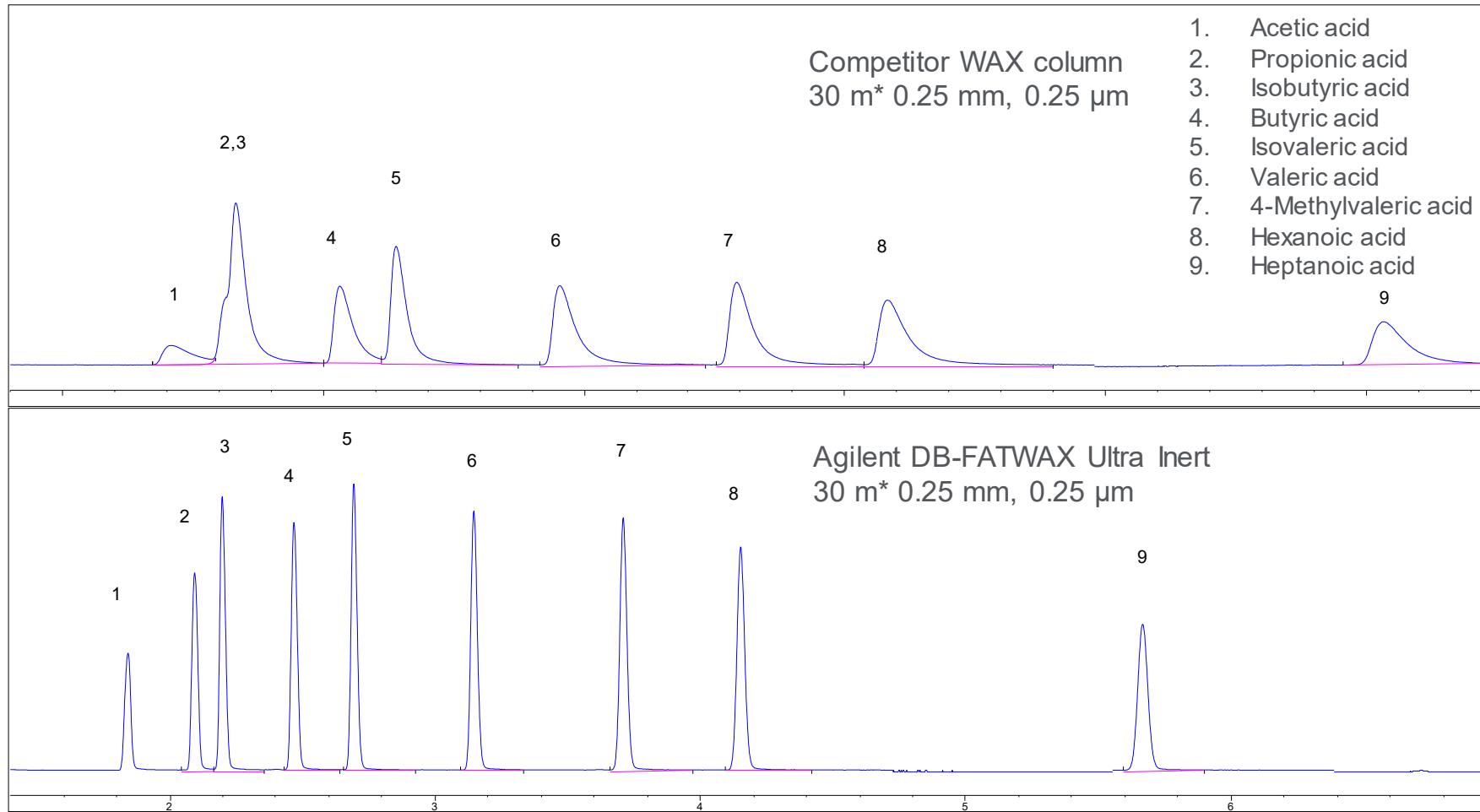
- Peak Number
- 1. Acetone
 - 2. Formic acid
 - 3. Acetic acid
 - 4. Propionic acid
 - 5. Isobutyric acid
 - 6. Butyric acid
 - 7. Isovaleric acid
 - 8. Valeric acid
 - 9. Isocaproic acid
 - 10. Caproic acid
 - 11. Heptanoic acid
 - 12. Octanoic acid
 - 13. Decanoic acid
 - 14. Dodecanoic acid
 - 15. Tetradecanoic acid
 - 16. Hexadecanoic acid
 - 17. Octadecanoic acid
 - 18. Arachidic acid



Separation of Short-Chain Volatile Organic Acids in Water

Using a traditional WAX column and DB-FATWAX Ultra Inert

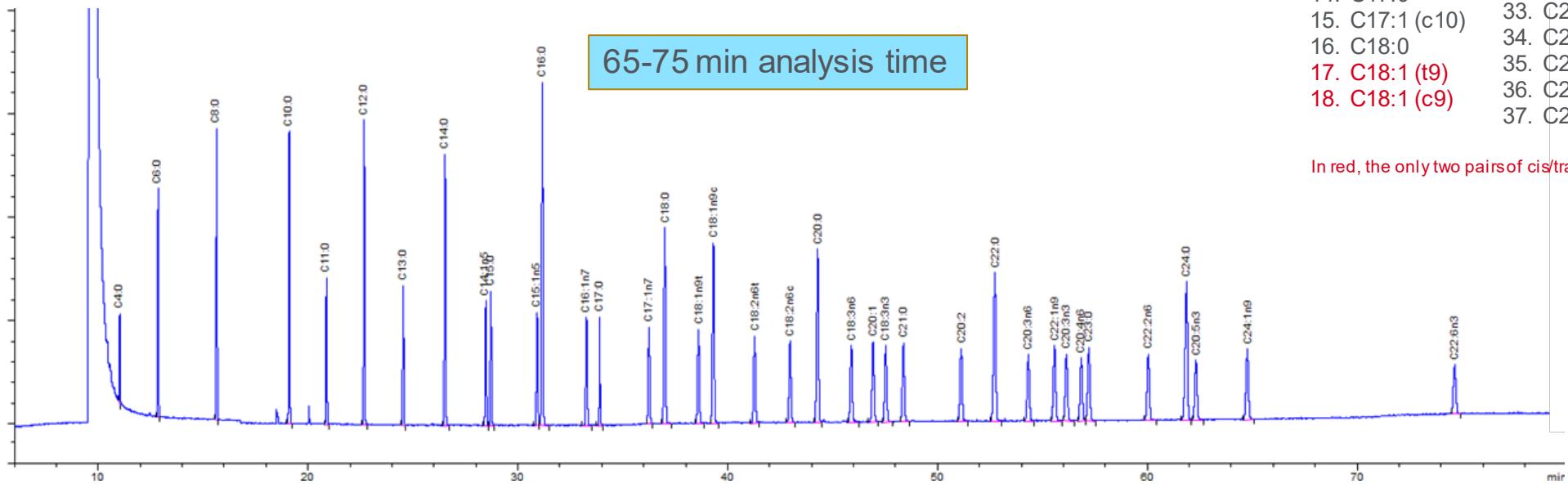
Standard WAX columns don't have the inertness to separate most organic acids



Traditional FAME Analysis on a CP-Sil 88

- Most common method, suggested by AOAC 996.06 and AOCS Ce 1j-07
- Cyanopropyl columns (100 m) are optimized for positional isomer separation of critical cis/trans FAMEs
- Analysis time is typically in the 65 to 76 minute range

