Fatty Acid and FAME Analysis Using State-of-the-Art Gas Chromatography

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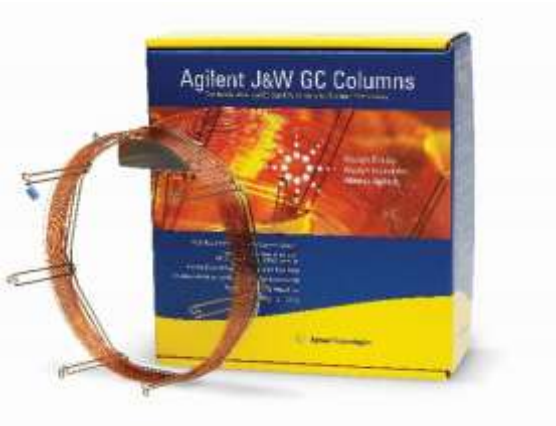

Agilent CrossLab
From Insight to Outcome

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Outline

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- Analysis of Fats and Oils and Column Selection
- DB-FATWAX Ultra Inert
- DB-FastFAME
- DB-HeavyWAX
- Conclusions

The importance of fatty acid analysis

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Quality

Labeling/Regulatory

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 660mg	**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 2,000 calorie diet. 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		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

Adulteration

EMA: Economically Motivated Adulteration

Process Monitoring/Product and assuring economics

Fatty acids commonly found in food samples

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- Milk fat (C4-C24 saturated/unsaturated FAMEs)

- Vegetable oil (hydrogenated and nonhydrogenated)
  - Palm oil, rapeseed oil, olive oil, soybean oil
  - Cooking oils

- Fish oil and meat fat

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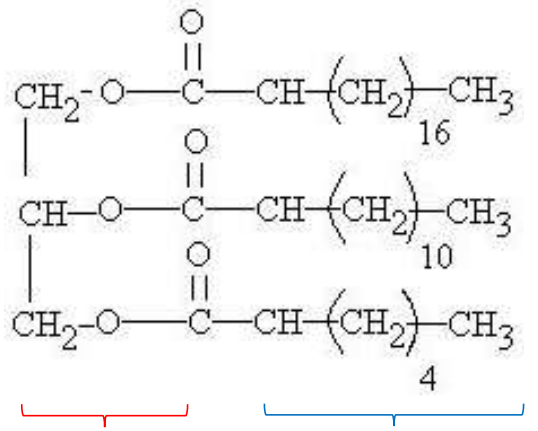
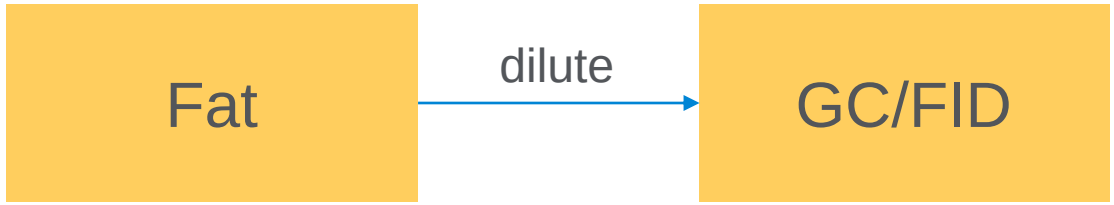
Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

Fat and Oil Analysis - Triglycerides

- Used to identify composition and percentage of each TG present in the sample
- Very easy to prepare and analyze, typically, only a dilution of the oil is required
- Cold on-column injection is required due to the low volatility of the triglycerides
- A typical TG analysis is olive oil adulteration with cheaper seed oils





Triglycerides analysis of butter fat

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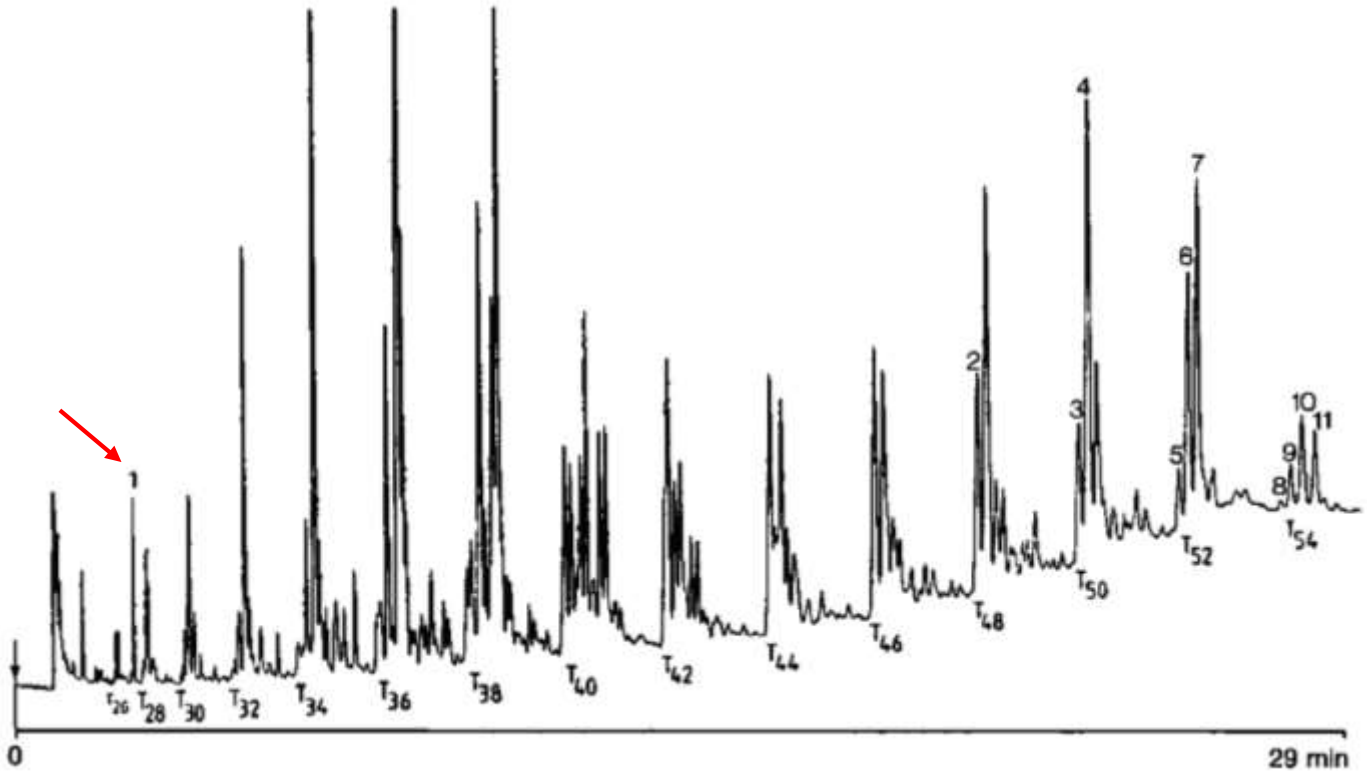
Column	**CP-TAP CB**, 25m, 0.25mm, 0.10µm (p/n CP7483)
Inlet	on-column
Carrier	Hydrogen, 100kPa (1 bar, 15 psi)
Oven	280 ºC (1 min) to 355 ºC at 3 ºC/min
Detector	FID
Injection	0.2 uL
Sample	0.05% butter fat in hexane

CP-TAP CB for Triglycerides

P: Palmitic acid, (hexadecanoic acid)	C16:0
S: Stearic acid (octadecanoic acid)	C18:0
O: Oleic acid (cis-9-octadecenoic acid)	C18:1

Peak No

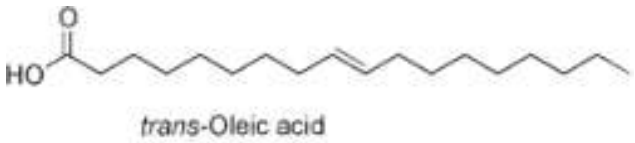
1. **Cholesterol**
2. PPP
3. PPS
4. PPO
5. PSS
6. PSO
7. POO
8. SSS
9. SSO
10. SOO
11. OOO



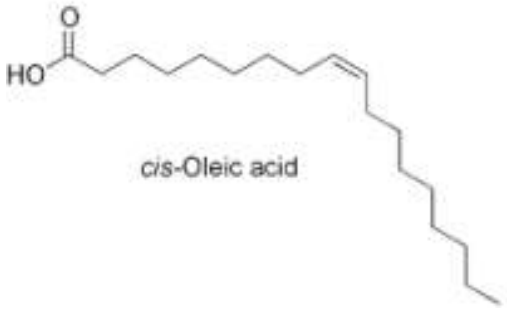
Application Note: A0223

Fat and Oil Analysis – Free Fatty Acids

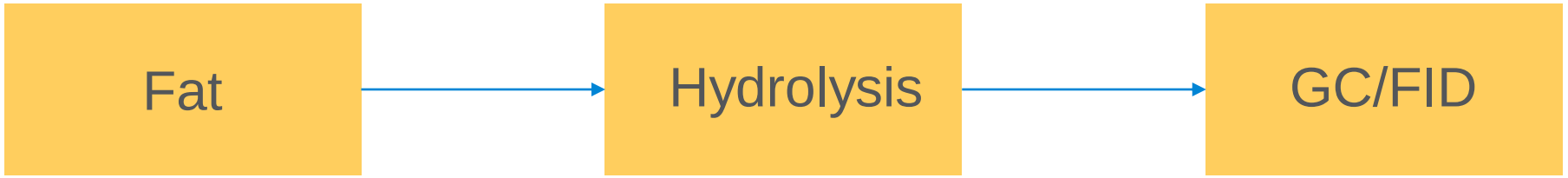
- Faster and easier sample preparation
- Traditionally small-chain or volatile fatty acids are analyzed in free form
- Usually difficult to analyze due to the carboxylic acid
- Typically requires specialized column such as acid-modified wax phases



