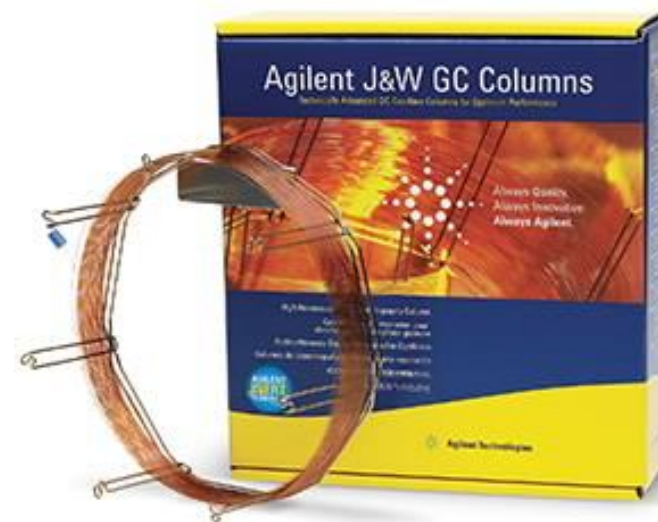


An overview of GC columns and new technologies for the analysis of FAMEs

Gustavo Serrano Izaguirre, Ph.D.
Global Product Manager – GC Columns

Saurabh Patel, Ph.D.
Applications support Engineer



What Are We Testing?

Fat profiles:

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

Free fatty acids

Triglycerides

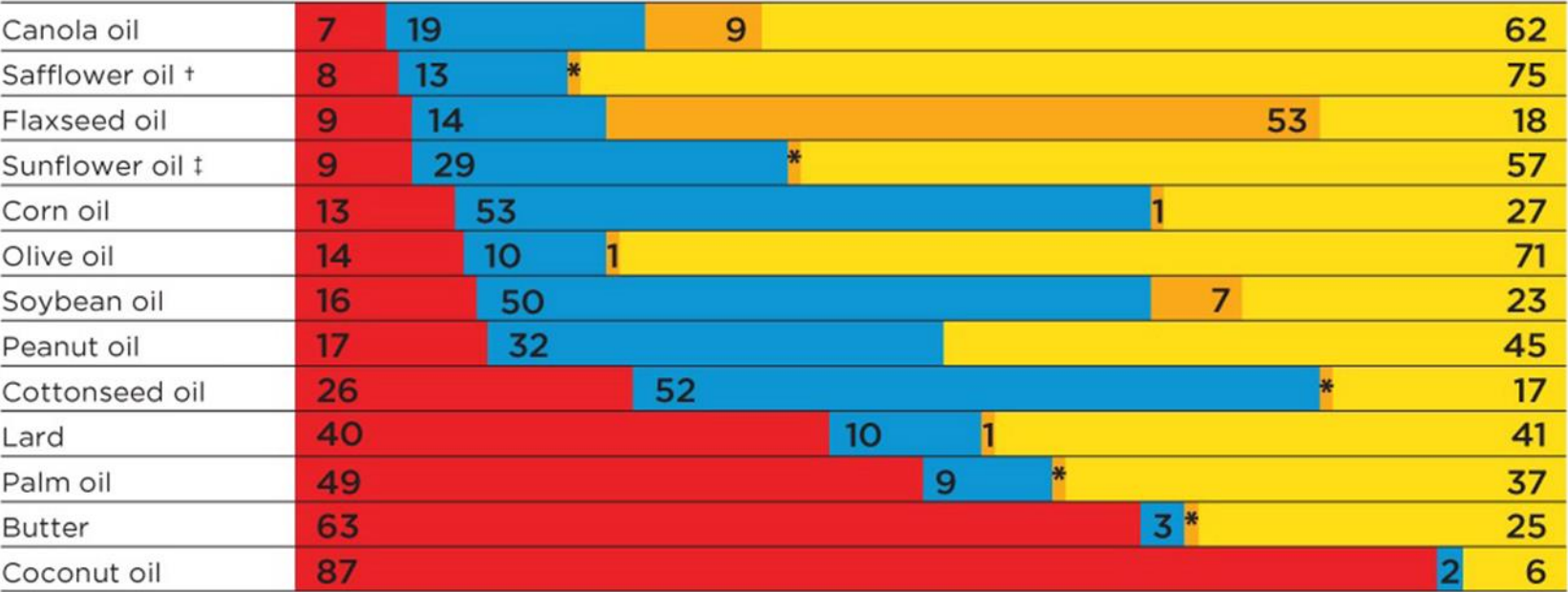
Omega 3 and 6 fatty acids



What Are We Testing?

Comparison of Dietary Fats

Dietary Fat



SOURCES: CANADIAN NUTRIENT FILE AND USDA NATIONAL NUTRIENT DATABASE. ACCESSED MAY 2016

Saturated Fat	Polyunsaturated Fat		Monounsaturated Fat
	linoleic acid (an omega-6 essential fatty acid)	alpha-linolenic acid (an omega-3 essential fatty acid)	oleic acid (an omega-9 fatty acid)

† High Oleic ‡ Mid Oleic * Trace

Fatty acid content expressed as g/100g fat

CanolaInfo. Fat Chart & Nutritional Analysis, <https://www.canolainfo.org>

Markets Where FAMES in Food are Analyzed



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

Nutrition Facts			
Serving Size 1 cup (228g)			
Servings Per Container 2			
Amount Per Serving			
Calories 260	Calories from Fat 120		
% Daily Value*			
Total Fat 13g	20%		
Saturated Fat 5g	25%		
Trans Fat 2g			
Cholesterol 30mg	10%		
Sodium 650mg	28%		
Total Carbohydrate 31g	10%		
Dietary Fiber 0g	0%		
Sugars 5g			
Protein 5g			
Vitamin A 4%	Vitamin C 2%		
Calcium 15%	Iron 4%		
*Percent Daily Values are based on a 			
Your Daily Values may be higher or lower depending on your calorie needs.			
	Calories	2,000	2,500
Total Fat	Less Than	65g	80g
Sat Fat	Less Than	20g	25g
Cholesterol	Less Than	300mg	300mg
Sodium	Less Than	2,400mg	2,400mg
Total Carbohydrate	Less Than	300g	375g
Dietary Fiber	25g	30g	
Calories per gram:			
Fat	9	Carbohydrate	4
		Protein	4

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



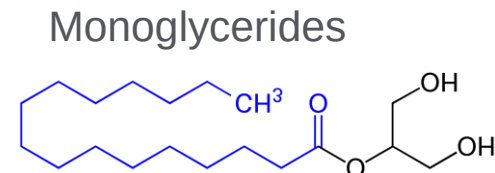
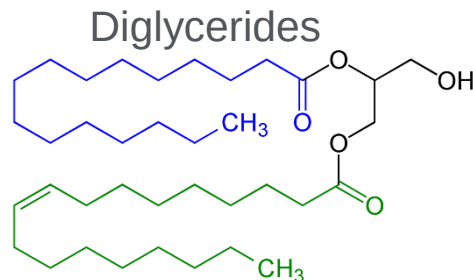
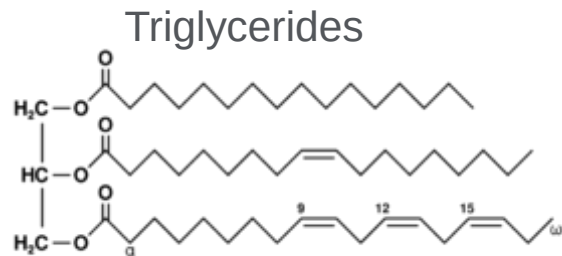
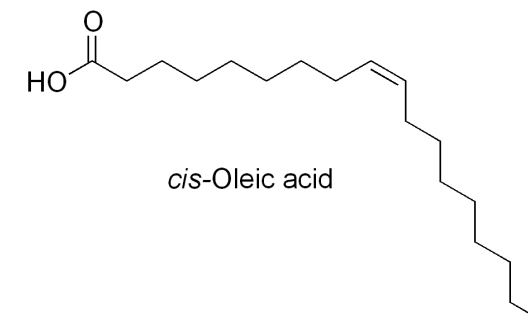
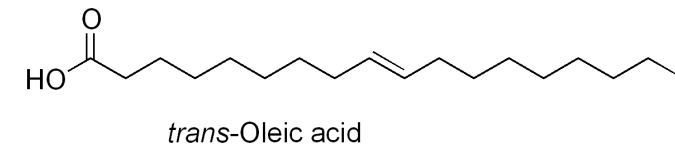
- Omega 3 and 6 supplements
- QA/QC, process control



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

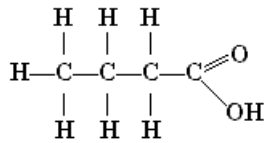
A triglyceride is called a fat if it is a solid at 25 °C; it is called an oil if it is a liquid at that temperature. These differences in melting points reflect differences in the degree of unsaturation and number of carbon atoms in the constituent fatty acids.

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):

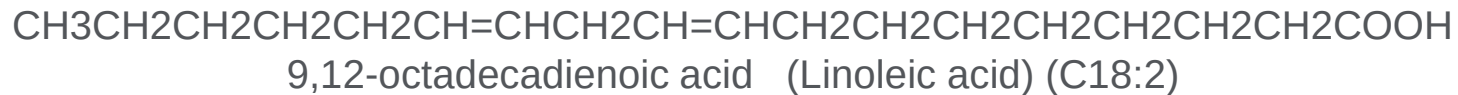
Have all the hydrogen that the carbon atoms can hold and, therefore, have no double bonds between carbon atoms.



or $\text{CH}_3\text{-CH}_2\text{-CH}_2\text{-COOH}$ or $\text{CH}_3\text{-(CH}_2\text{)}_2\text{-COOH}$
Butyric acid

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

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



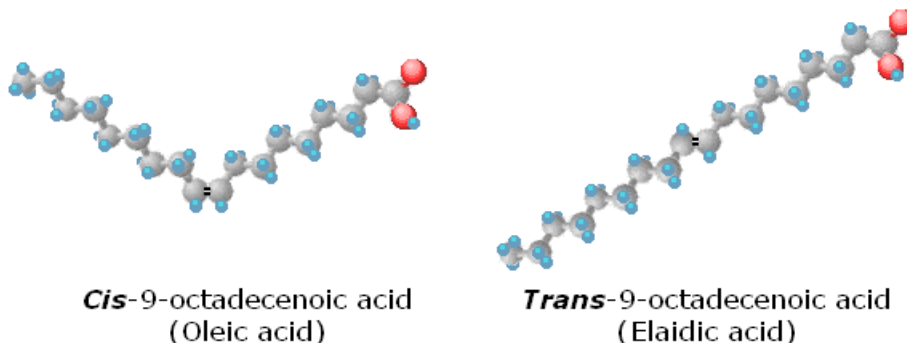
Which would be abbreviated as:
 $\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$

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.