FAMES by AOAC 996.06 using traditional 100 m cyanopropylphase



Peak Number

1. C4:0
2. C6:0
3. C8:0
4. C10:0
5. C11:0
6. C12:0
7. C13:0
8. C14:0
9. C14:1 (c9)
10. C15:0
11. C15:1
12. C16:0
13. C16:1 (c9)
14. C17:0
15. C17:1 (c10)
16. C18:0
17. C18:1 (t9)
18. C18:1 (c9)
19. C18:2 (t9, t12)
20. C18:2 (c9, c12)
21. C20:0
22. C18:3 (c6,c9,c12)
23. C20:1 (c11)
24. C18:3 (c9, c12, c15)
25. C21:0
26. C20:2 (c11, c14)
27. C22:0
28. C20:3 (c8, c11, c14)
29. C22:1 (c13)
30. C20:3 (c11, c14, c17)
31. C23:0
32. C20:4 (c5,c8,c11,c14)
33. C22:2 (c13, c16)
34. C24:0
35. C20:5 (c5,c8,c11,c14,c17)
36. C24:1 (c15)
37. C22:6 (c4,c7,c10,c13,c16,c19)

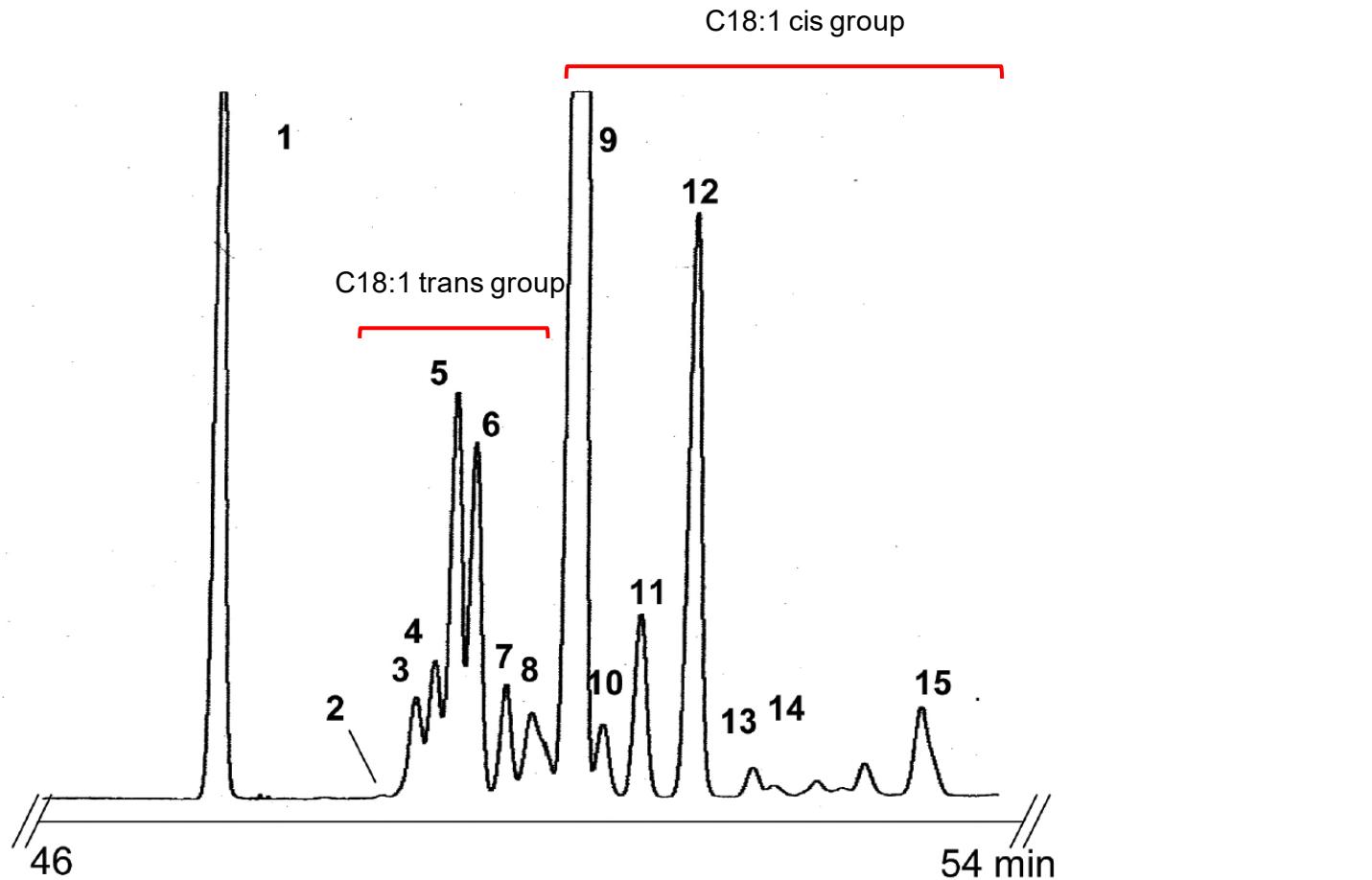
Detailed Analysis of Cis/Trans FAMEs C18:1 Positional Isomers

Column **Select FAME**, 200 m, 0.25 mm id (p/n CP7421)
Inlet 250 °C, split mode, split ratio 1:20
Carrier Helium, 520 kPa
Oven 185 °C
FID 250 °C
Injection 0.5 µL

Select-FAME

Peak Number

1. C18:0
2. C18:1 7 trans
3. C18:1 8 trans
4. C18:1 9 trans
5. C18:1 10 trans
6. C18:1 11 trans
7. C18:1 12 trans
8. C18:1 trans + ?
9. C18:1 9 cis
10. C18:1 10 cis
11. C18:1 11 cis
12. C18:1 12 cis
13. C18:1 13 cis
14. C18:1 14 cis
15. C18:1 15 cis