trans-Oleic acid



cis-Oleic acid



Analysis of free fatty acids

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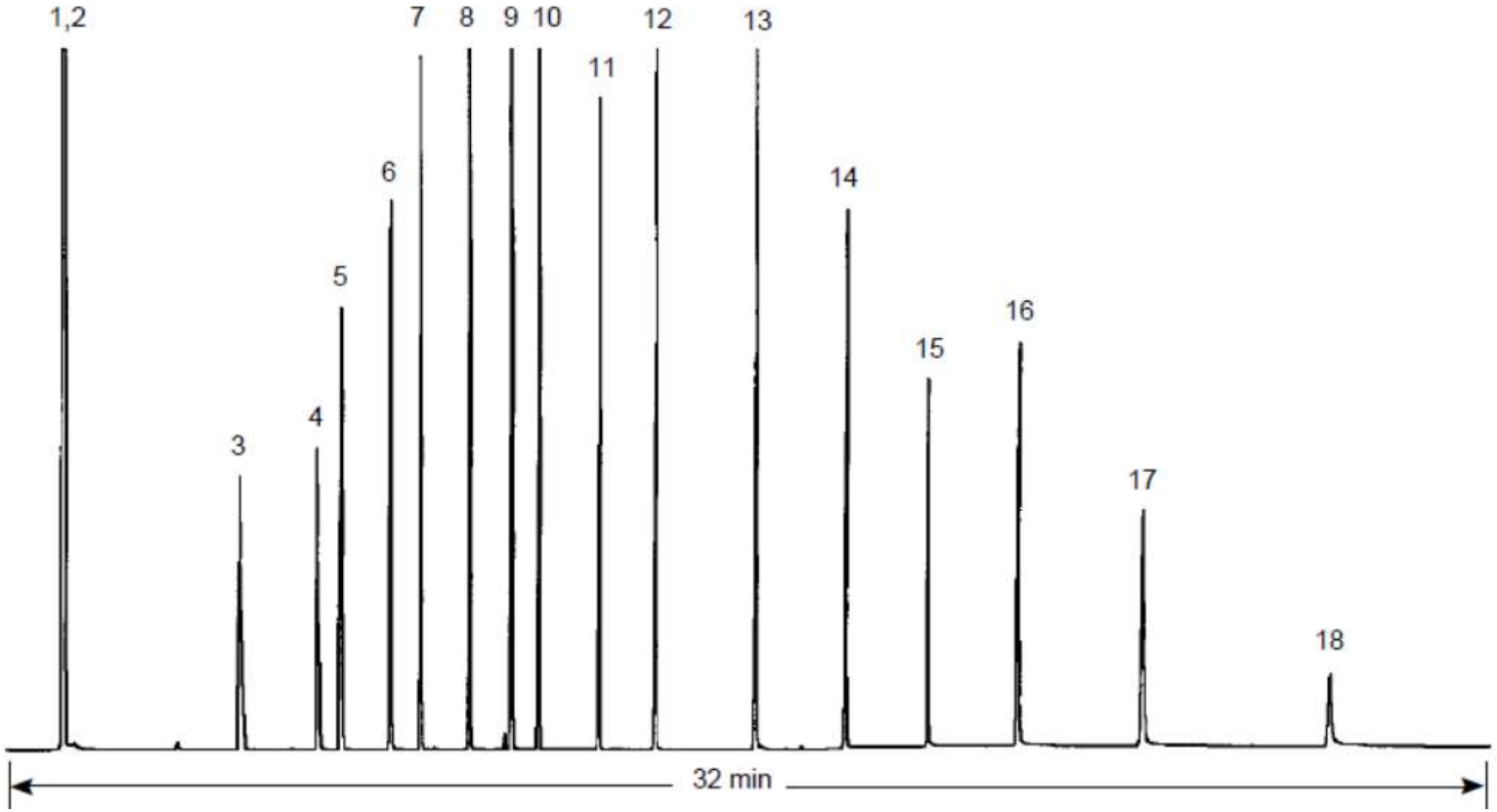
Column	**DB-FFAP**, 30m, 0.25mm, 0.25µm (p/n 122-3232)
Carrier	Helium at 40 cm/sec, measured at 100 °C
Oven	100 ºC (5 min) to 250 ºC (12 min) at 10 ºC/min
Detector	FID, 300 °C
	Nitrogen makeup gas at 30 mL/min
Injector	Split 1:50, 250 °C

DB-FFAP

Chromatogram C077

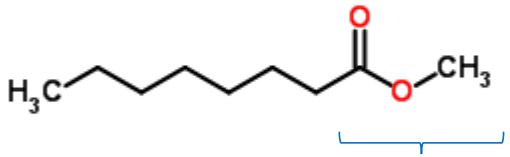
Peak No

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

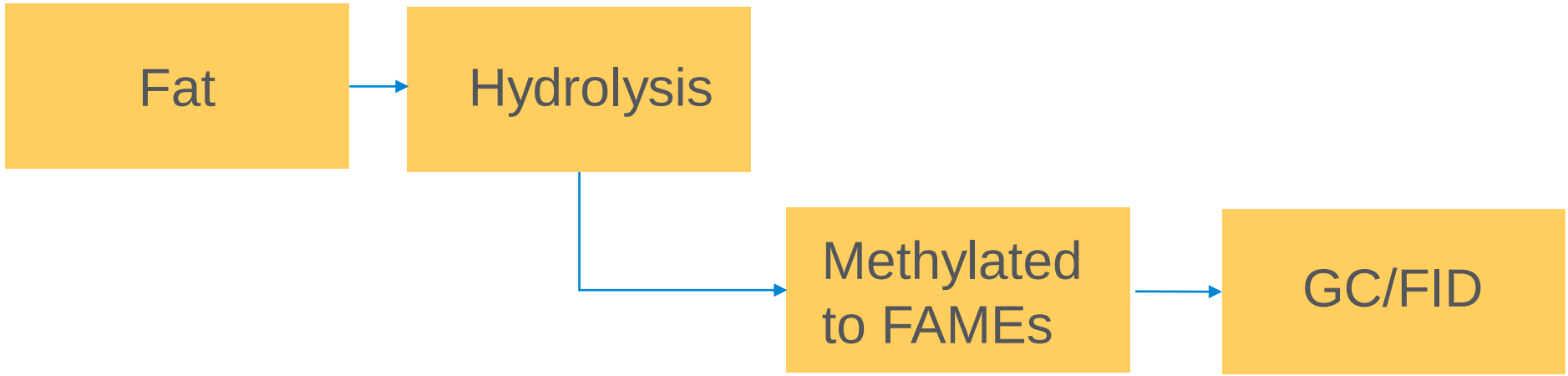


Fat and Oil Analysis - FAME

- Transesterification of free fatty acids is the most preferred method for fat analysis
- Allows to distinguish slight differences among unsaturated fatty acids (e.g. positional geometric isomers)
- Generally, sample preparation requires a few complex steps



ester group



Traditional FAME analysis on a CP-Sil 88

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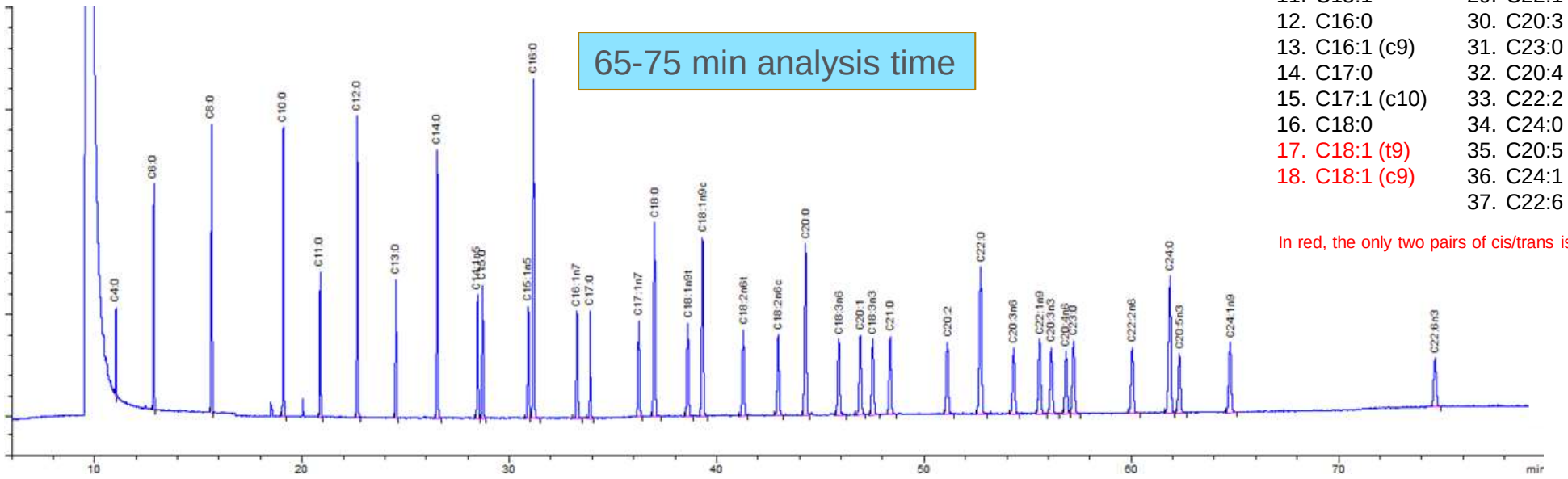
- Most common method, suggested by AOAC 996.06 and AOCS Ce 1j-07
- 100-m cyanopropyl columns are optimized for positional isomer separation of critical cis/trans FAMEs
- Analysis time typically in the 65-76 min range

CP-Sil 88

FAMES by AOAC 996.06 using 100-m CP-Sil 88



Peak No

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)