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 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)

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

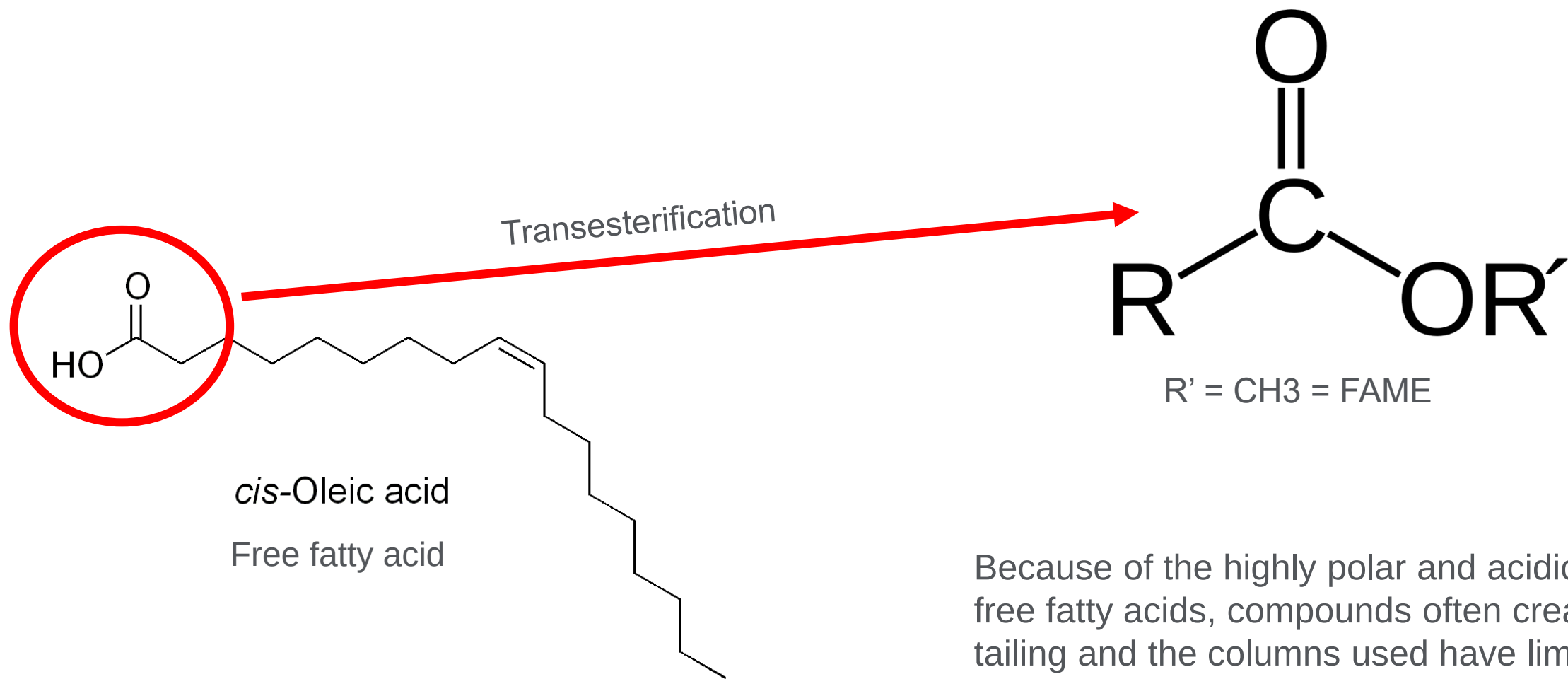
^c Total contents of 9c 12t 15t C18:3 and 9c12c 15t C18:3

^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

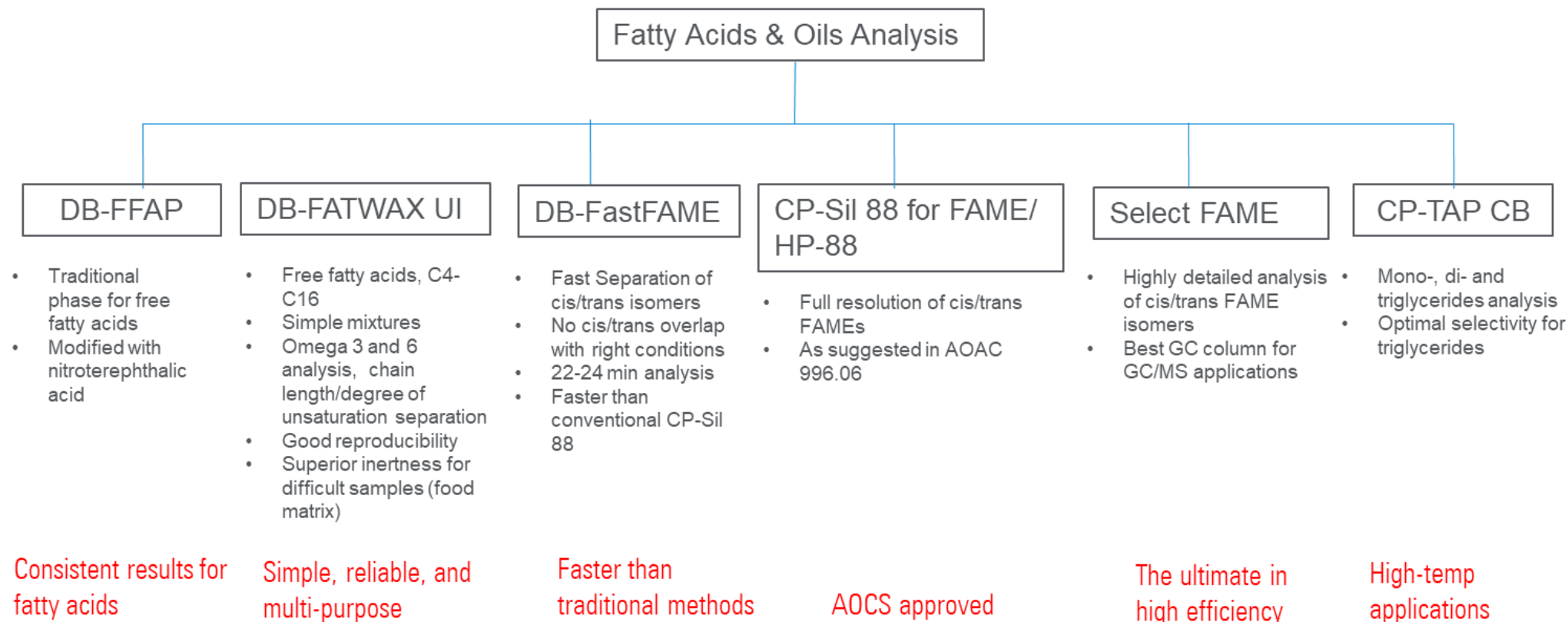
Why Do We Analyze FAMES?



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

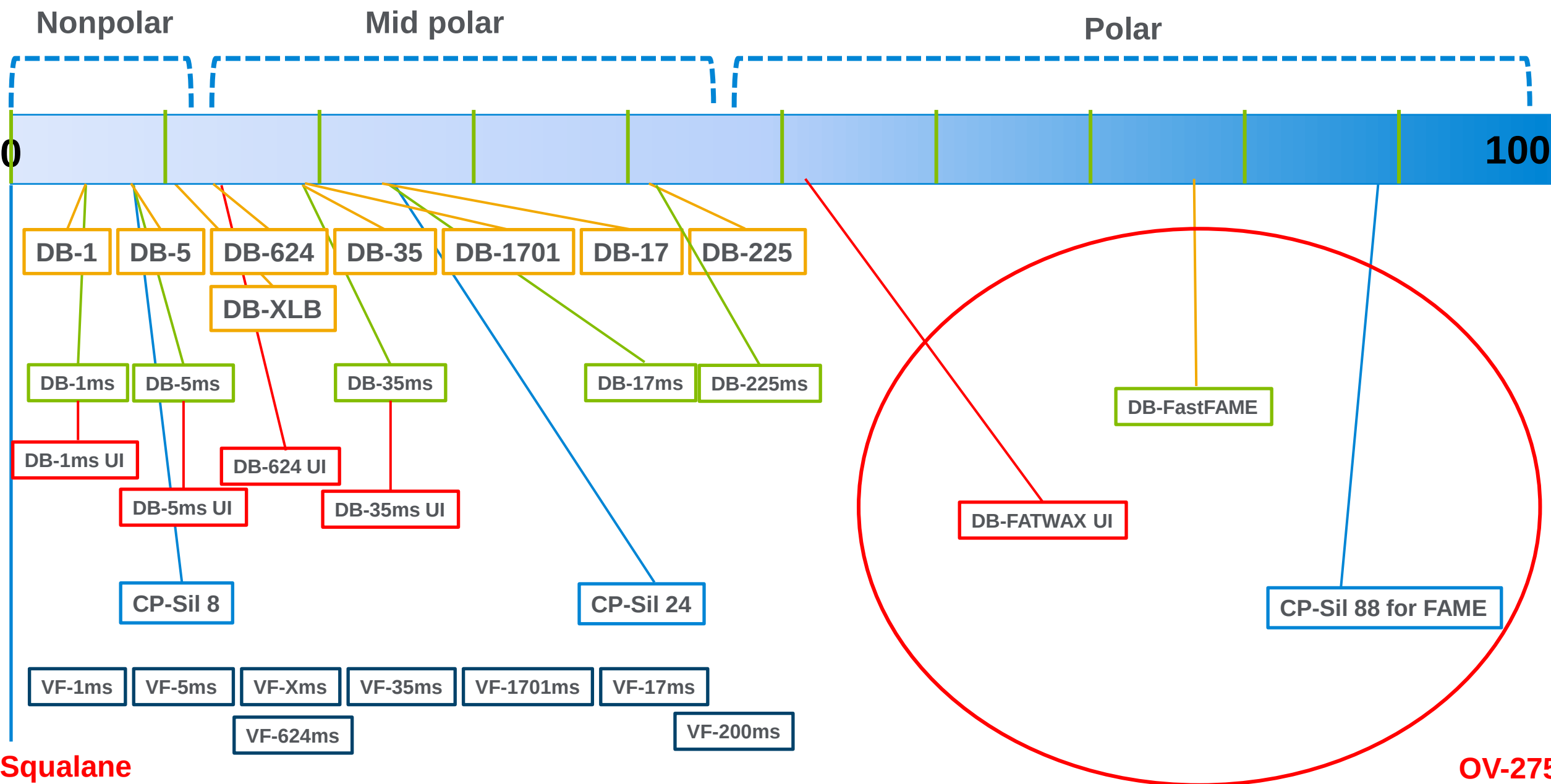
Comprehensive Portfolio for Fatty Acids, FAME, and Oils Analysis