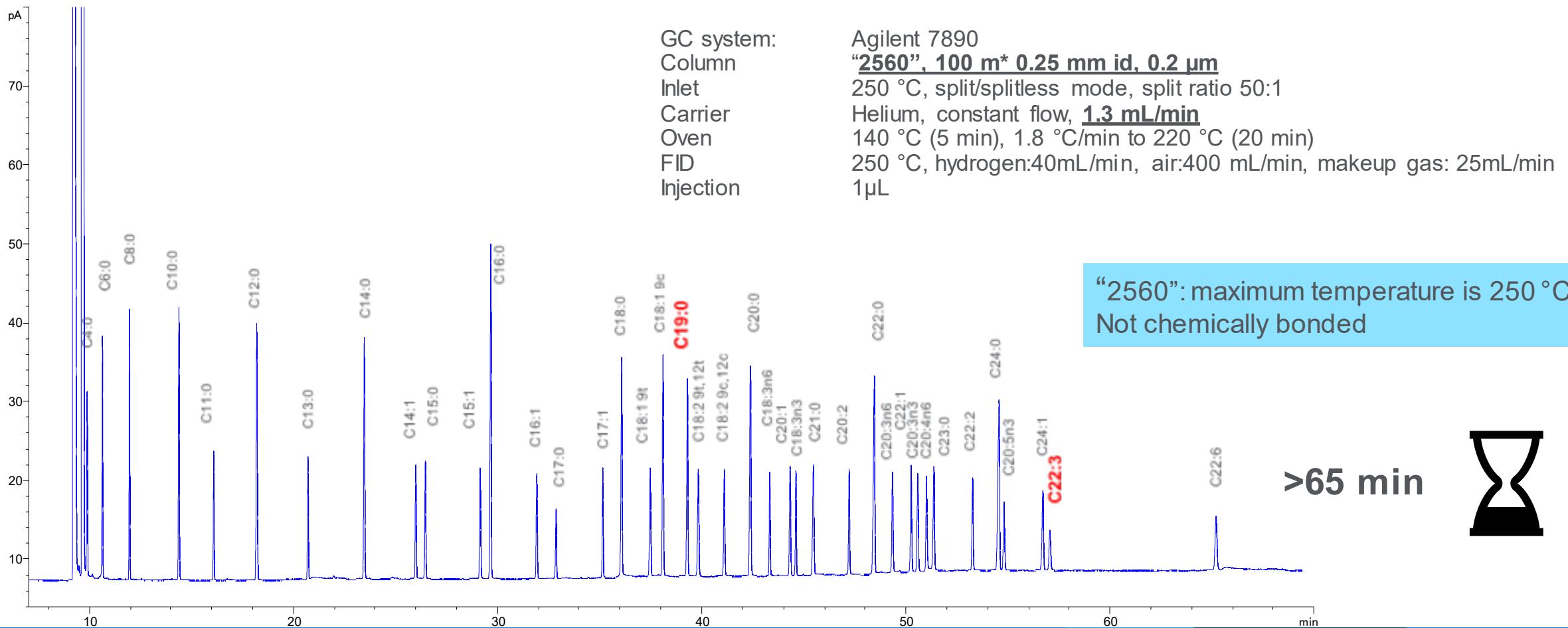


Introducing High-Resolution DB-FastFAME

- Fast separation of cis/trans isomers
 - No cis/trans overlap with right conditions
 - 22 to 24 minute analysis (<37 FAMES)
-
- Faster than conventional 88-type columns
 - Improve throughput
 - Reduced column cost
 - Reduced run time
 - Improved column longevity/durability

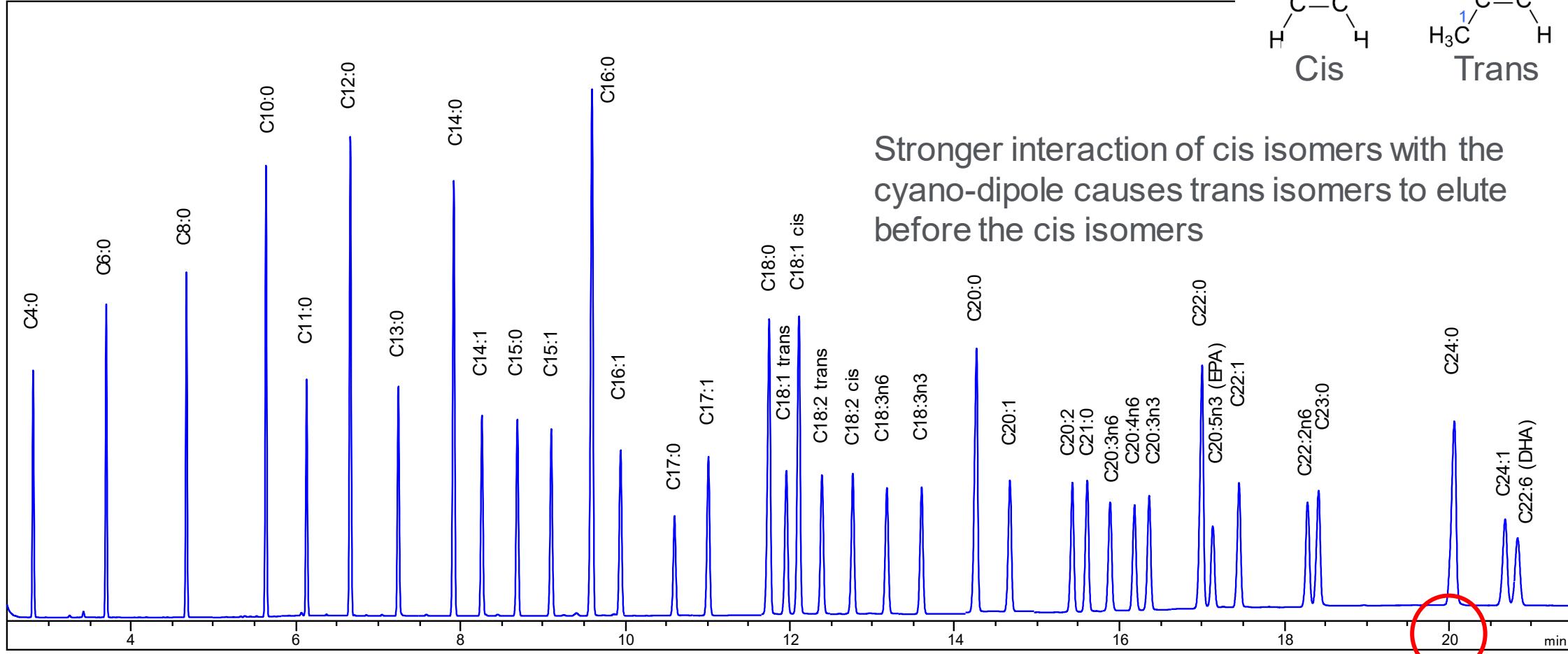
Traditional FAME Analysis on “2560” Column

- 37-FAME mix plus C19:0 and C22:3
- Analysis time is typically in the 65 to 76 min range, following AOAC 996.02 method
- Cyanopropyl column (100 m) is the ‘go-to’ column for positional cis/trans isomers



DB-FastFAME (30 m x 0.25 mm x 0.25 µm)