In red, the only two pairs of cis/trans isomers

Detailed Analysis of cis/trans FAMEs C18:1 positional isomers

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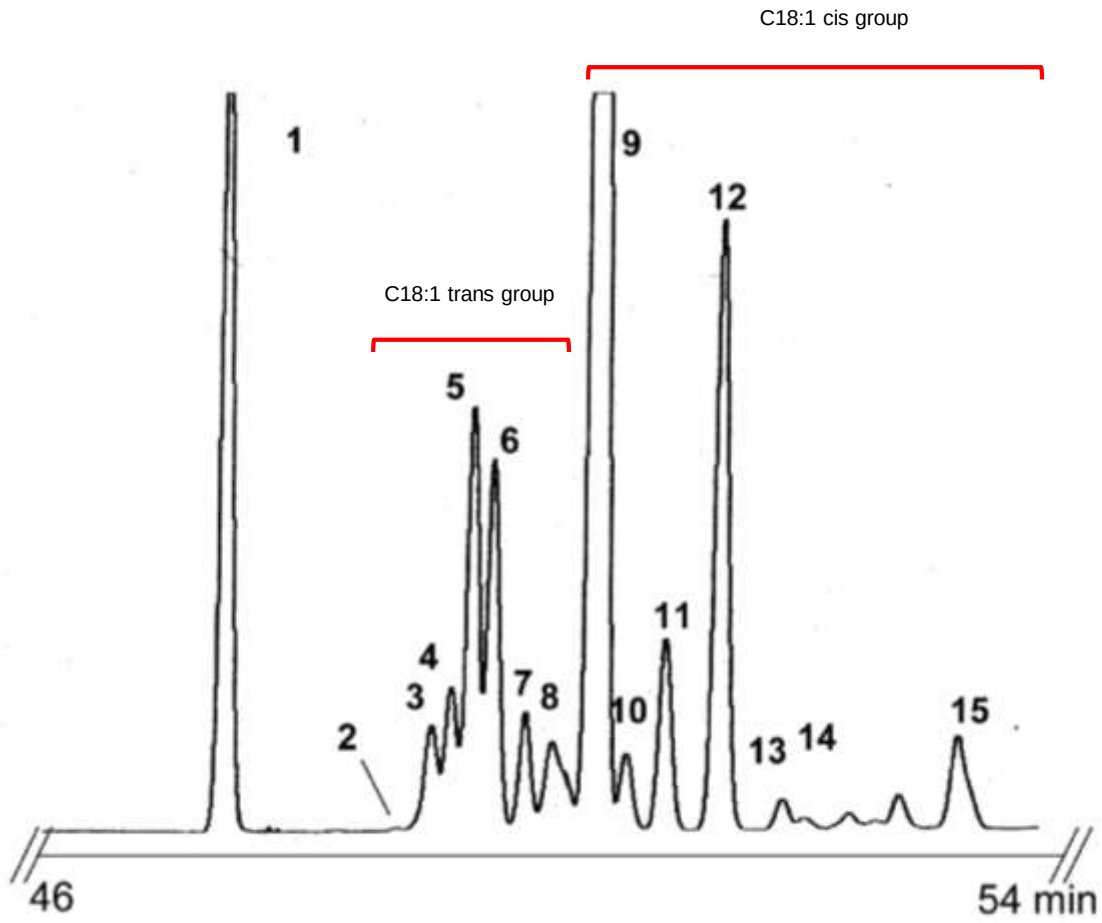
Column	**Select FAME, 200-m,** 0.25 mm i.d. (P/N CP7421)
Inlet	250°C, split mode, split ratio 1:20
Carrier	Helium, 520 kPa
Oven	185°C
FID	250°C
Injection	0.5 µL

Select-FAME

Peak No

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



Comprehensive Portfolio for Fatty Acids, FAME and Oils Analysis

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Fatty Acids & Oils Analysis

DB-FFAP

- Traditional phase for free fatty acids
- Modified with nitroterephthalic acid

Consistent results for fatty acids

DB-FATWAX UI

- Free fatty acids, C4-C16
- Simple mixtures
- Omega 3 and 6 analysis, chain length/degree of unsaturation separation
- Good reproducibility
- Superior inertness for difficult samples (food matrix)

Simple, reliable, and multi-purpose

DB-FastFAME

- Fast Separation of cis/trans isomers
- No cis/trans overlap with right conditions
- 22-24 min analysis
- Faster than conventional CP-Sil 88

Faster than traditional methods

CP-Sil 88 for FAME/ HP-88

- Full resolution of cis/trans FAMEs
- As suggested in AOAC 996.06

AOCS approved

Select FAME

- Highly detailed analysis of cis/trans FAME isomers
- Best GC column for GC/MS applications

The ultimate in high efficiency

CP-TAP CB

- Mono-, di- and triglycerides analysis
- Optimal selectivity for triglycerides

High-temp applications

DB-FATWAX Ultra Inert

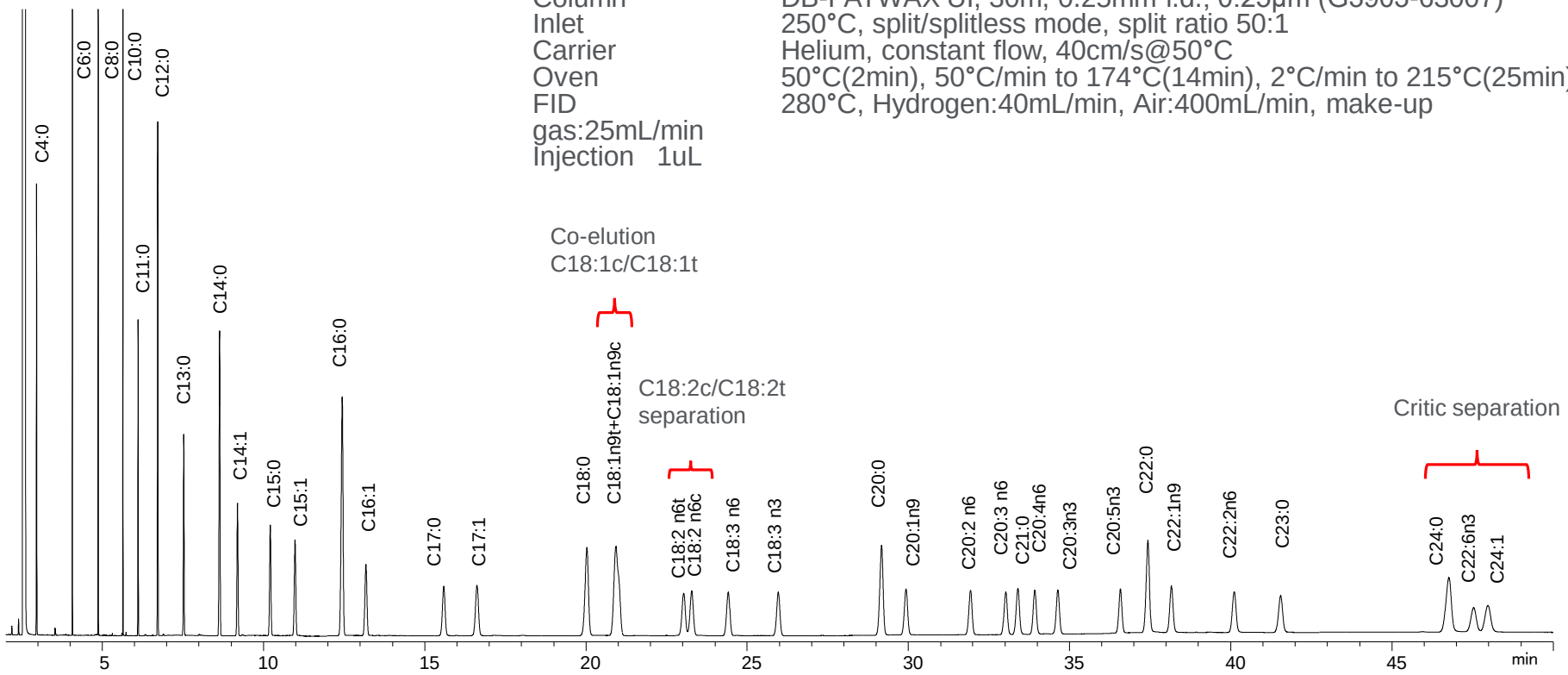
For the analysis of:

- FAMEs including Omega 3 and Omega 6
- Small organic acids and free fatty acids
- Applications in fish oil and animal fat analysis

37-FAME mix analysis with DB-FATWAX Ultra Inert

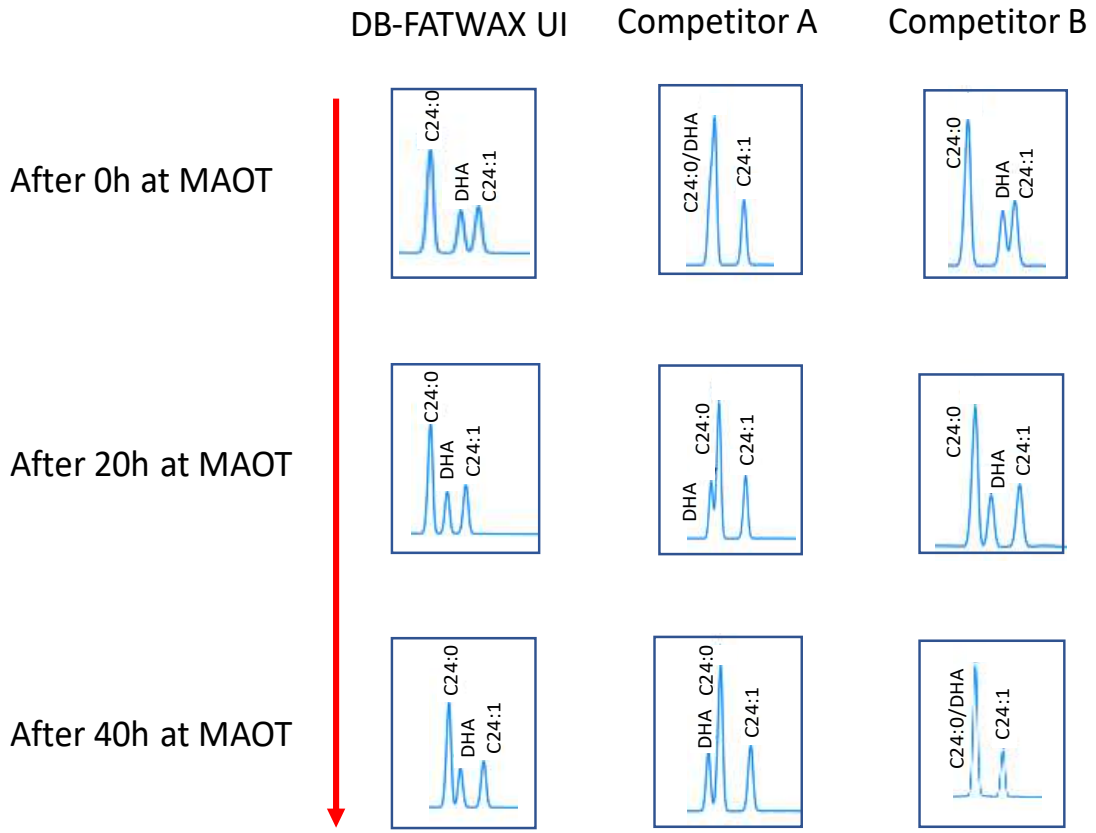
- Best option for saturated and polyunsaturated FAMEs, including Omega 3 and Omega 6
- WAX-type selectivity not ideal for cis/trans separation

GC System	Agilent 7890B
Column	DB-FATWAX UI, 30m, 0.25mm i.d., 0.25µm (G3903-63007)
Inlet	250°C, split/splitless mode, split ratio 50:1
Carrier	Helium, constant flow, 40cm/s@50°C
Oven	50°C(2min), 50°C/min to 174°C(14min), 2°C/min to 215°C(25min)
FID	280°C, Hydrogen:40mL/min, Air:400mL/min, make-up
gas:25mL/min	
Injection 1uL	



Comparison study of the C24:0/C22:6n3 (DHA) critical pair

- DB-FATWAX UI fully resolves the C24:0/DHA critical pair
- It remains stable with no change in selectivity or retention time even after 40 h at MAOT.