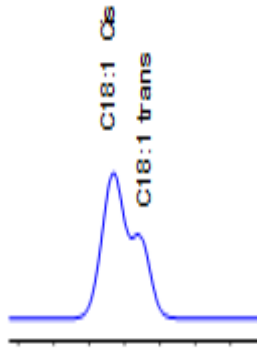
FFA

FAME

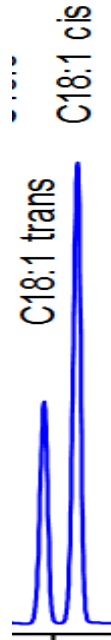
TG



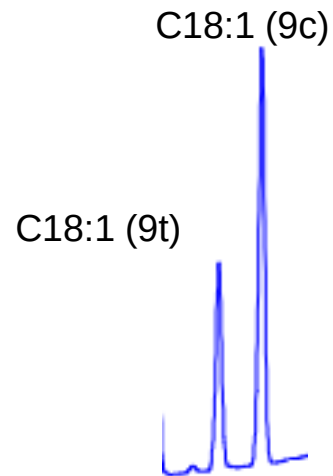
Column Polarity/Interaction Effects – FAMES



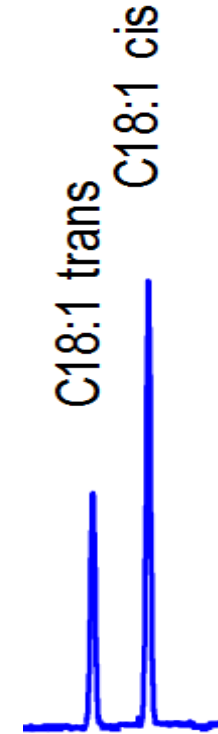
DB-FATWAX UI



DB-FastFAME, 30 m



DB-FastFAME, 90 m



CP-Sil 88 FAME

Traditional GC Columns

For fatty acids, FAMES and triglycerides

Triglycerides Analysis of Palm Oil

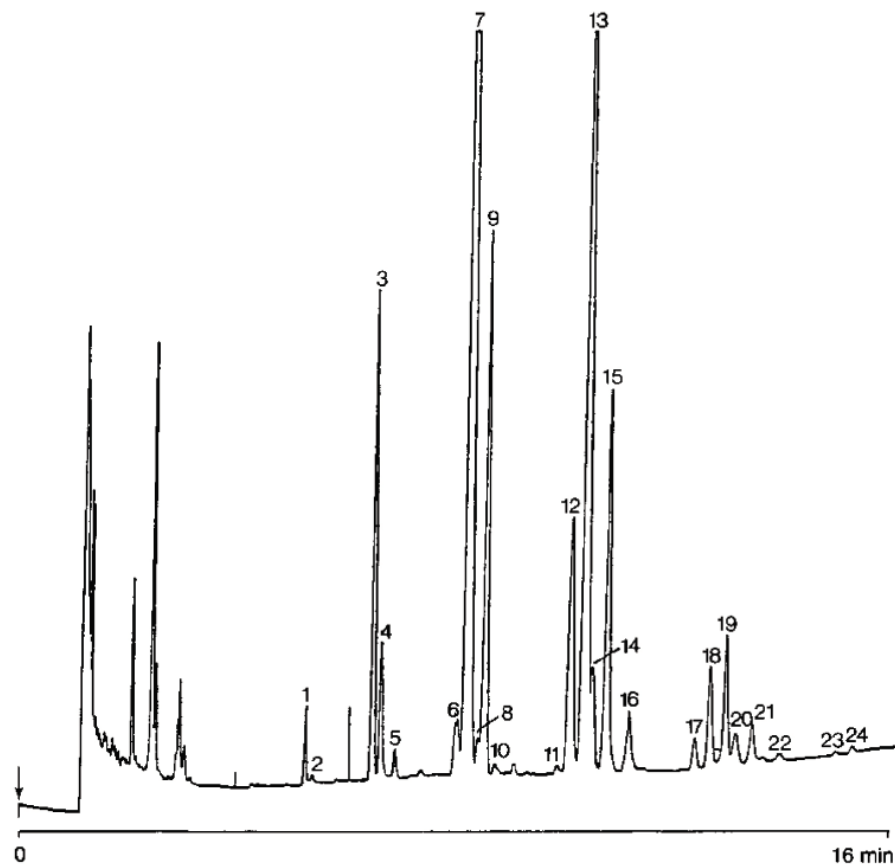
CP-TAP CB for triglycerides

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 hexan

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

	Carbon No.
1. MPP	46
2. MOM	46
3. PPP	48
4. MOP	48
5. MLP	48
6. PPS	50
7. POP	50
8. MOO	50
9. PLP	50
10. MLO	50
11. PSS	52
12. POS	52
13. POO	52
14. PLS	52
15. PLO	52
16. PLL	52
17. SOS	54
18. SOO	54
19. OOO	54
20. SLO	54
21. OLO	54
22. OLL	54
23. SOA	56
24. AOO	56

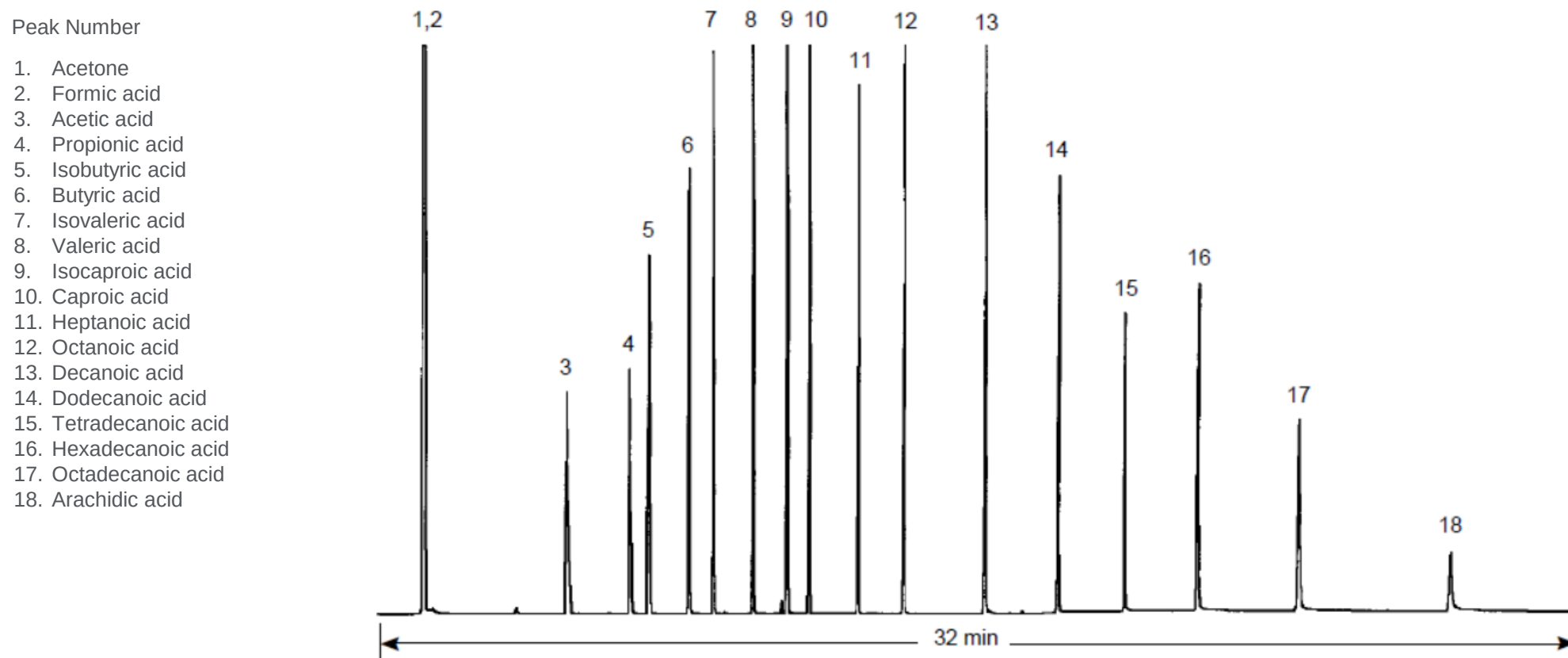


Analysis of Free Fatty Acids

Column **DB-FFAP**, 30 m, 0.25 mm, 0.25 μ m (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