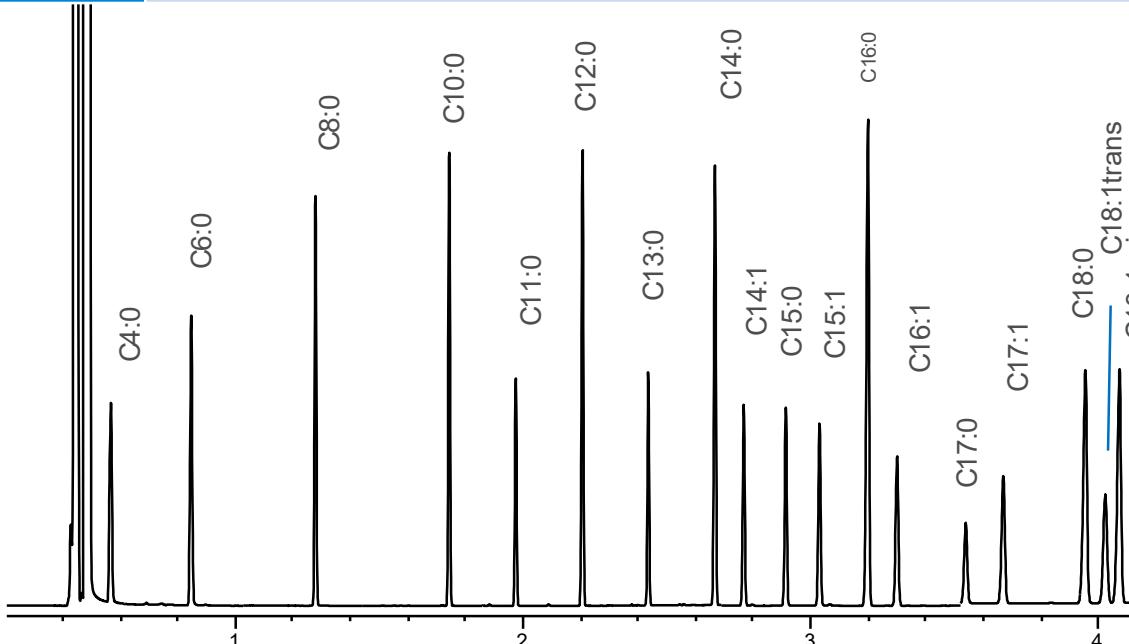
Technical note 5991-8706EN



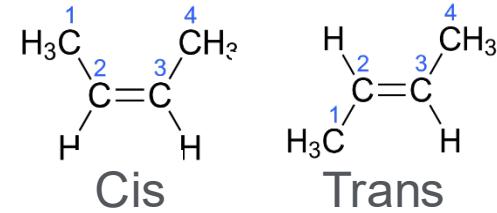
DB-FastFAME

20 m x 0.18 mm x 0.20 μm

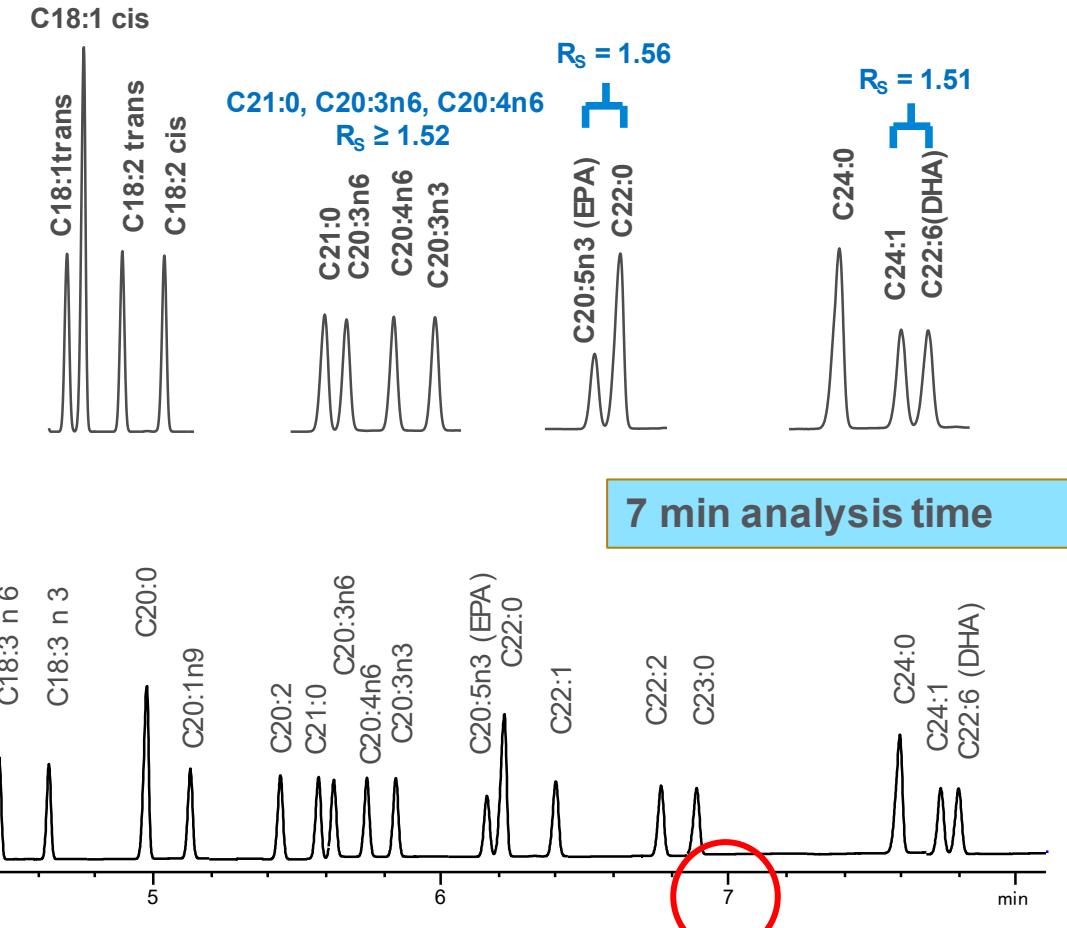
Column	Agilent J&W DB-FastFAME, 20 m x 0.18 mm, 0.20 μm
Gas	Hydrogen, 28 psi, constant pressure mode
Inlet	Split/splitless, 250 °C, split ratio 50:1
Oven	80 °C (0.5 min), 65 °C/min to 175 °C, 10 °C/min to 185 °C (0.5 min), 7 °C/min to 230 °C
FID	280 °C, hydrogen: 40 mL/min; air: 400 mL/min; makeup gas: 25 mL/min.
Injection	1 μL



Strong interaction between cis isomers and the dipoles of the cyanopropyl ligands. This allows the trans to elute after the cis isomers.



$R_s \geq 1.95$ for cis/trans isomers



7 min analysis time

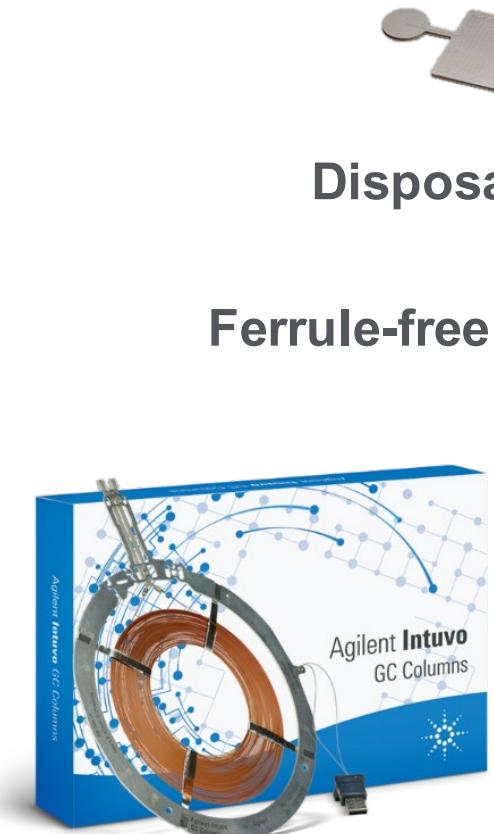
Agilent Intuvo 9000

- New design
- New column connection
- Guard chip
- New column heating
- Application to FAME analysis



Intelligent, intuitive, innovative. Intuvo.