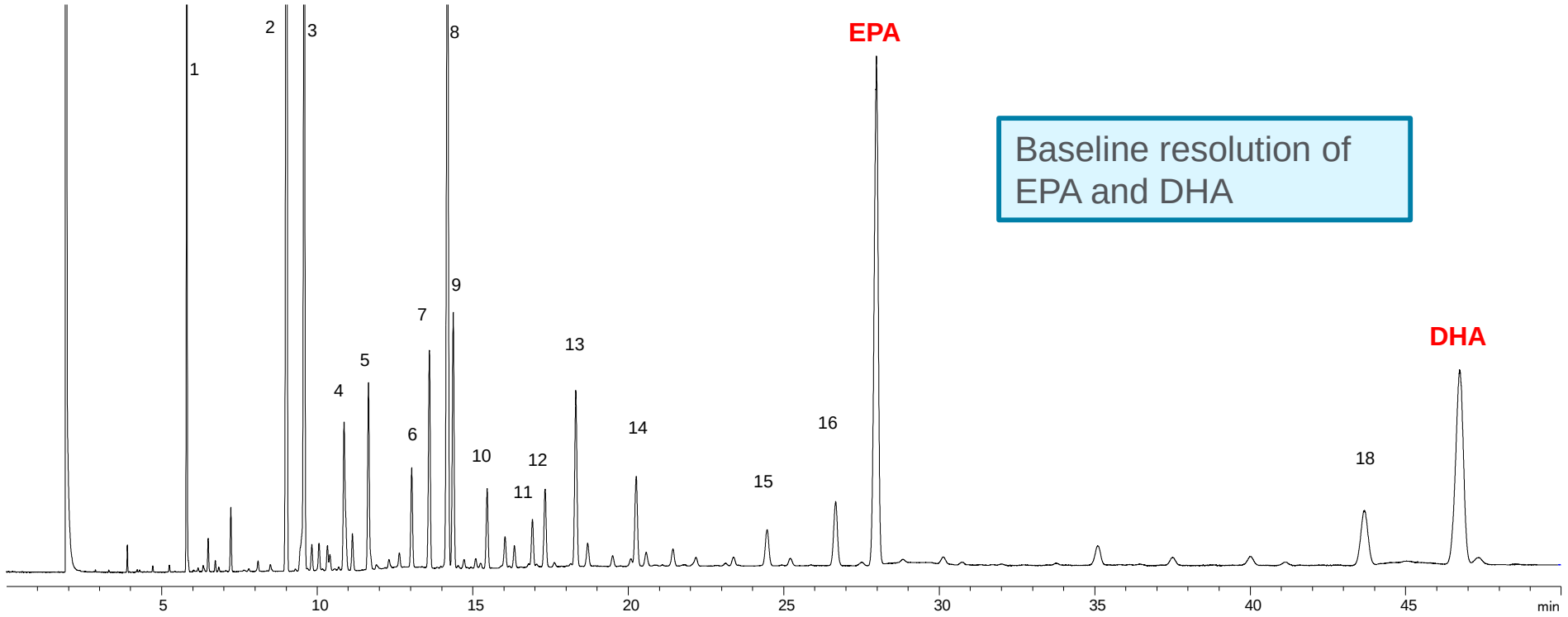


*MAOT: maximum allowable operating temperature

Analysis of real samples

Column	DB-FATWAX UI, **30m, 0.25mm i.d., 0.25µm** (G3903-63007)
Inlet	250°C, split/splitless mode, split ratio 100:1
Carrier	Helium, constant flow, 30cm/s@180°C
Oven	180°C(2min), 2°C/min to 210°C(35min)
FID	280°C, Hydrogen:40mL/min, Air:400mL/min, make-up gas:25mL/min
Injection	1uL
Sample	PUFA No.3 (diluted)

Menhaden Oil analysis following AOAC method



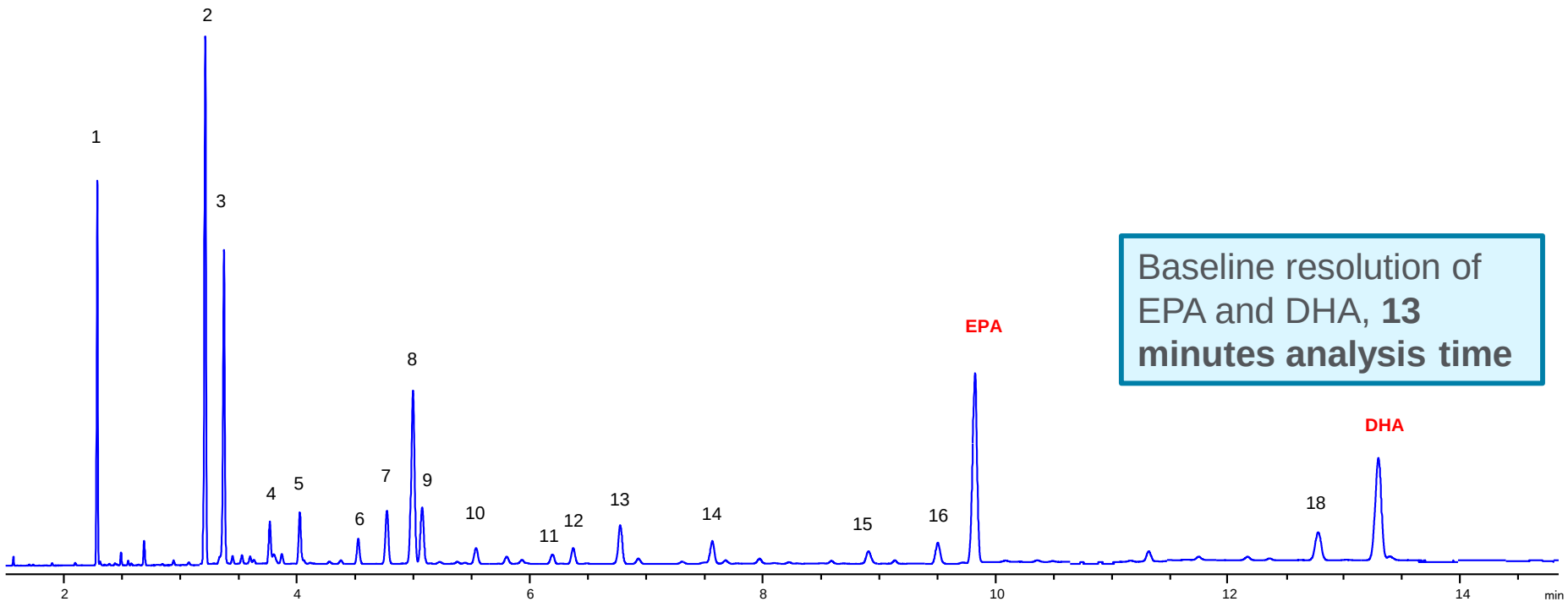
Baseline resolution of EPA and DHA

1. C14:0
2. C16:0
3. C16:1n7
4. C16:2n4
5. C16:3n4
6. C16:4n1
7. C18:0
8. C18:1n9
9. C18:1n7
10. C18:2n6
11. C18:3n3
12. C18:3n4
13. C18:4n3
14. C20:1n9
15. C20:4n6
16. C20:4n3
17. C20:5n3 (EPA)
18. C22:5n3
19. C22:6n3 (DHA)

Analysis of real samples

Column	DB-FATWAX UI, **20m*0.18mm I.D, 0.18um**
Inlet	250°C, split/splitless mode, split ratio 100:1
Carrier	Hydrogen, constant flow, 2mL/min
Oven	140°C,20°C/min to 190°C(3min), 5°C/min to 220°C(15min)
FID	250°C, Hydrogen:40mL/min, Air:400mL/min, make-up gas:25mL/min
Injection	1uL