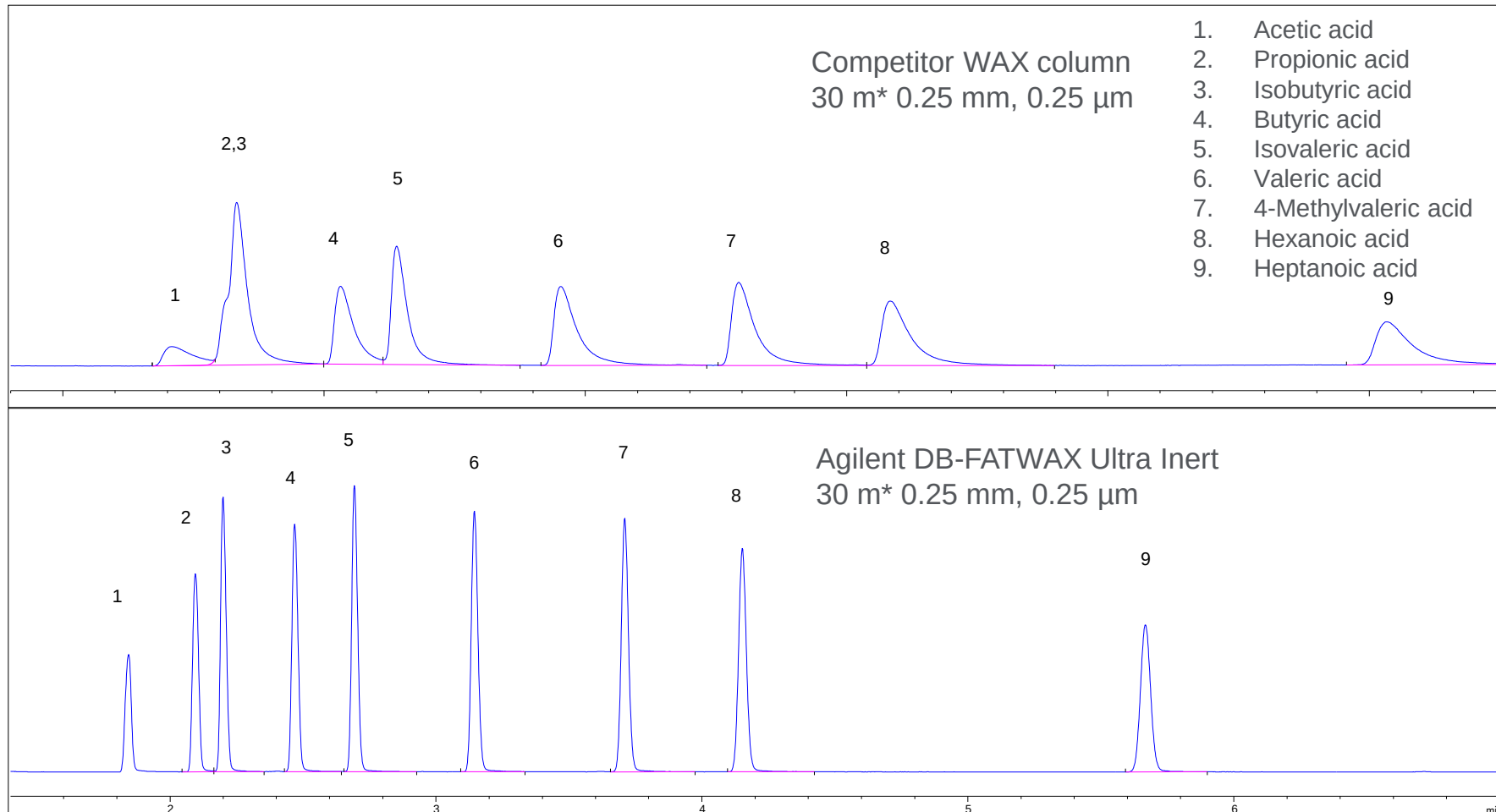
Chromatogram C077



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



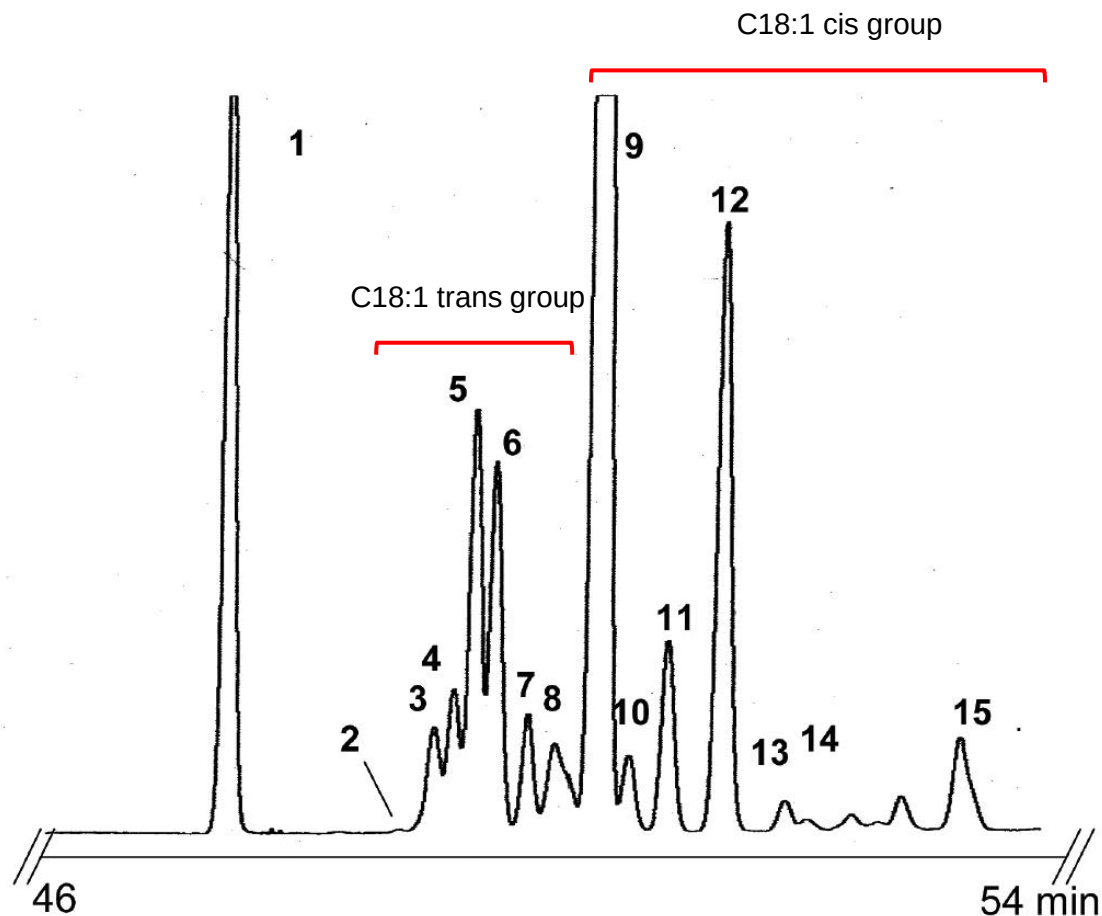
Detailed Analysis of Cis/Trans FAMES C18:1 Positional Isomers

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

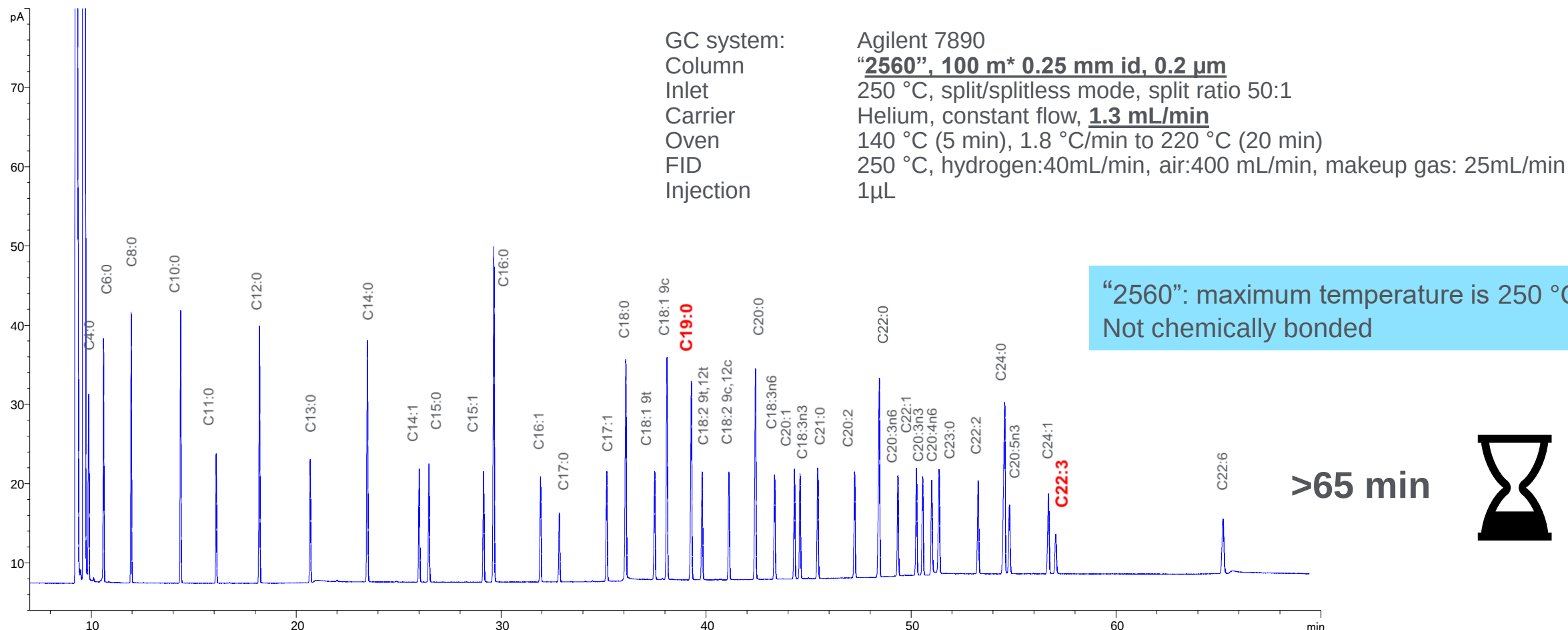


Introducing High-Resolution DB-FastFAME

- Fast separation of cis/trans isomers
- No cis/trans overlap with right conditions
- 22 to 24 minute analysis (<37 FAMES)

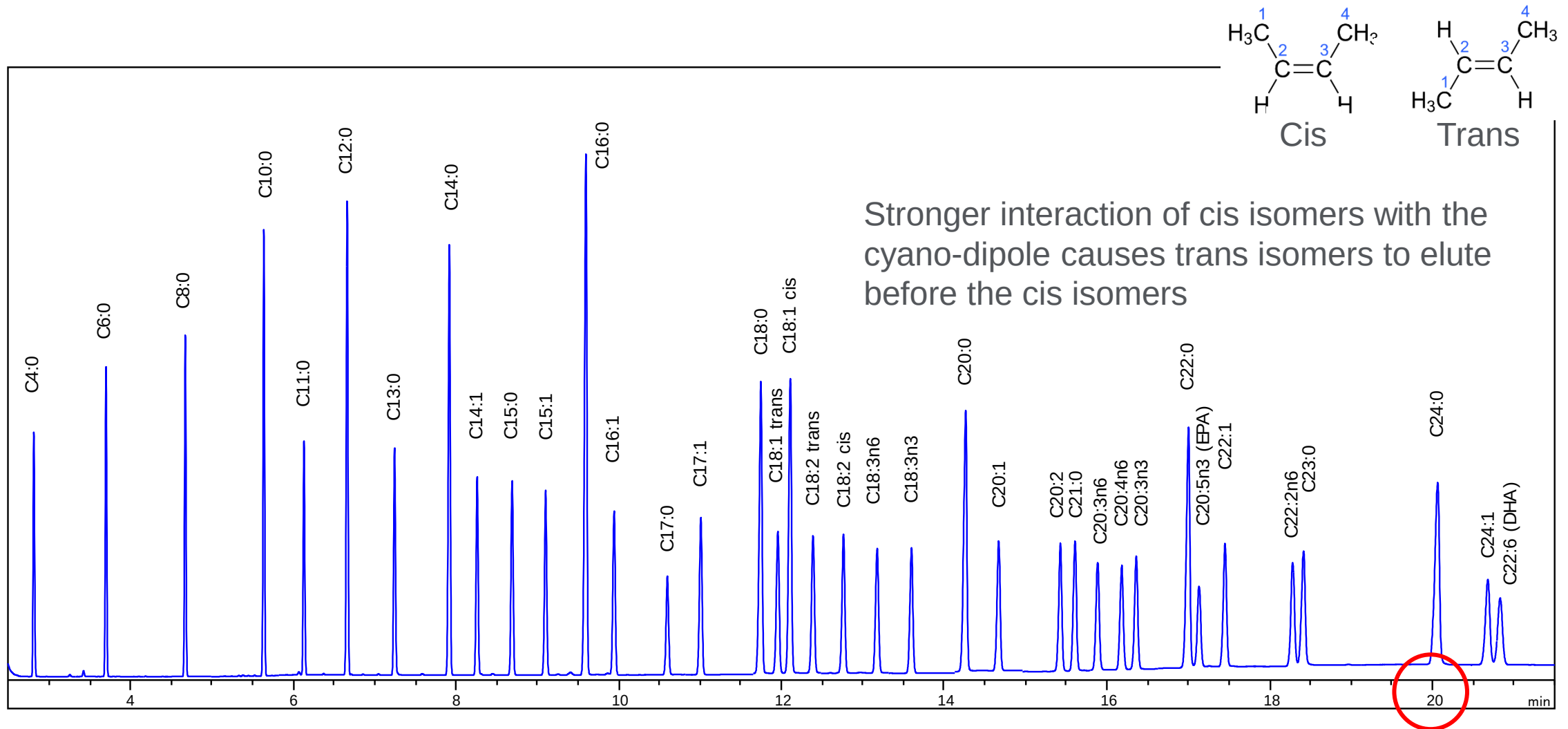
Traditional FAME Analysis on “2560” Column