Innovating a New Path to GC Productivity

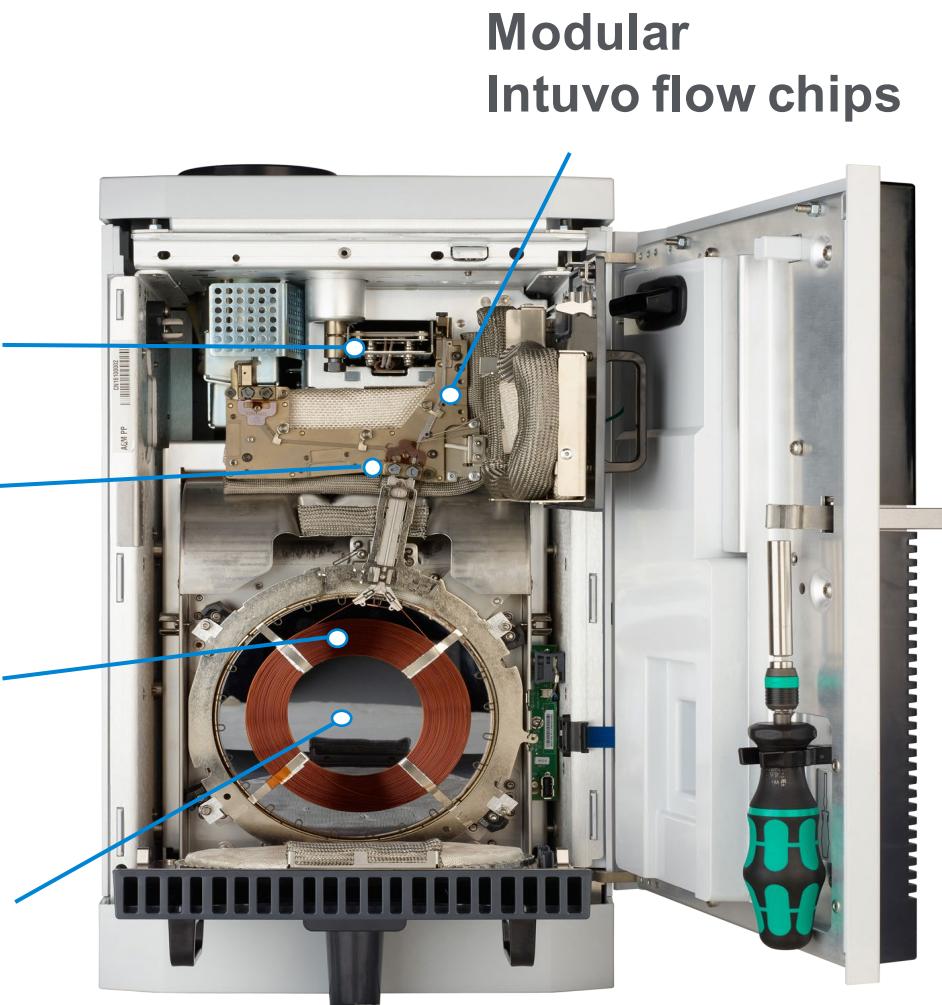


Disposable Guard chip

Ferrule-free click-and-run connections

No-trim column

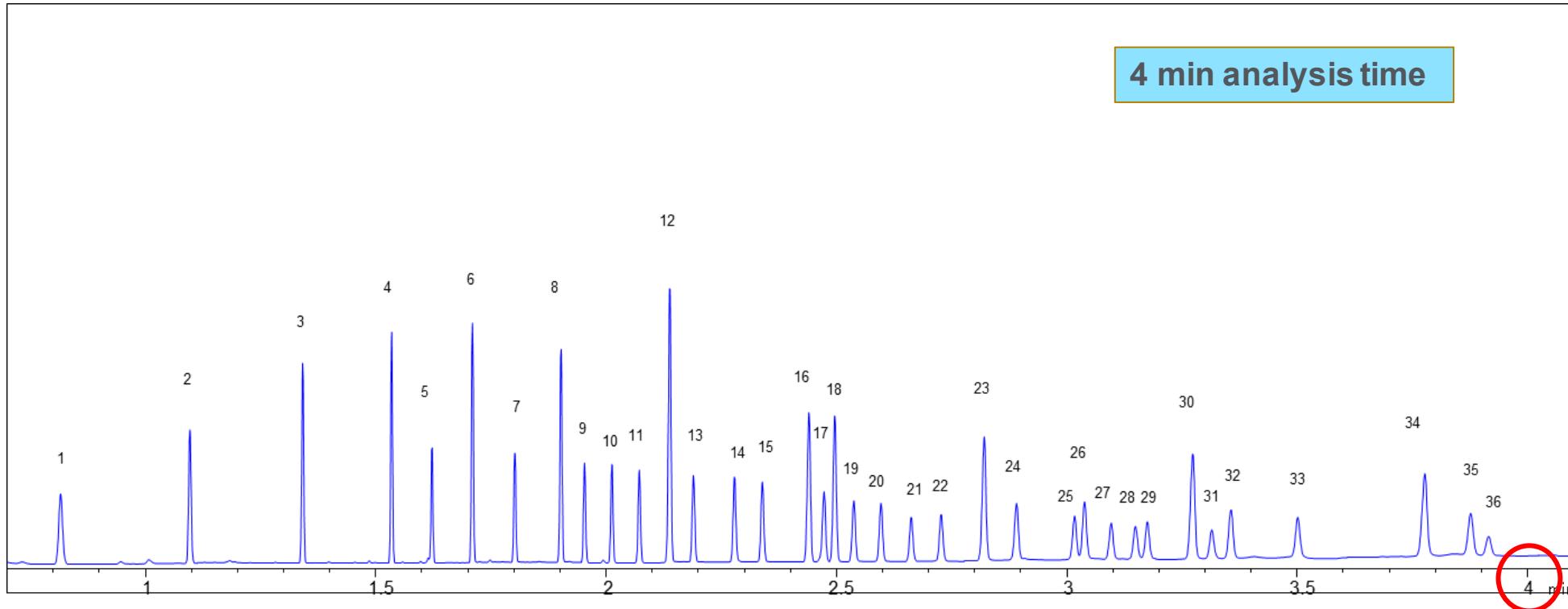
Direct heating



Fast Analysis of a 36-FAME Mix (AOAC 2012.13)

With the Intuvo 20-m DB-FastFAME

GC system: Agilent Intuvo 9000 GC system
Column DB-FastFAME, 20 m* 0.18 mm id, 0.20 µm, Intuvo module
Inlet 250 °C, split/splitless mode, split ratio 100:1
Intuvo Guard chip 200 °C
Carrier Hydrogen, constant pressure, 28 psi
Oven 50 °C (0.3 min), 200 °C/min to 200 °C (0.4 min), 20 °C/min to 240 °C (1 min)
FID 260 °C, hydrogen:40 mL/min, air: 400 mL/min, makeup gas: 25 mL/min
Injection 1 µL



How About More Complex FAME Samples like PHVO?

New high-resolution DB-FastFAME GC columns

New Agilent J&W DB-FastFAME High-Resolution GC Columns

Highlights

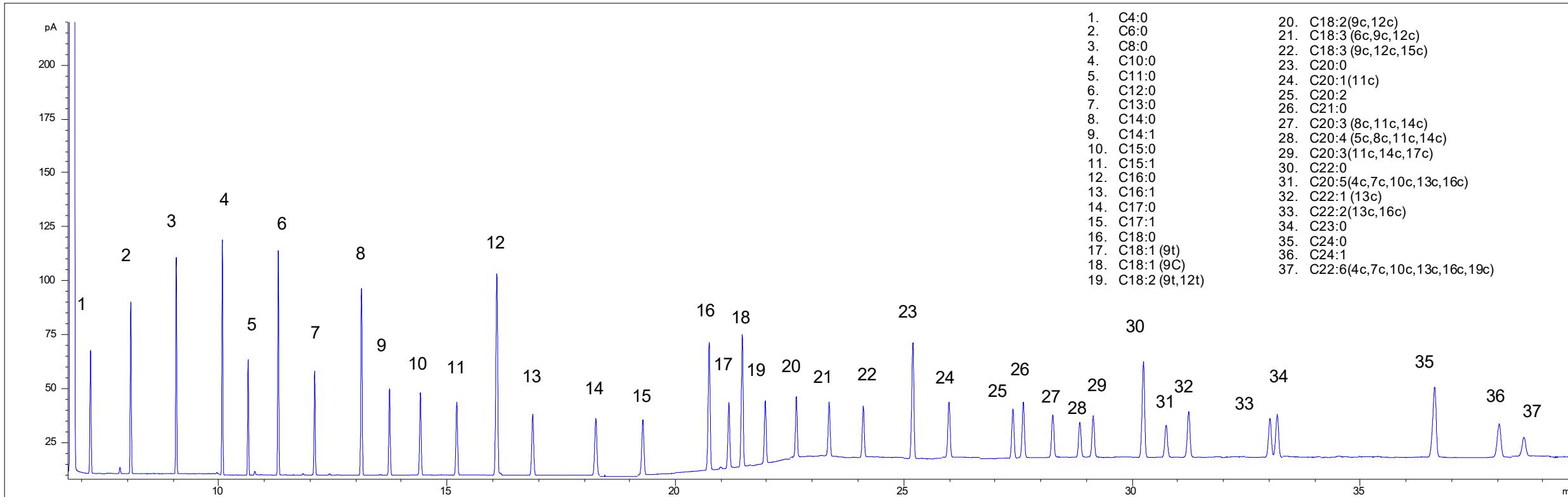
- The new 90-meter DB-FastFAME separates complex positional cis/trans FAME isomers with similar or better resolution than SP-2560, in <48 minutes
- A 60-meter DB-FastFAME is available for less demanding separations, and Intuvo applications
- Resolves critical fatty acids in food samples, making it easy to comply with labeling requirements
- Provides increased thermal stability and extend column lifetime over nonbonded cyano phases
- Same micro-union developed for Intuvo is used to assemble the 90-meter DB-FastFAME