Optimize separation of Menhaden Oil using a fast GC method



Baseline resolution of EPA and DHA, **13 minutes analysis time**

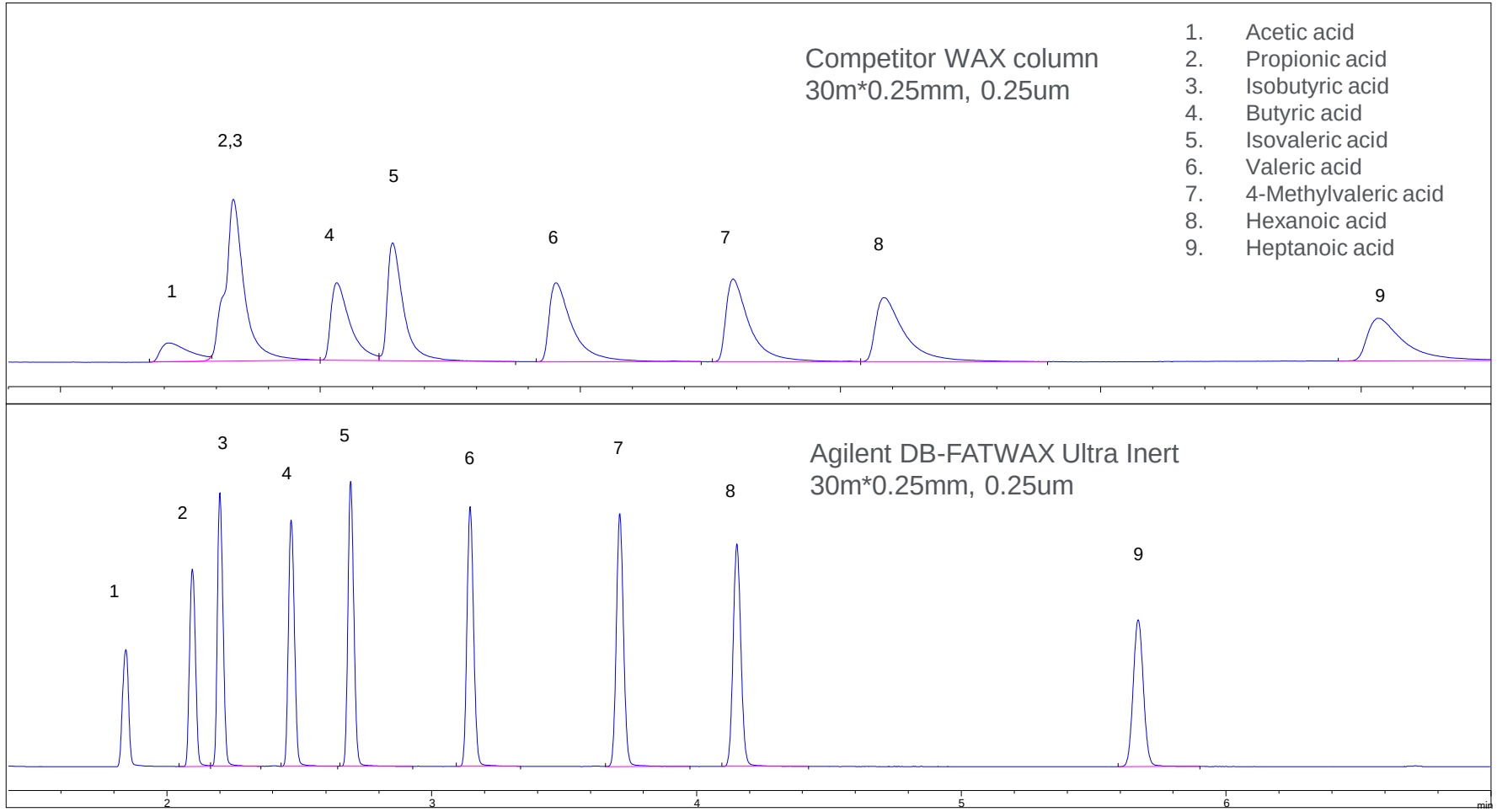
1. C14:0
2. C16:0
3. C16:1n7
4. C16:2n4
5. C16:3n4
6. C16:4n1
7. C18:0
8. C18:1n9
9. C18:1n7
10. C18:2n6
11. C18:3n3
12. C18:3n4
13. C18:4n3
14. C20:1n9
15. C20:4n6
16. C20:4n3
17. C20:5n3
18. C22:5n3
19. C22:6n3

DB-FATWAX UI provides the desire inertness and thermal stability to separate challenge organic acids and fatty acids

- Great need in the food, forensic and cosmetic industries to monitor the content of free and natural occurring fatty acids.
- Analysis of underivatized organic acids and free acids is desirable to eliminate the problems associated with derivatization, extraction, and cleanup procedures
- Volatile organic acids and fatty acids are difficult to quantify accurately by standard WAX columns. These acids often elute as tailing or poorly resolved peaks. For some acids, adsorption can become irreversible.
- Normally:
  - Fatty acids are derivatized to the methyl ester (FAME)
  - Free fatty acids are analyzed using acid-deactivated wax columns. The acid modifier, nitroterephthalic acid, however, reduces thermal stability, operating temperature and reacts with humidity, reducing column life time

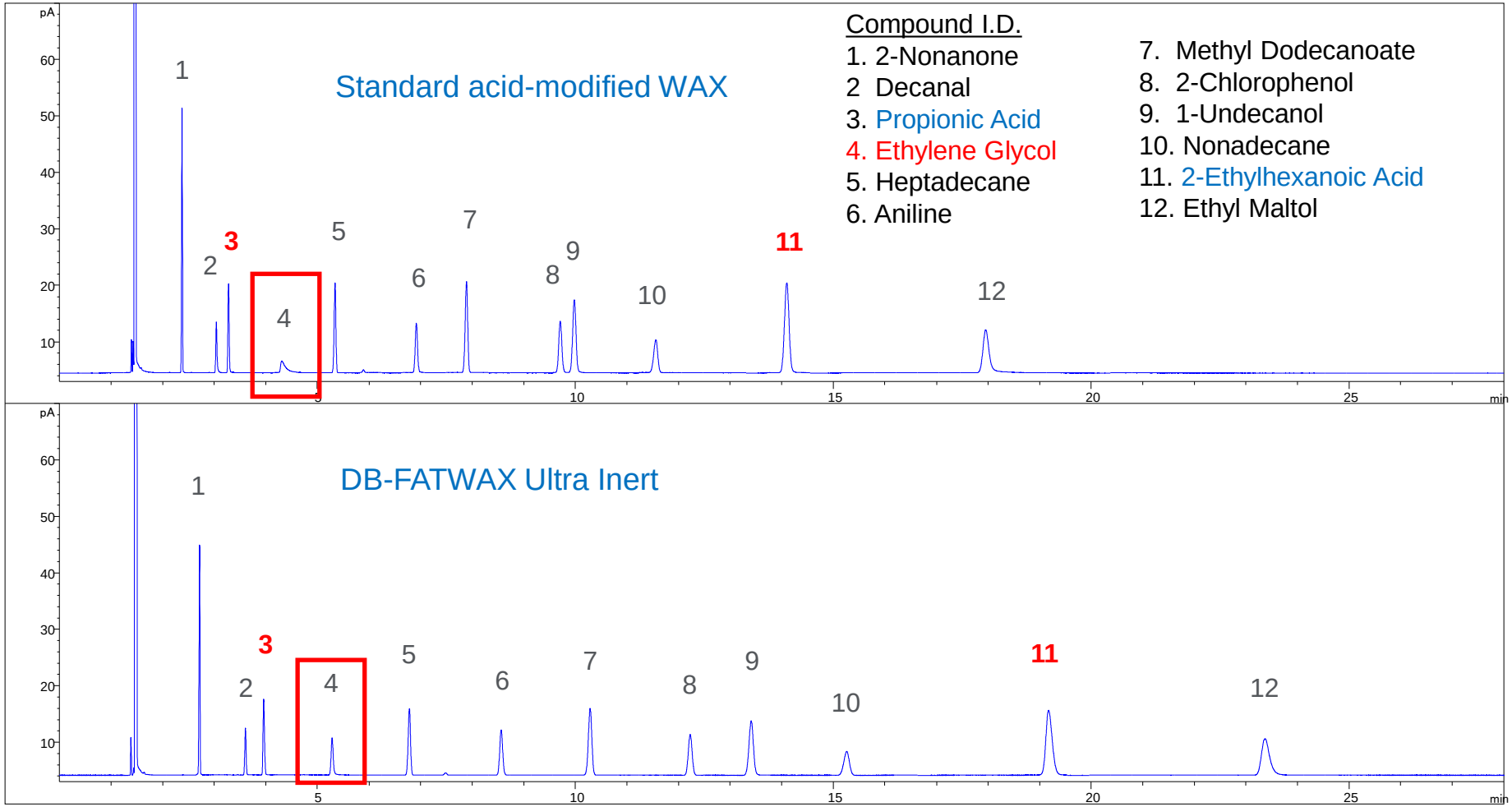
Separation of Short-chain volatile organic acids in water using a competitor WAX column and DB-FATWAX Ultra Inert

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



Comparison between acid-modified WAX and DB-FATWAX UI after 50h at 250 ºC

DB-FATWAX Ultra Inert shows superior inertness and thermal stability than acid-modified WAX

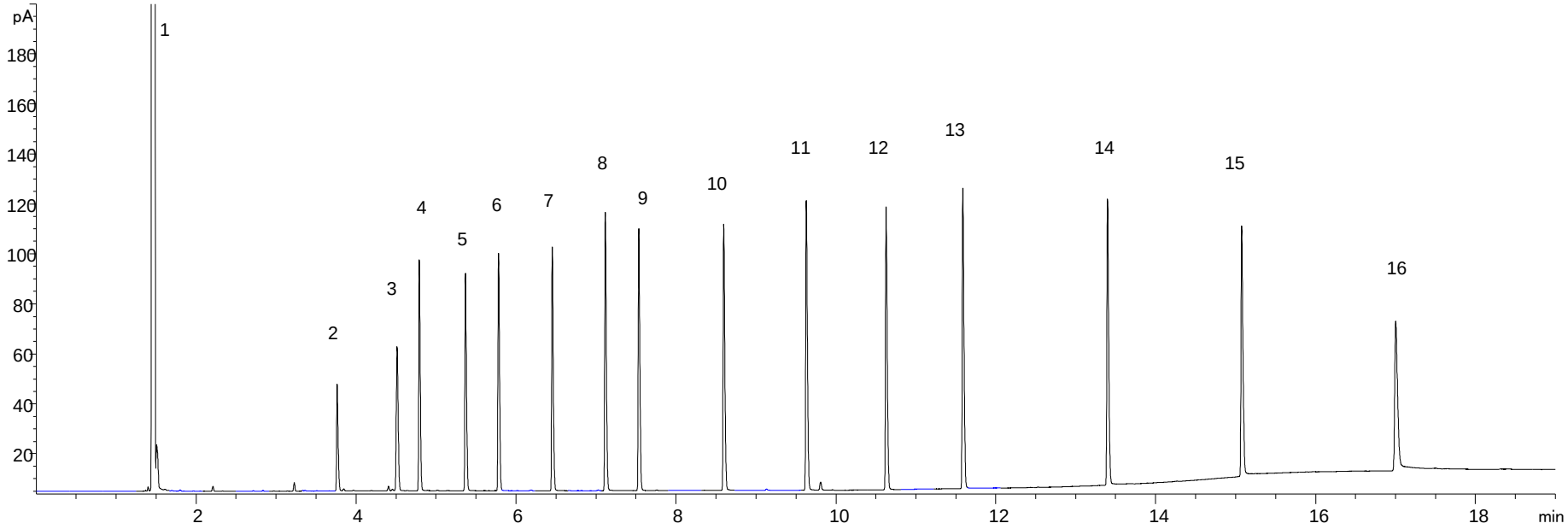


Analysis of Fatty acids (FA)

- Analysis of most FA and FAMEs without the need of an acid-modified WAX phase

1. Acetone
2. Acetic acid
3. Propionic acid
4. Isobutyric acid
5. Butyric acid
6. Isovaleric acid
7. Valeric acid
8. 4-Methylvaleric acid
9. Hexanoic acid
10. Heptanoic acid
11. Octanoic acid
12. Nonanoic acid
13. Decanoic acid
14. Lauric acid
15. Myristic acid
16. Palmitic acid