- 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

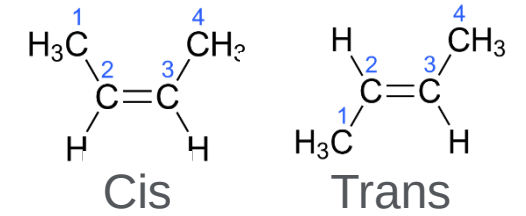


DB-FastFAME

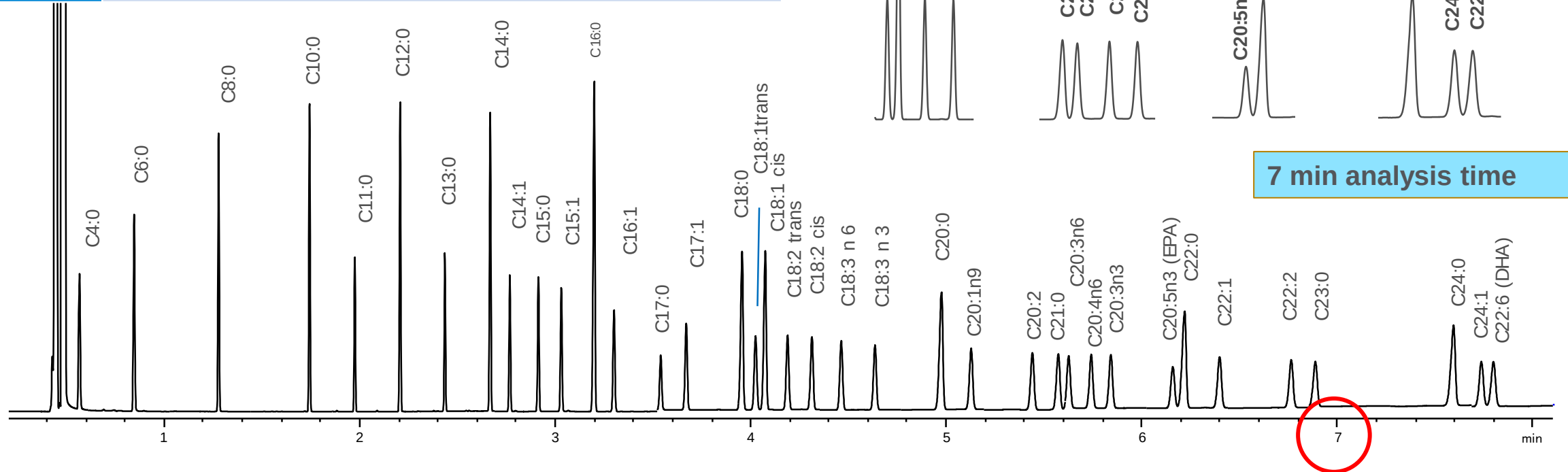
20 m x 0.18 mm x 0.20 µm

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

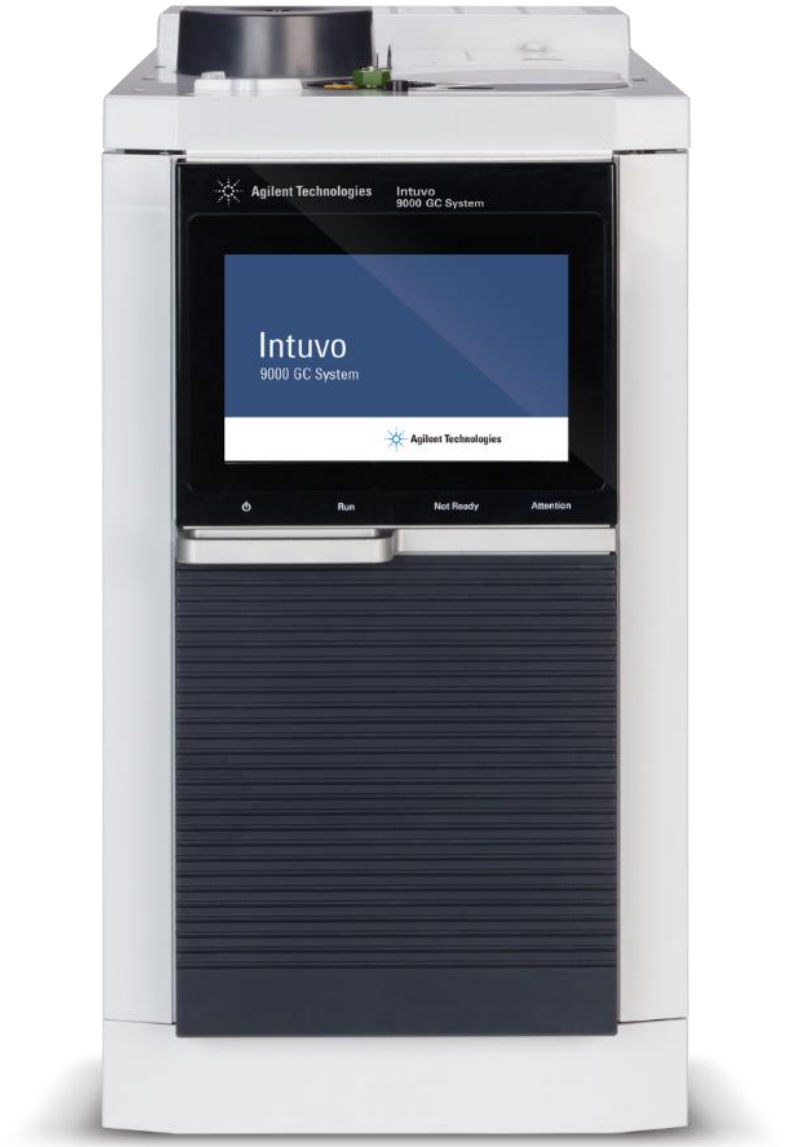


7 min analysis time

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

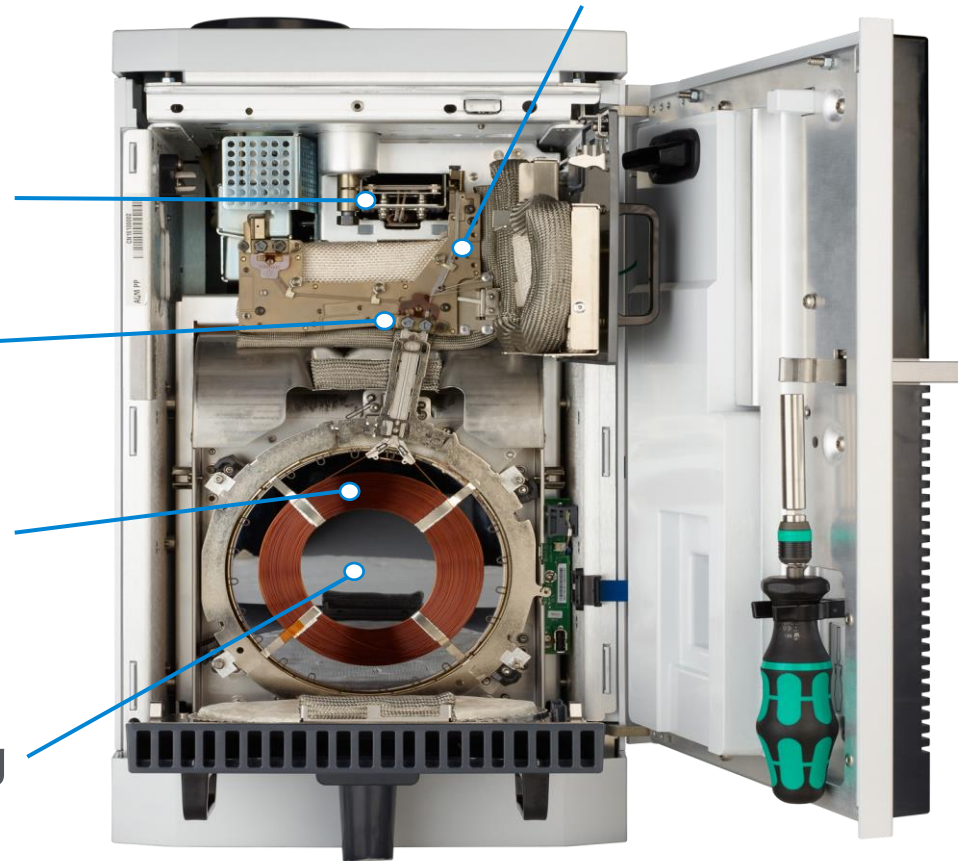
Ferrule-free click-and-run connections



No-trim column

Direct heating

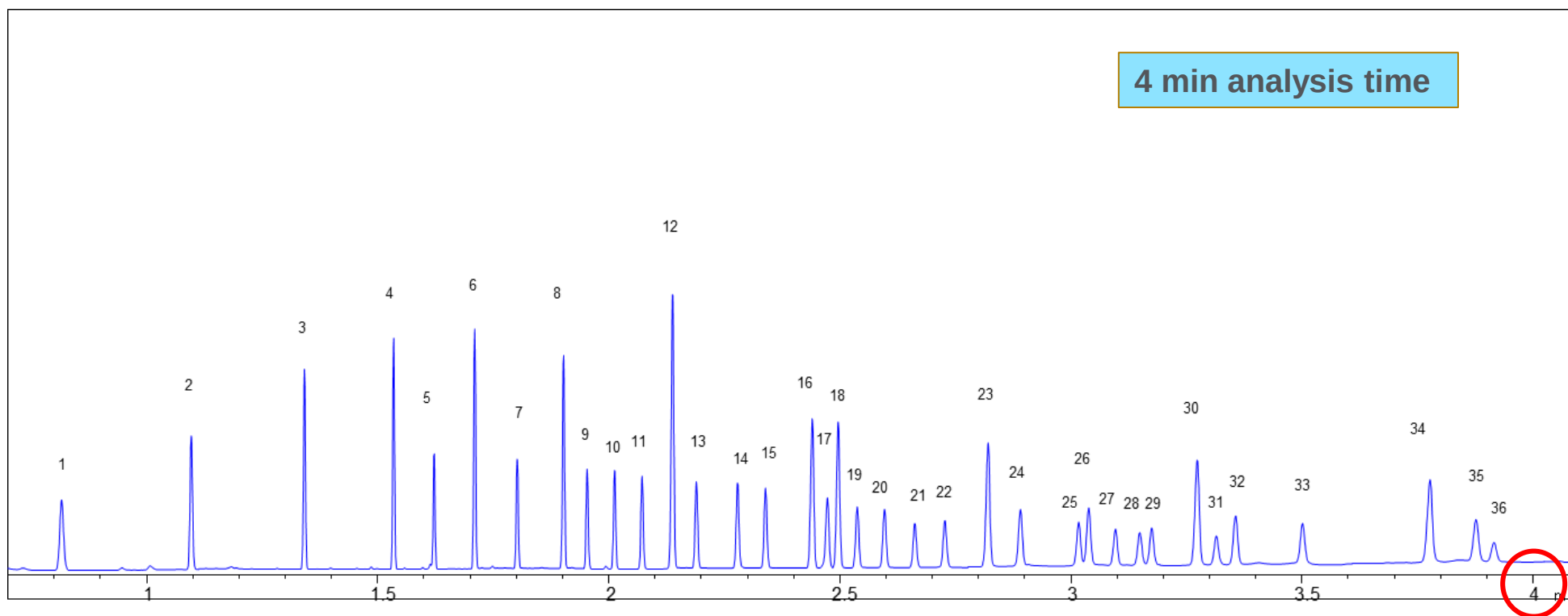
Modular Intuvo flow chips



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

With the Intuvo 20-m DB-FastFAME

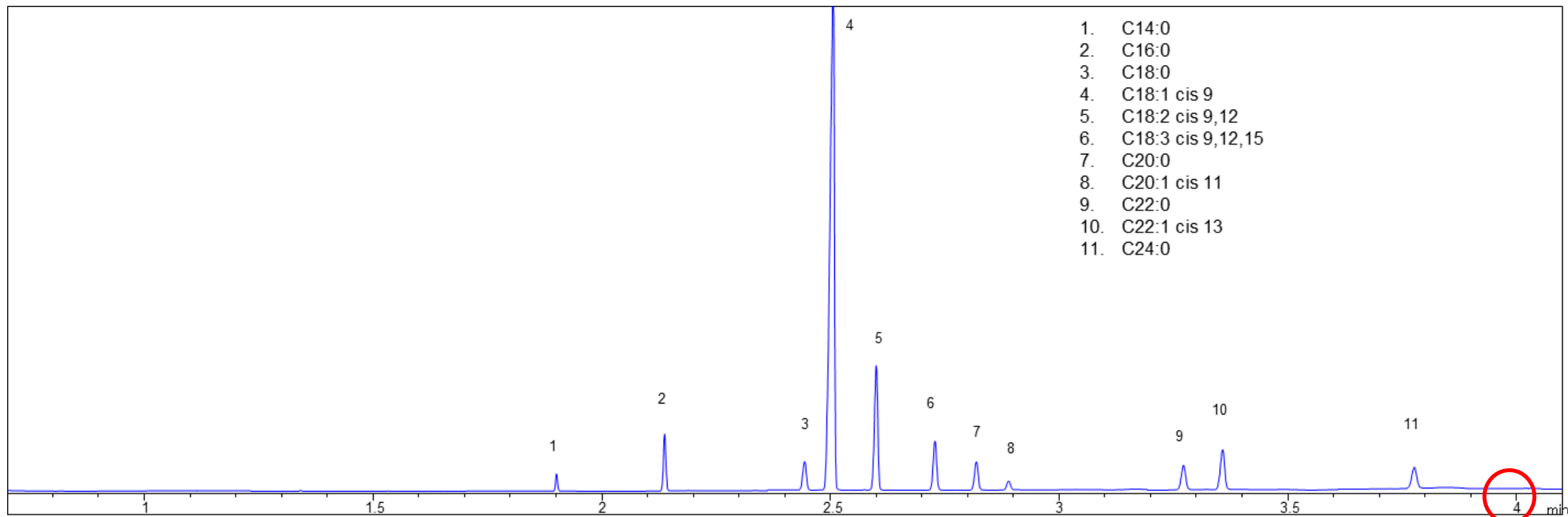
GC system: Agilent Intuvo 9000 GC system
Column DB-FastFAME, 20 m* 0.18 mm id, 0.20 µm, Intuvo module
Inlet 250 °C, split/splitless mode, split ratio 100:1
Intuvo Guard chip 200 °C
Carrier **Hydrogen**, constant pressure, 28 psi
Oven 50 °C (0.3 min), 200 °C/min to 200 °C (0.4 min), 20 °C/min to 240 °C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



Fast analysis of rapeseed oil

With the Intuvo 20-m DB-FastFAME

GC System: Agilent Intuvo 9000 GC system
Column DB-FastFAME, 20m*0.18mm I.D, 0.20um, Intuvo module