37-Component FAMEs Standard Mixture on a DB-FastFAME GC Column Under 40 minutes

GC system: Agilent 8890
Column DB-FastFAME, 90 m* 0.25 mm id, 0.25 µm (p/n G3903-63013Z,s/n T009721Z)
Inlet 260 °C, split/splitless mode, split ratio 50:1
Carrier Helium, constant pressure 48 psi
Oven 80 °C (1 min), 40 °C/min to 200 °C (15 min), 10 °C/min to 235 °C (25 min)
FID 260 °C, hydrogen: 30 mL/min, air: 300 mL/min, makeup gas: 25 mL/min
Injection 1 µL

Application Note: 5994-1862EN



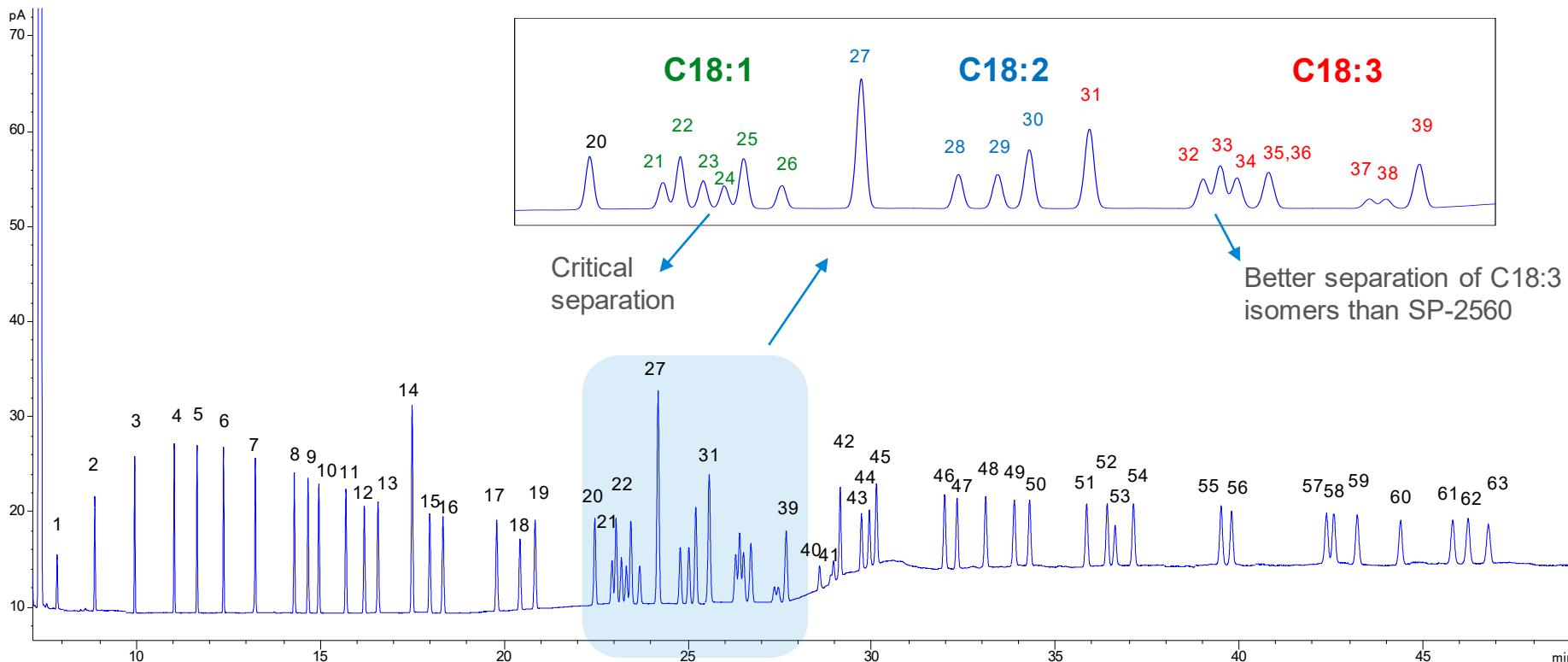
63-Component FAME Mixture Analysis

In <48min using a 90m x 0.25mm DB-fastFAME column

GC system: Agilent 8890
Column: DB-FastFAME, 90 m* 0.25 mm id, 0.25 μ m (p/n G3903-63013Z, s/n T009721Z)
Inlet: 260 °C, split/splitless mode, split ratio 30:1
Carrier: Helium, constant pressure 44 psi
Oven: 75 °C (1 min), 35 °C/min to 200 °C (14 min), 2.5 °C/min to 210 °C (5 min), 12 °C/min to 230 °C (20 min)
°C/min to FID: 260 °C, hydrogen: 30 mL/min, air: 300 mL/min, makeup gas: 25 mL/min
Injection: 1 μ L

- Separation of critical C18:1 isomers
- R_S of C18:1 (11t)/c18:1(6c) >1.40 (baseline resolution)
- Separation of C18:2 isomers
- Separation of C18:3 isomers except for one pair (same as SP-2560)
- Analysis time <48min

Application Note: 5994-1862EN



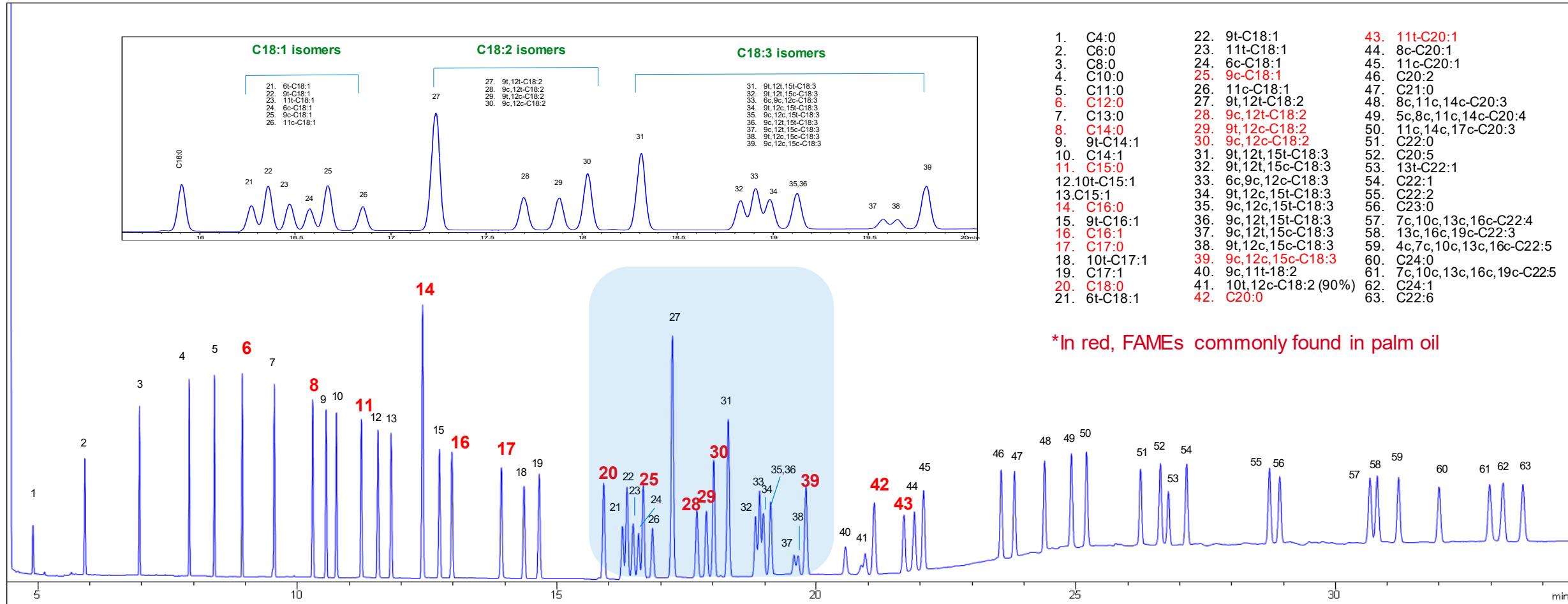
1. C4:0
2. C6:0
3. C8:0
4. C10:0
5. C11:0
6. C12:0
7. C13:0
8. C14:0
9. C14:1(9t)
10. C14:1
11. C15:0
12. C15:1
13. C15:1 (10t)
14. C16:0
15. C16:1 (9t)
16. C16:1
17. C17:0
18. C17:1(10t)
19. C17:1
20. C18:0
21. C18:1(6t)
22. C18:1(9t)
23. C18:1(11t)
24. C18:1(6C)
25. C18:1(9C)
26. C18:1(11C)
27. C18:2(9t,12t)
28. C18:2(9c,12t)
29. C18:2(9t,12c)
30. C18:2(9c,12c)
31. C18:3(9t,12t,15t)
32. C18:3(9t,12t,15c)
33. C18:3(6c,9c,12c)
34. C18:3(9t,12c,15t)
35. C18:3(9c,12c,15t)
36. C18:3(9c,12t,15t)
37. C18:3(9c,12t,15c)
38. C18:3(9t,12c,15c)
39. C18:3(9c,12c,15c)
40. C18:2(t9,c11)
41. C18:2(t10,c12)(90%)
42. C20:0
43. C20:1(11t)
44. C20:1(8c)
45. C20:1(11c)
46. C20:2
47. C21:0
48. C20:3(8c,11c,14c)
49. C20:4(5c,8c,11c,14c)
50. C20:3(11c,14c,17c)
51. C22:0
52. C20:5(4c,7c,10c,13c,16c)
53. C22:1(13t)
54. C22:1(13c)
55. C22:2(13c,16c)
56. C23:0
57. C22:4(7c,10c,13c,16c)
58. C22:3(13c,16c,19c)
59. C22:5(4c,7c,10c,13c,16c)
60. C24:0
61. C22:5(7c,10c,13c,16c,19c)
62. C24:1
63. C22:6(4c,7c,10c,13c,16c,19c)