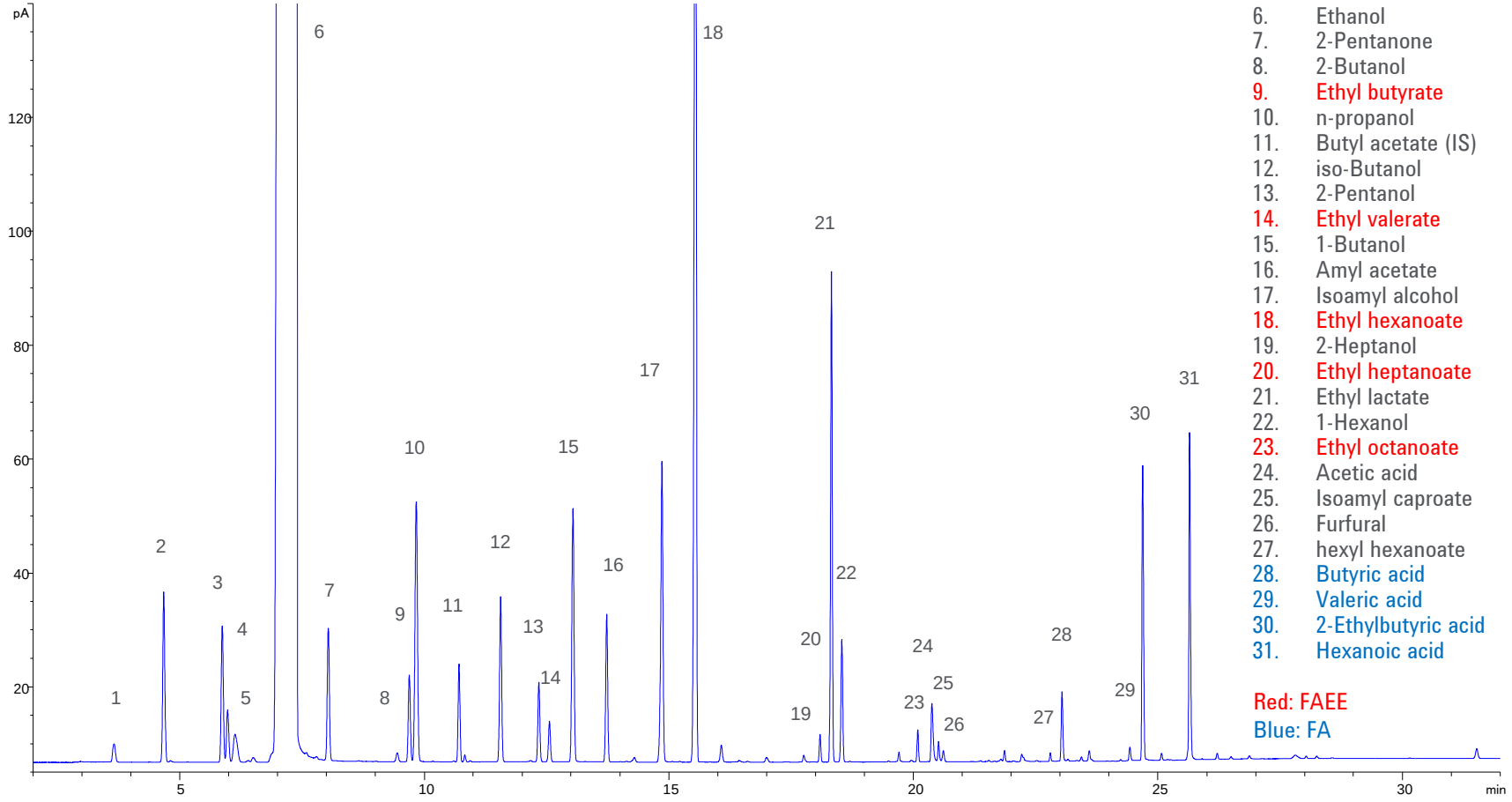
Column: Agilent DB-FATWAX UI, 30m, 0.25mm, 0.25µm (G3903-63007)
Carrier gas: Helium, 40cm/s @ 100°C
Oven: 100°C, to 250°C @10°C/min; 250°C (10min)
Inlet: 250°C,split ratio= 50:1,
FID: 280°C
Injection vol.: 1ul



Fatty acids and naturally ocurring fatty acid ethyl esters analysis in alcoholic beverages

Chinese liquor sample

62% aqueous sample
Good resolution and peak shape



Peak No	Compounds
1.	Acetaldehyde
2.	Acetone
3.	Ethyl acetate
4.	Acetal (acetaldehyde diethyl acetal)
5.	Methanol
6.	Ethanol
7.	2-Pentanone
8.	2-Butanol
9.	Ethyl butyrate
10.	n-propanol
11.	Butyl acetate (IS)
12.	iso-Butanol
13.	2-Pentanol
14.	Ethyl valerate
15.	1-Butanol
16.	Amyl acetate
17.	Isoamyl alcohol
18.	Ethyl hexanoate
19.	2-Heptanol
20.	Ethyl heptanoate
21.	Ethyl lactate
22.	1-Hexanol
23.	Ethyl octanoate
24.	Acetic acid
25.	Isoamyl caproate
26.	Furfural
27.	hexyl hexanoate
28.	Butyric acid
29.	Valeric acid
30.	2-Ethylbutyric acid
31.	Hexanoic acid

Red: FAEE
Blue: FA

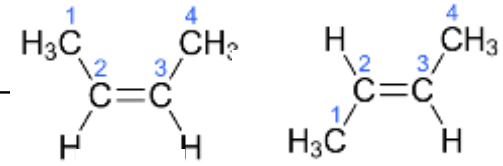
DB-FastFAME

For the analysis of:

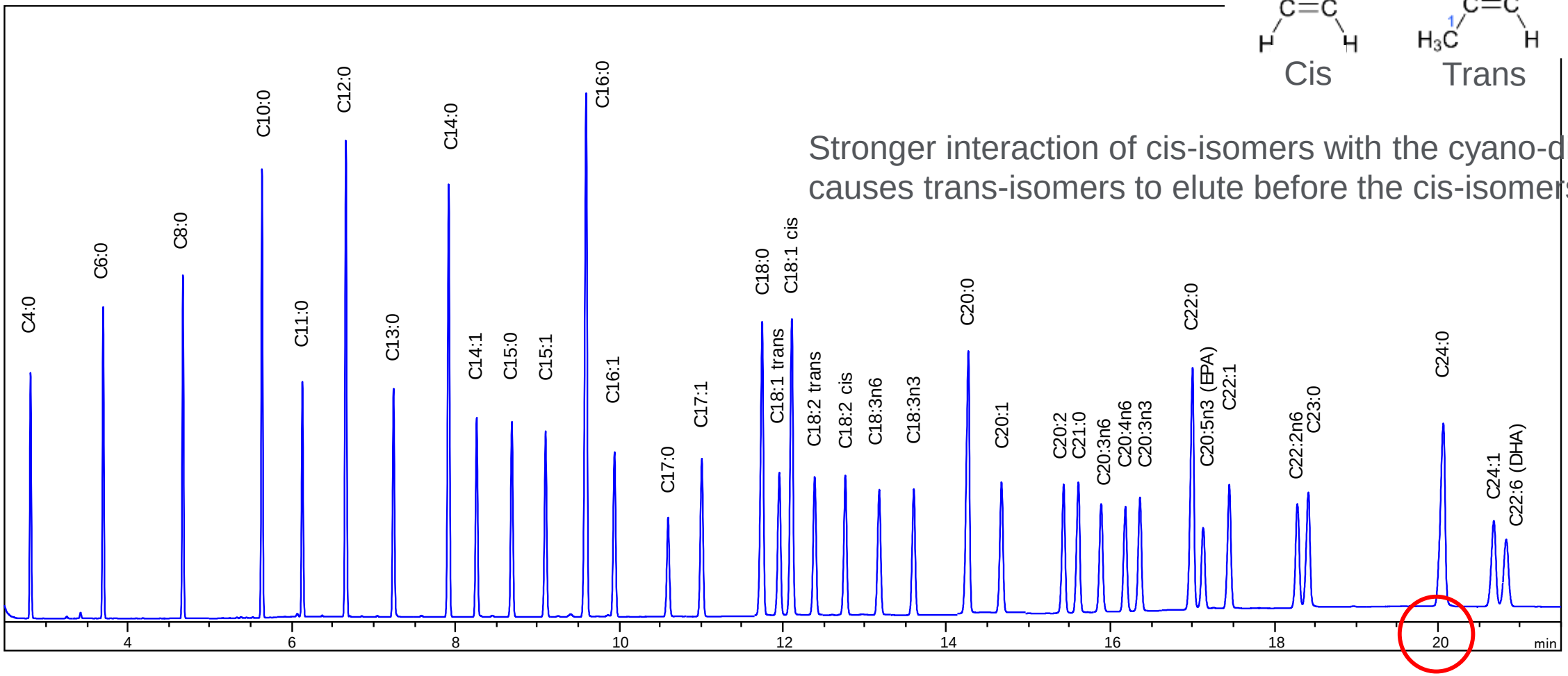
- Ultra fast FAME analysis
- < 10 min analysis time for complex FAME mixtures

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

Technical note 5991-8706EN



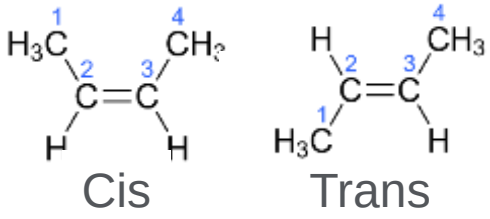
Stronger interaction of cis-isomers with the cyano-dipole causes trans-isomers to elute before the cis-isomers