Inlet 250°C, split/splitless mode, split ratio 100:1
Intuvo Guard chip 200°C
Carrier **Hydrogen**, constant pressure, 28psi
Oven 50°C(0.3min), 200°C/min to 200°C(0.4min), 20°C/min to 240°C(1min)
FID 260°C, Hydrogen:40mL/min, Air:400mL/min, make-up gas:25mL/min
Injection 1uL



How About More Complex FAME Samples like PHVO?

Agilent J&W High-Resolution DB-FastFAME GC columns

Highlights

- The 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



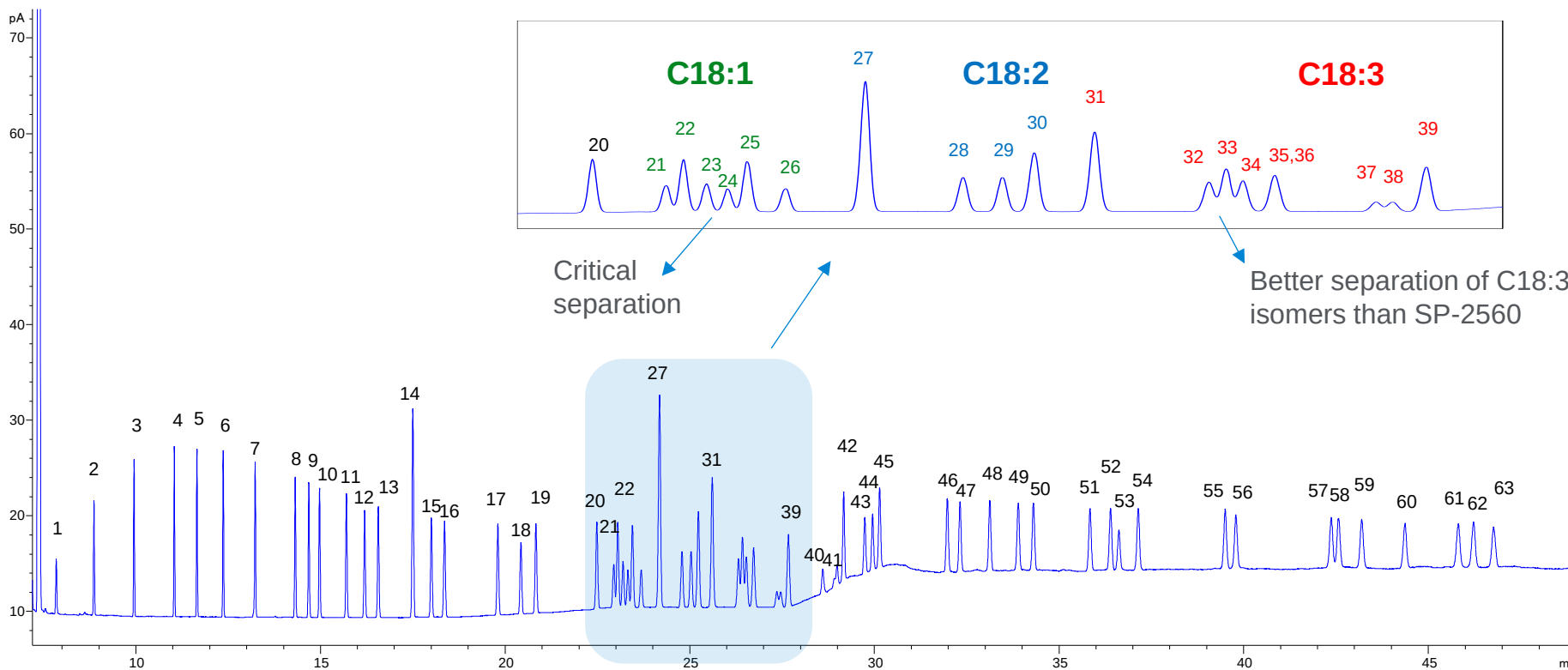
63-Component FAME Mixture Analysis

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 µm (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)
FID: 260 °C, hydrogen: 30 mL/min, air: 300 mL/min, makeup gas: 25 mL/min
Injection: 1 µL

- 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



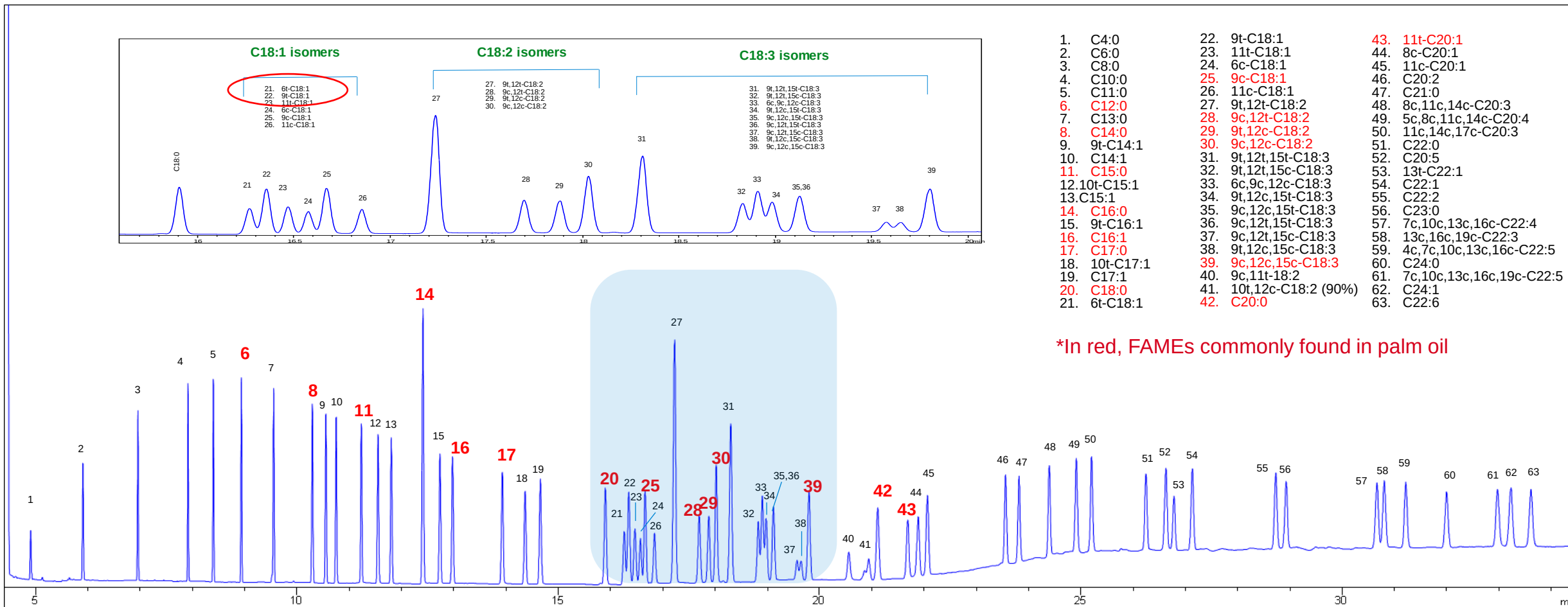
1. C4:0	33. C18:3 (6c,9c,12c)
2. C6:0	34. C18:3 (9t,12c,15t)
3. C8:0	35. C18:3 (9c,12c,15t)
4. C10:0	36. C18:3 (9c,12t,15t)
5. C11:0	37. C18:3 (9c,12t,15c)
6. C12:0	38. C18:3 (9t,12c,15c)
7. C13:0	39. C18:3 (9c,12c,15c)
8. C14:0	40. C18:2 (t9,c11)
9. C14:1(9t)	41. C18:2 (t10,c12) (90%)
10. C14:1	42.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