GC/FID Chromatogram of 63-Component FAMEs Standard Mixture

On an Agilent J&W DB-FastFAME column using hydrogen_as carrier gas

<40 min

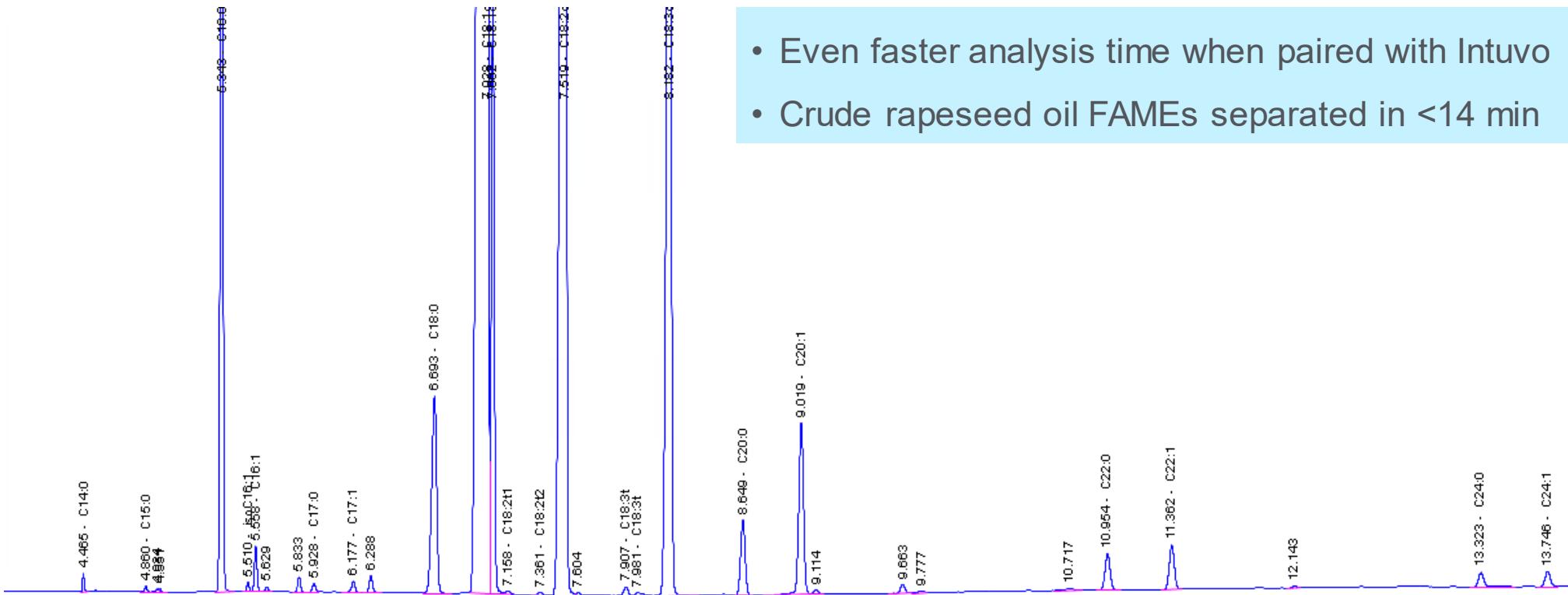
Application note: 5994-1862EN



Crude Rapeseed Oil FAMEs with Intuvo 60 m DB-FastFAME GC column

Column
Inlet
Guard Chip
Carrier
Oven
FID
Injection

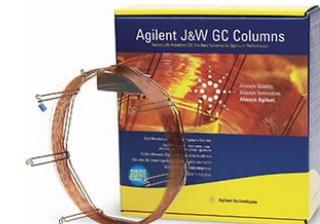
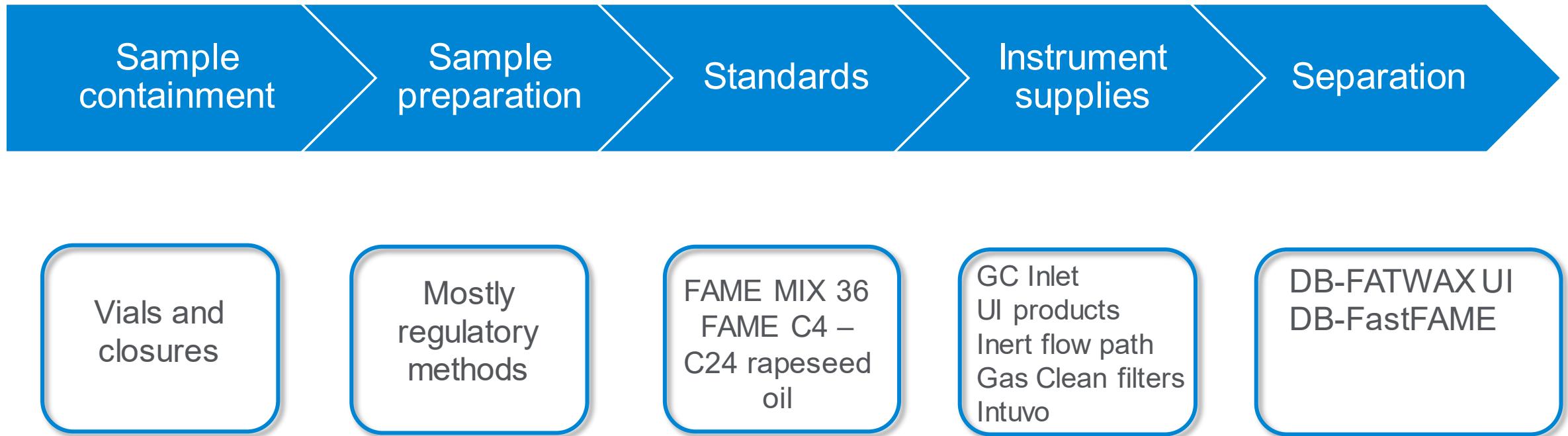
DB-FastFame, 60 m* 0.25 mm id, 0.25 µm (p/n G3909-63007)
250 °C, split mode, split ratio 100:1
SSL guard chip in track oven mode
Hydrogen, constant flow mode, 50 cm/s avg linear velocity
120 °C (1 min), 100 °C/min to 200 °C (5 min), 5 °C/min to 240 °C
250 °C, hydrogen: 40 mL/min, air: 400 mL/min, makeup gas: 25 mL/min
1 µL