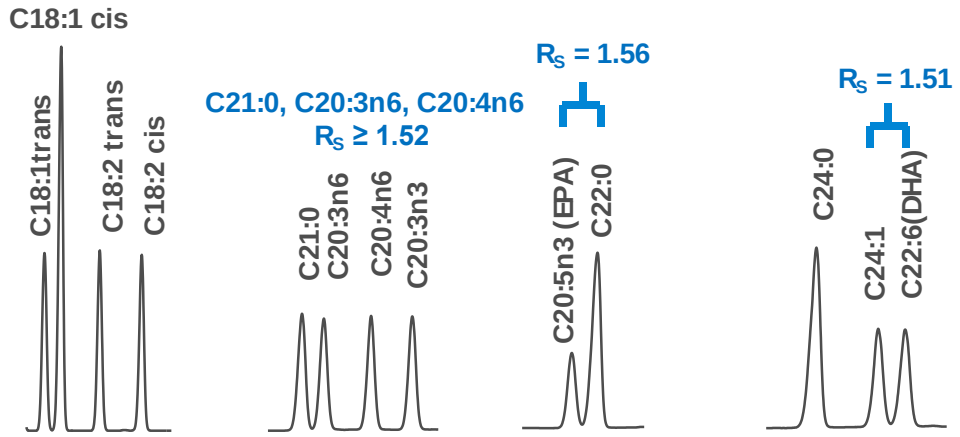
DB-FastFAME
20m x 0.18mm x 0.20µm

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; make-up gas: 25 mL/min.
Injection	1 µL

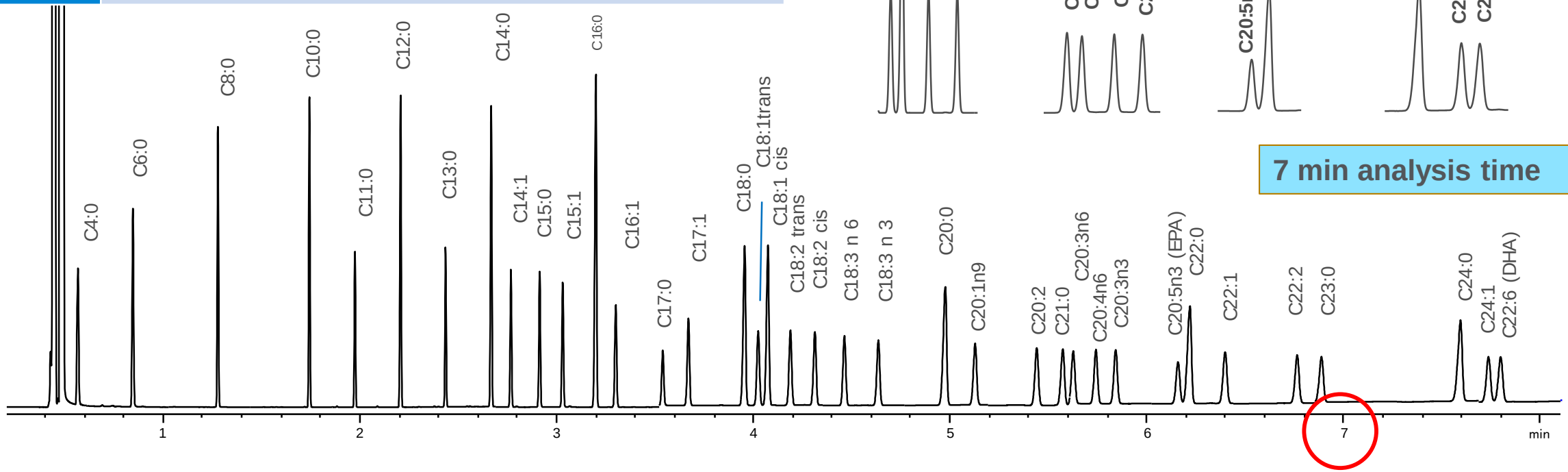
Strong interaction between cis isomers and the dipoles of the cyano propyl ligands. That allows the trans to elute after the cis isomers.



$R_S \geq 1.95$ 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

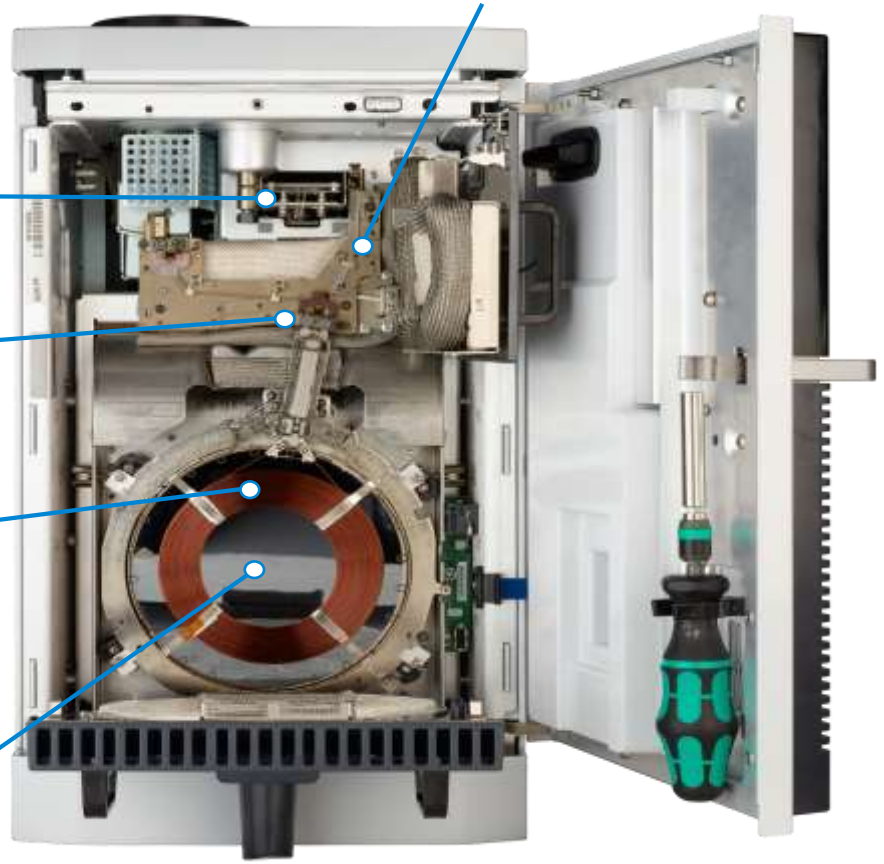
Disposable Guard chip

Ferrule-free click-and-run connections

No-trim column

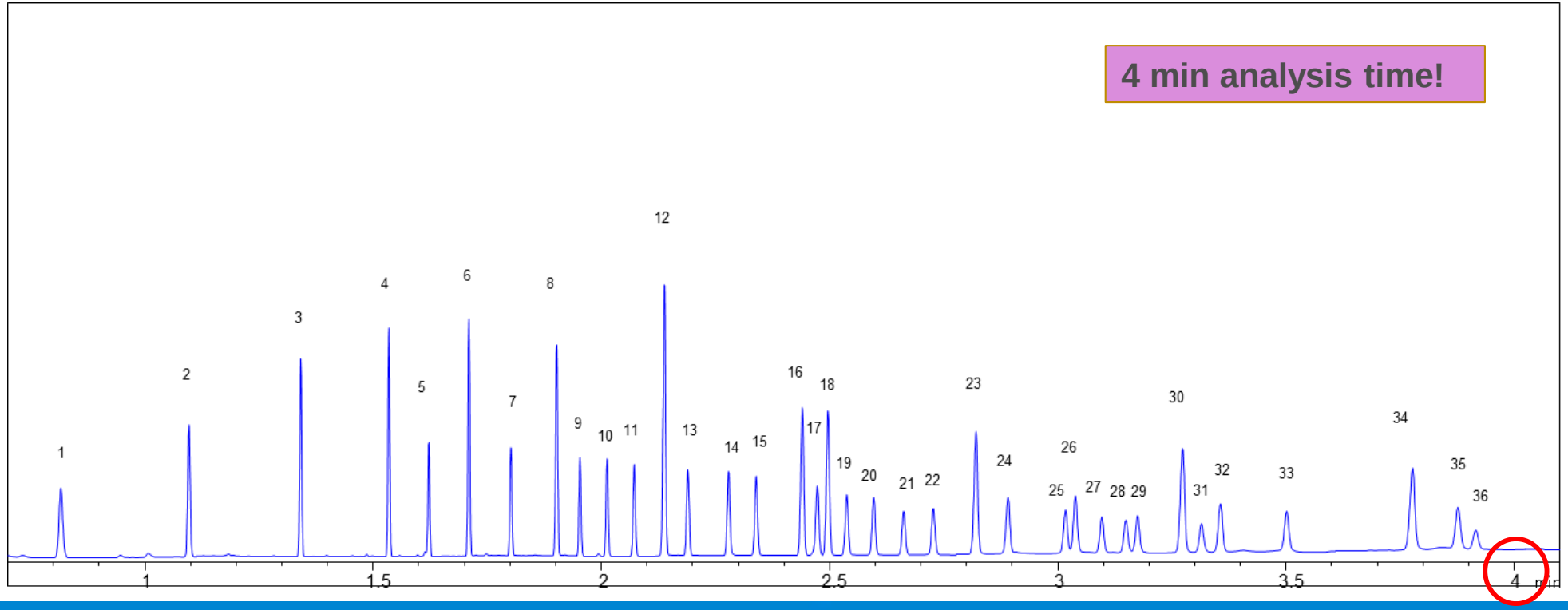
Direct heating

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, 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