GC/FID Chromatogram of 63-Component FAMES Standard Mixture

On an Agilent J&W DB-FastFAME column using **hydrogen** as carrier gas

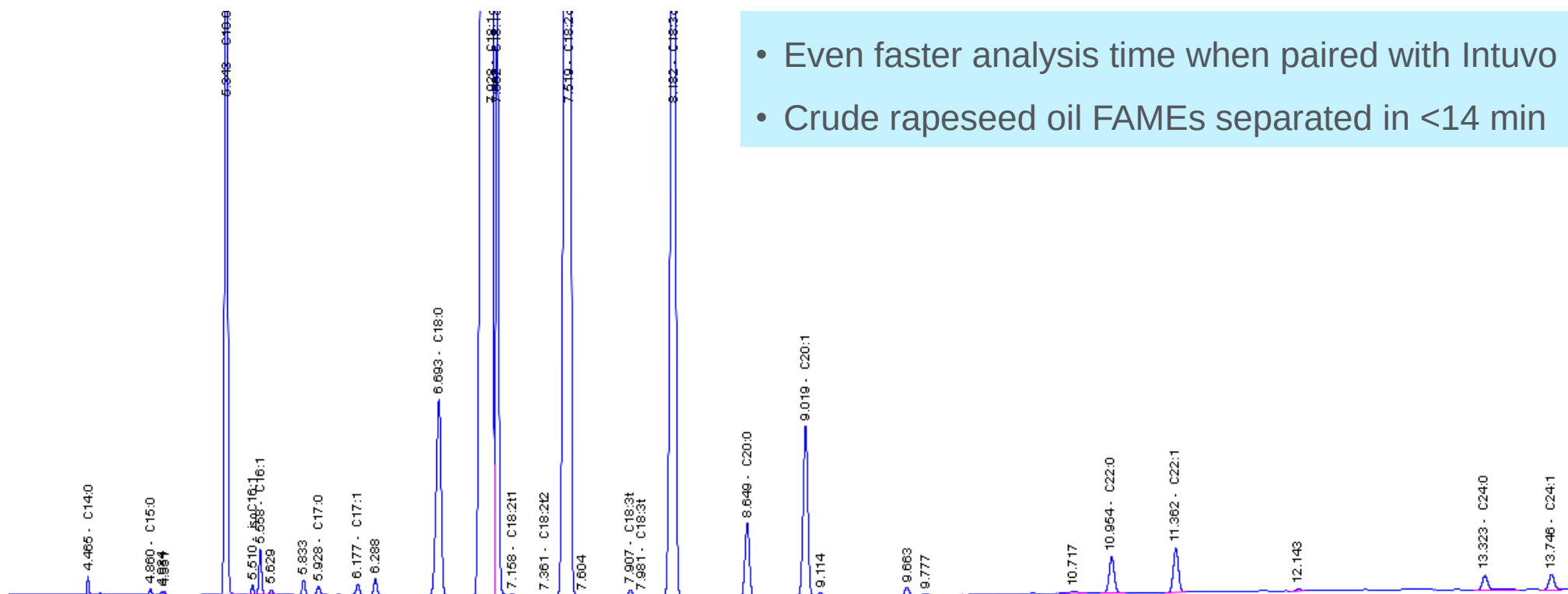
<40 min

Application note: 5994-1862EN



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

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



- Even faster analysis time when paired with Intuvo
- Crude rapeseed oil FAMES separated in <14 min



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

Introducing a novel detector for FAME analysis by GC

LUMA multi-channel vacuum ultraviolet
(VUV)



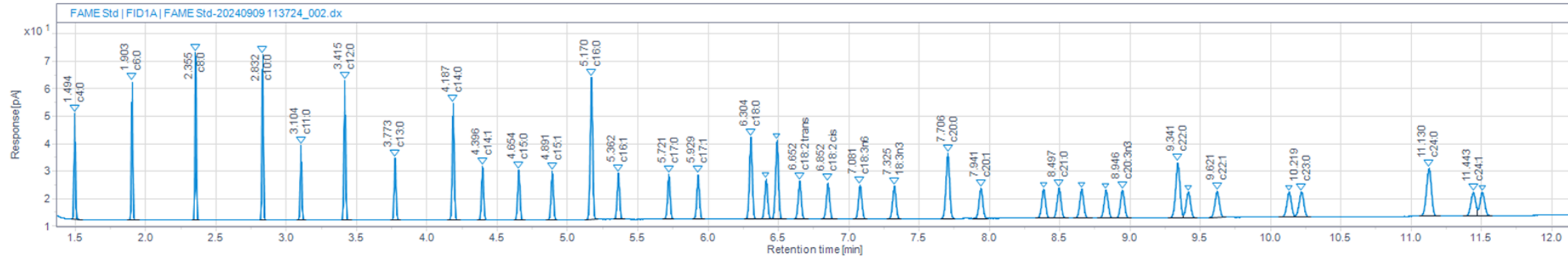
Some features of the LUMA detector

- Uses vacuum ultraviolet light for highly sensitive and selective detection of organic compounds (118-1050 nm over 12 channels)
- Capable of detecting a wide array of substances, including challenging compounds that are often difficult to identify with other detectors
- Excellent for complex matrices, offering clear, interpretable results by effectively ignoring common interferences
- Delivers detection down to parts per billion (ppb), making it ideal for trace-level analysis

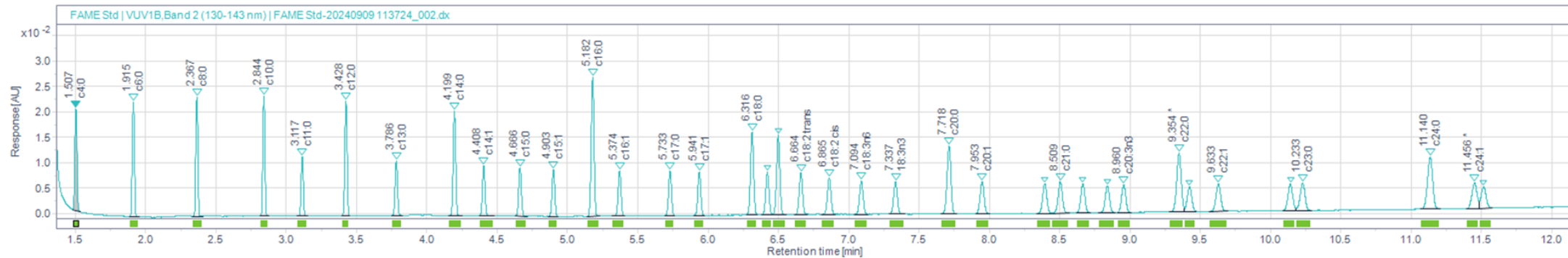


A 37-FAME mix analyzed simultaneously on an FID and LUMA VUV

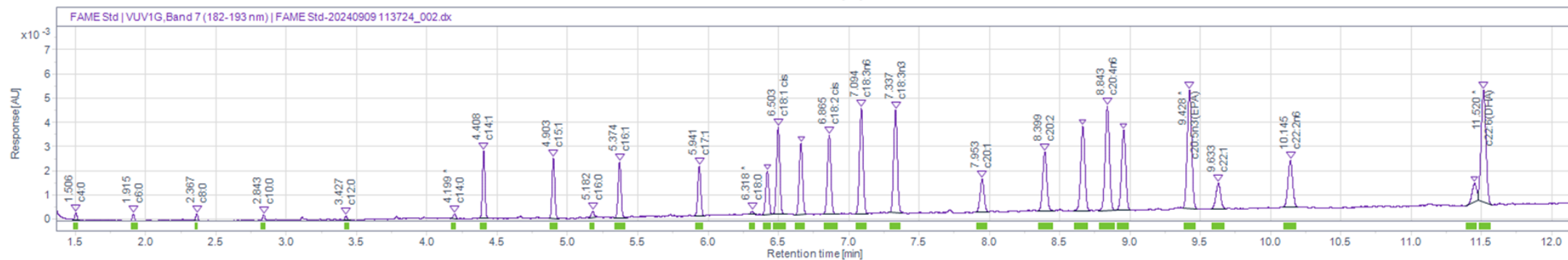
FID



LUMA Band 2
(130 to 143 nm)



LUMA Band 7
(186 to 198 nm)

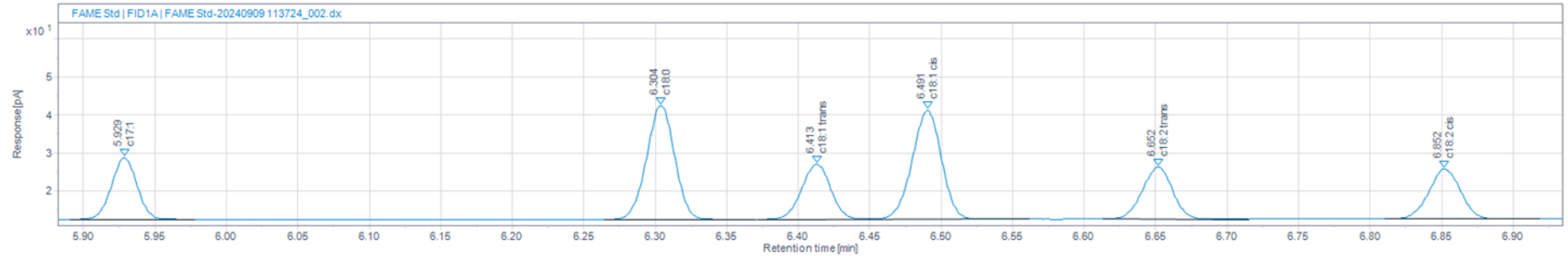


This band shows stronger absorbance for unsaturated fats

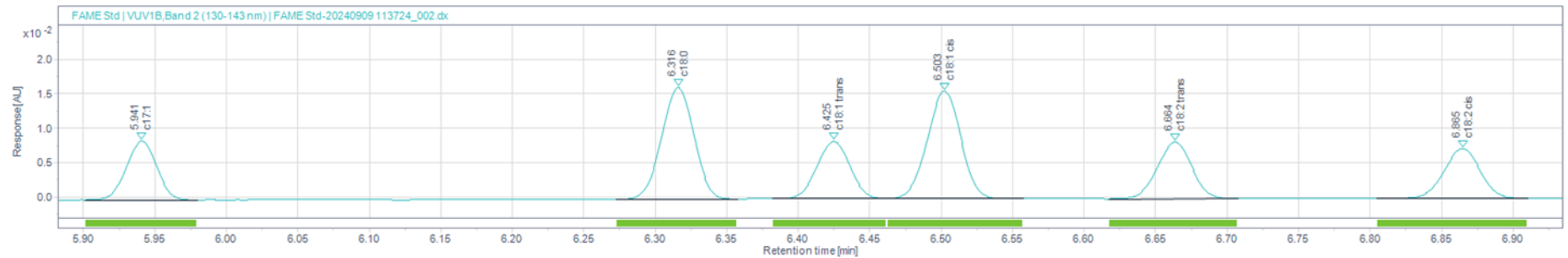
Zoomed-in chromatograms of the C17:1 to C18:3n6 FAMES