The High-Resolution Columns are a DB-FastFAME Product Line Extension

DB-FastFAME Product Line	Features and Benefits	Applications
20 m x 0.18 mm x 0.20 µm, 7 in cage and Intuvo	Faster separation of FAMEs than any other column on the market	Simpler FAME mixture (<37 FAMEs) in 8 minutes (for example, vegetable and extra virgin oils)
30 m x 0.25 mm x 0.25 µm, 7 in cage and Intuvo	Good balance between speed and capacity	Simpler FAME mixture (<37 FAMEs) in 25 minutes (for example, vegetable and extra virgin oils)
60 m x 0.25 mm x 0.25 µm, 7 in cage and Intuvo	Ideal for specific FAME applications (CLAs, C18:1 groups, C18:3 groups), and Intuvo applications	Intuvo applications CLA isomers per new GB method 55 FAMEs <38 minutes
90 m x 0.25 mm x 0.25 µm, 7 in cage only	High detail analysis of positional cis/trans isomers with 30-35% faster analysis time than SP-2560	All purpose FAME column (milk fat, cooking oil, trans fats) 63 FAMEs <48 minutes

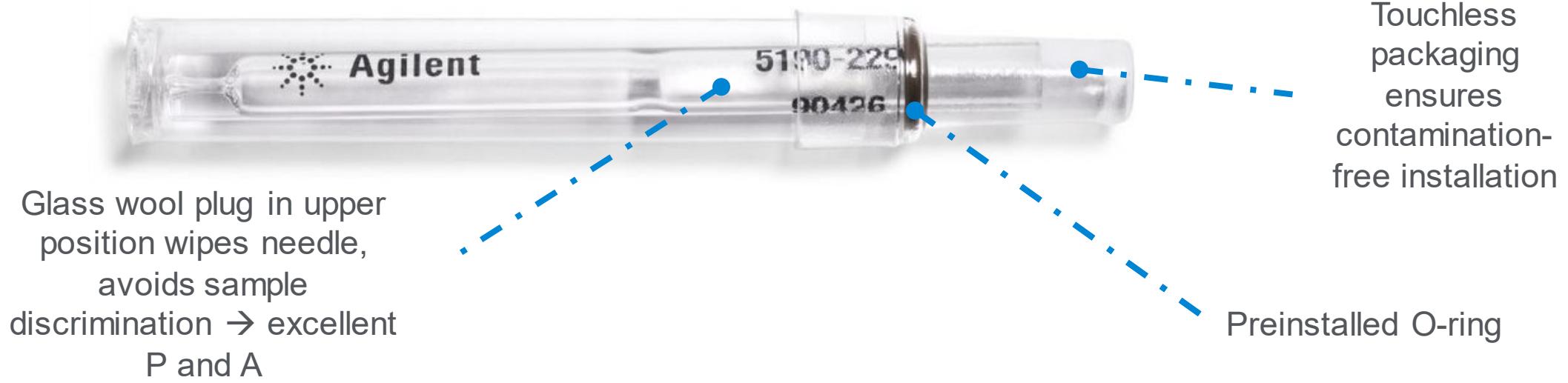
Workflow



Sample Introduction is a Critical Step in FAME Analysis

Most FAMEs and FFA methods use split injection

- Potential exists for sample discrimination between low and high boiling components
 - Leads to poor precision and accuracy
 - FAMEs → varying carbon chain length → various boiling points
 - Using a **split** liner with wool is important (do **not** use a splitless liner)
 - **Ultra Inert** liners enable excellent peak shapes for tricky analytes like FAMEs and FFAs
- 5190-2295 is a recommended liner



Other Opportunities for GC Sample Intro and Supplies

In split analyses, especially high matrix samples, timely replacement of the **split vent trap** is key to avoiding ghost peaks and carryover.

1/pk: [5188-6495](#) 2/pk: [G1544-80530](#)



Premium Blue Line **syringes** offer excellent precision and accuracy. Recommend a PTFE tip for high matrix samples.

G4513-80204- 10 μ L tapered, fixed 23-26s/42/HP
G4513-80203- 10 μ L tapered, fixed, PTFE tip 23-26s/42/HP



FFAs and FAMEs Product Line Workflow

Sample Containment	Sample Preparation	Standards	Instrument Supplies/Sample Introduction	Columns	Regulatory	Applications/Selection Guides/User Tools
<ul style="list-style-type: none">• Vials 2 mL• Amber• Write on spot• PTFE/Silicone screw cap• Standard/Certified• UPL• ValueLab• Crimpers	<ul style="list-style-type: none">• AOAC and AOCS regulatory methods• QuEChERS• Bond Elut SPE• Chem Elut• Captiva EMR-Lipid	<ul style="list-style-type: none">• FAME Mix – 36:• FAME Mix C4-C24:• Rapeseed oil	<ul style="list-style-type: none">• Inlet supplies• Liner UI: Split w/ glass wool• Septa: BTO• Inlet seal: UI• Split vent trap• Inert flow path• Gas Clean filters• Intuvo• Self tightening nuts• Ferrules• Syringes	<ul style="list-style-type: none">• DB FATWAX UI GC• DB FastFame• Multiple dimension for standard GCs• Intuvo columns	<ul style="list-style-type: none">• AOCS Official Method of Analysis (2000), method Ce 2-66• IUPAC, Standard methods for Analysis of Oils, Fats and Derivatives, Blackwell Scientific Publications, IUPAC Method 2.301• AOAC 2012.13• AOAC 996.06• AOCS Ce 1-62• AOCS Ce 1b-89	<ul style="list-style-type: none">• Food safety brochures• FATWAX UI application notes• FastFAME application notes• FAME GC brochure

Collateral

LITERATURE	LINK/PUBLICATION NUMBER	
Improving the Analysis of 37 Fatty Acid Methyl Esters: Using Three Types of Capillary Columns	5991-8706EN	Application note
Analysis of Omega 3 and Omega 6 FAMEs in Fish Oil and Animal Fat Using an Agilent J&W DB-FATWAX Ultra Inert GC Column	5991-8744EN	Application note
A comparison study of the analysis of volatile organic acids and fatty acids: Using DB-FATWAX Ultra Inert and other WAX GC columns	5991-9223EN	Application note
Comprehensive Analysis of FAMEs, Fatty Acids and Triglycerides	5991-8763EN	20 page brochure
Fast Analysis of FAMES on the Intuvo 9000	5991-9482EN	Application note
Rapid Separation of Fatty Acid Methyl Esters using DB-FastFAME Intuvo GC columns	5994-0116EN	Application note

Contact Agilent Chemistries and Supplies Technical Support



1-800-227-9770 Option 3, Option 3:

Option 1 for GC and GC/MS columns and supplies

Option 2 for LC and LC/MS columns and supplies

Option 3 for sample preparation, filtration, and QuEChERS

Option 4 for spectroscopy supplies

Option 5 for chemical standards

Available in the USA and Canada 8–5, all time zones



gc-column-support@agilent.com

lc-column-support@agilent.com

spp-support@agilent.com

spectro-supplies-support@agilent.com

chem-standards-support@agilent.com

Thanks for your attention

Any questions?