- The low absorbance of C18:0 is more evident

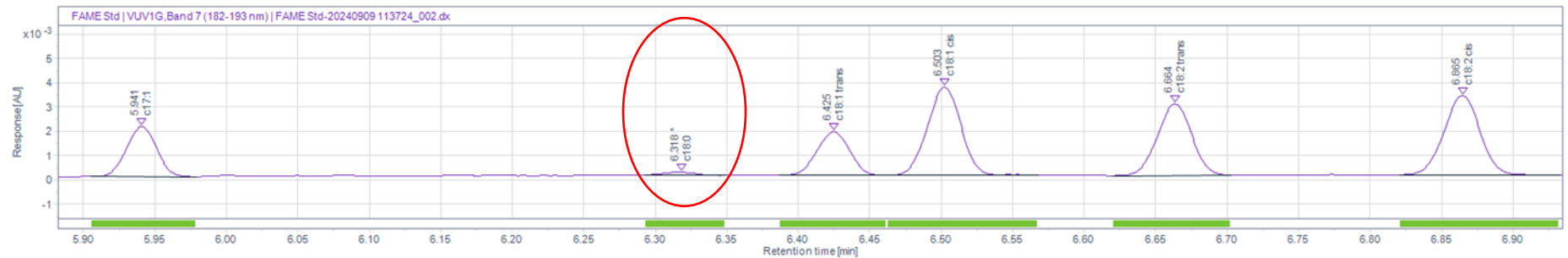
FID



Band 2



Band 7

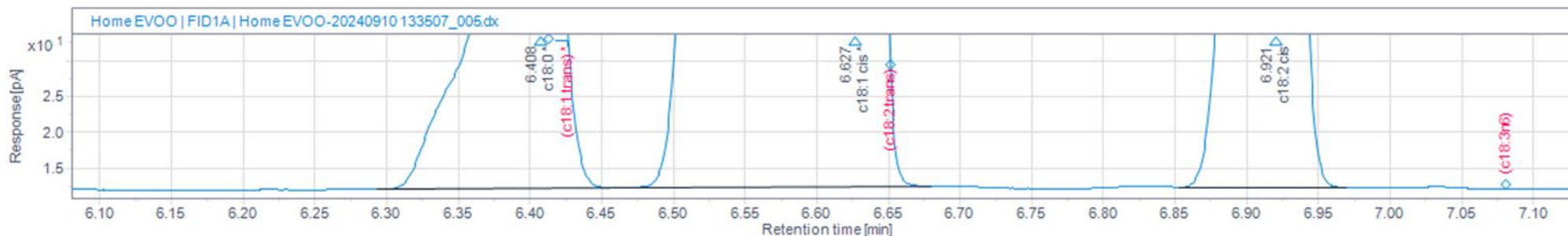


The benefits of the LUMA detector in real samples

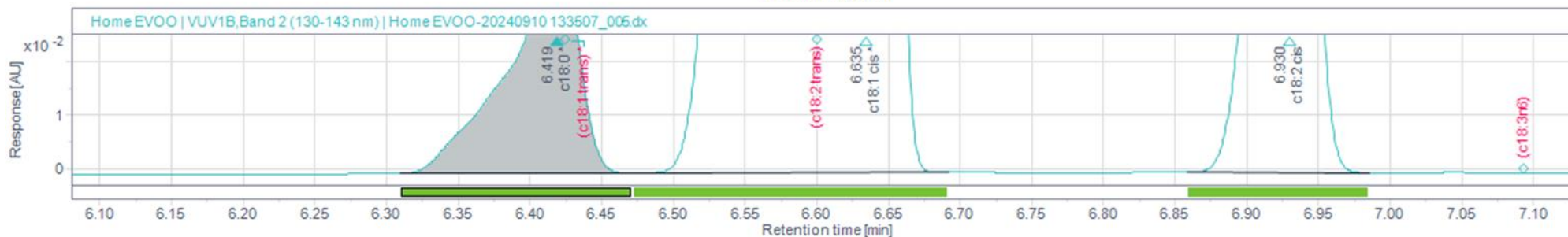
EVOO sample as analyzed on the FID and LUMA bands 2 and 7

- The sample shows a potential co-elution between C18:0 and C18:1t when using GC/FID

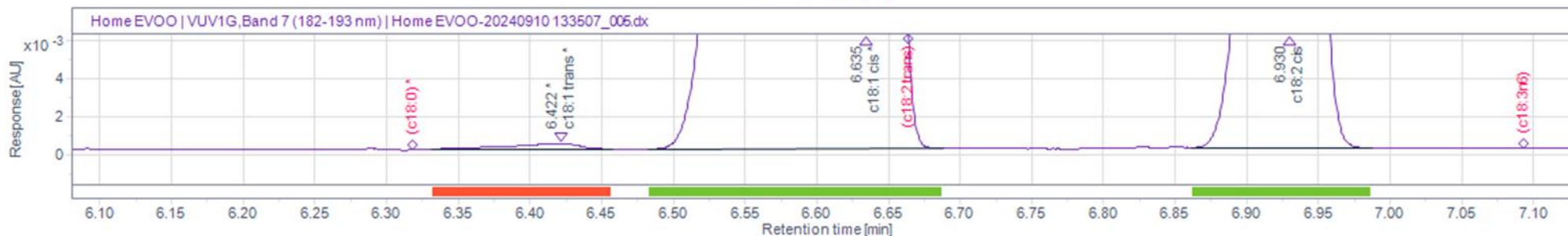
FID



Band 2

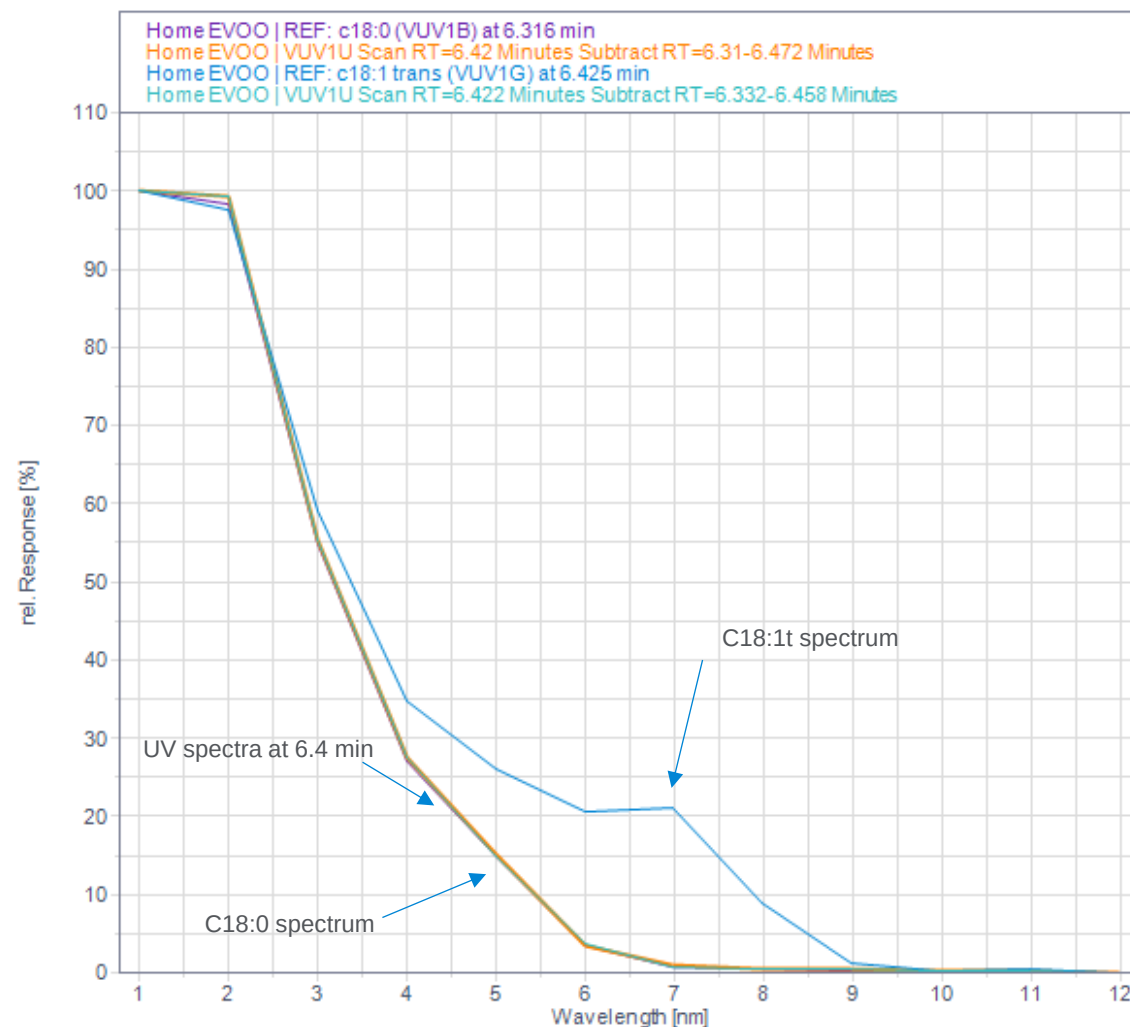


Band 7



The benefits of the LUMA detector in real samples

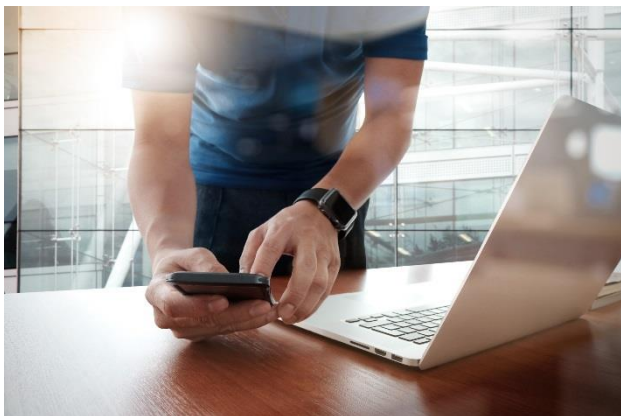
- We can use UV purity criteria for each compound using standards
- This is then match from the UV spectra from the sample at a specific time and compared
- Purity criteria matching is a power tool for peak deconvolution and can be used to confirm the presence of co-elution in the samples.



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
Rapid Separation of Fatty Acid Methyl Esters using DB-FastFAME Intuvo GC columns	5994-0116EN	Application note
Analysis of 37 Fatty Acid Methyl Esters on the Agilent 8890 GC using FID and LUMA detectors	5994-7849EN	Application brief

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?