Agilent CrossLab
From Insight to Outcome

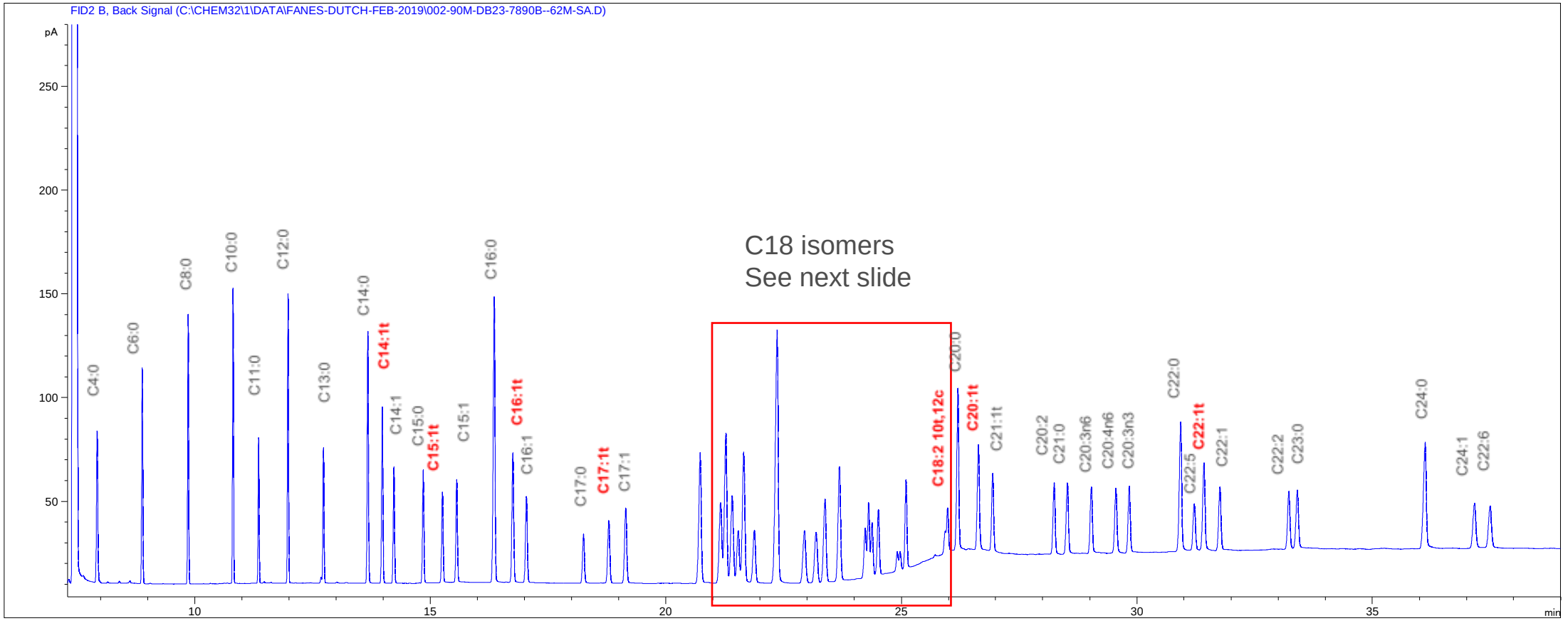
How about more complex FAME samples like PHVO?

New DB-FastFAME GC column dimensions coming soon…

Agilent

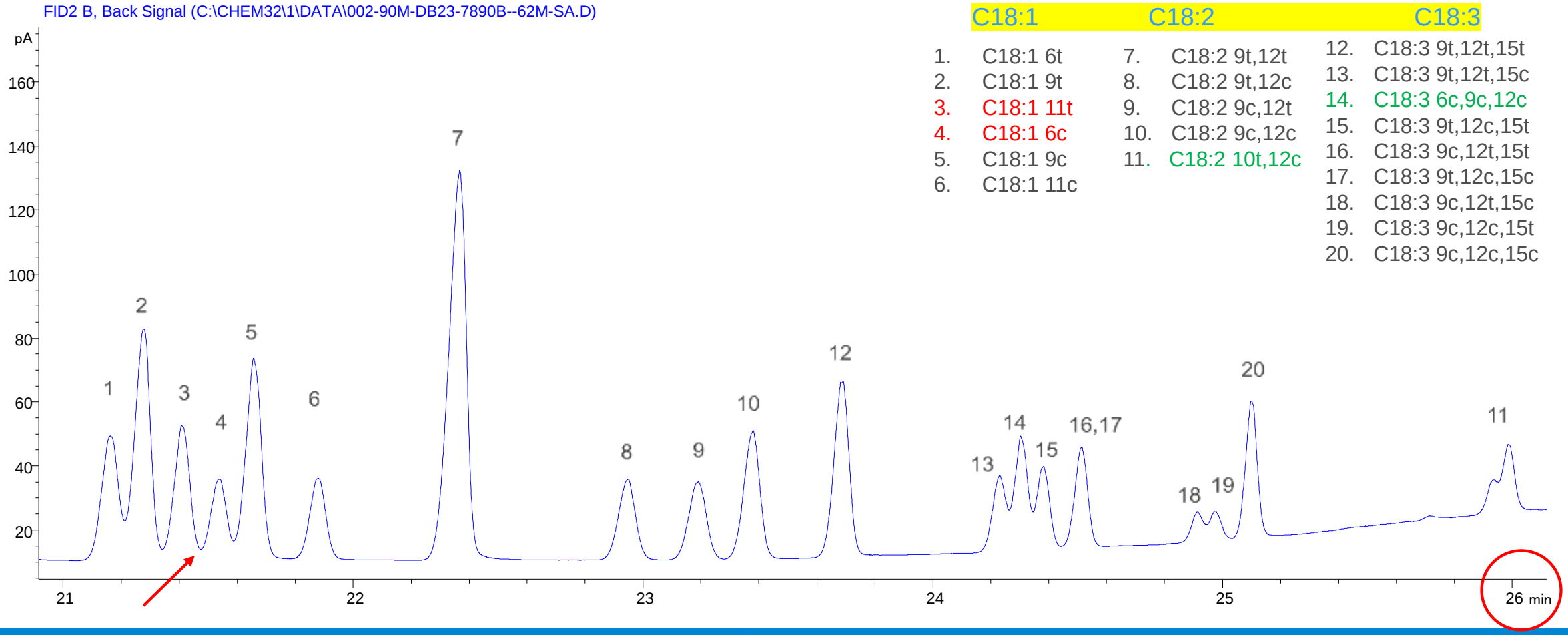
Analysis of 57-FAMEs using a 90m DB-FastFAME

GC System:	Agilent 7890B
Column	DB-FastFAME, 90m*0.25mm I.D, 0.25um
Inlet	260°C, split/splitless mode, split ratio 50:1
Carrier	Helium, constant pressure, **46psi**
Oven	70°C(1min), 40°C/min to 200°C(5 min), 2°C/min to 205°C(11.5 min); 15°C/min to 245°C(15 min)
FID	280°C, Hydrogen:40mL/min, Air:400mL/min, make-up gas:25mL/min
Injection	1uL



Expanded view of 57-FAME Chromatogram 90-m DB-FastFAME

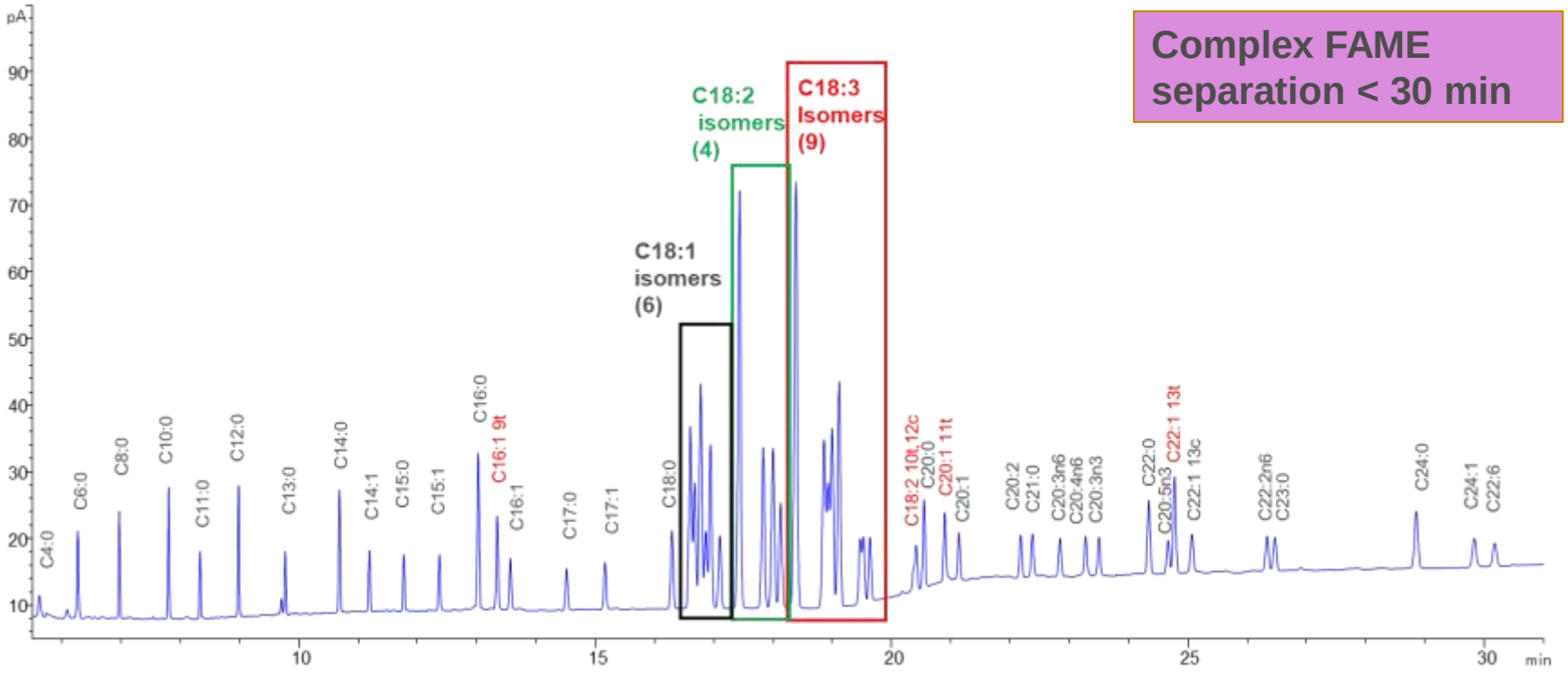
26 min analysis time!



C18:1	C18:2	C18:3
1. C18:1 6t	7. C18:2 9t,12t	12. C18:3 9t,12t,15t
2. C18:1 9t	8. C18:2 9t,12c	13. C18:3 9t,12t,15c
3. C18:1 11t	9. C18:2 9c,12t	14. C18:3 6c,9c,12c
4. C18:1 6c	10. C18:2 9c,12c	15. C18:3 9t,12c,15t
5. C18:1 9c	11. C18:2 10t,12c	16. C18:3 9c,12t,15t
6. C18:1 11c		17. C18:3 9t,12c,15c
		18. C18:3 9c,12t,15c
		19. C18:3 9c,12c,15t
		20. C18:3 9c,12c,15c

Fast Analysis of a 54-FAME mix
With the Intuvo 60-m DB-FastFAME (coming soon!)

GC System:	Agilent intuvo GC/FID
Column	DB-FastFAME Intuvo GC column, 60m*0.25mm I.D, 0.25um
Inlet	260°C, split/splitless mode, split ratio 100:1
Guard chip	200°C
Carrier	Helium, constant pressure, 30psi
Oven	70°C(1min), at 200°C/min to 175°C(2min); at 5°C/min to 210°C(8min); at15°C/min to 240°C(15min)
FID	260°C, Hydrogen:40mL/min, Air:400mL/min, make-up gas:25mL/min
Injection	1uL



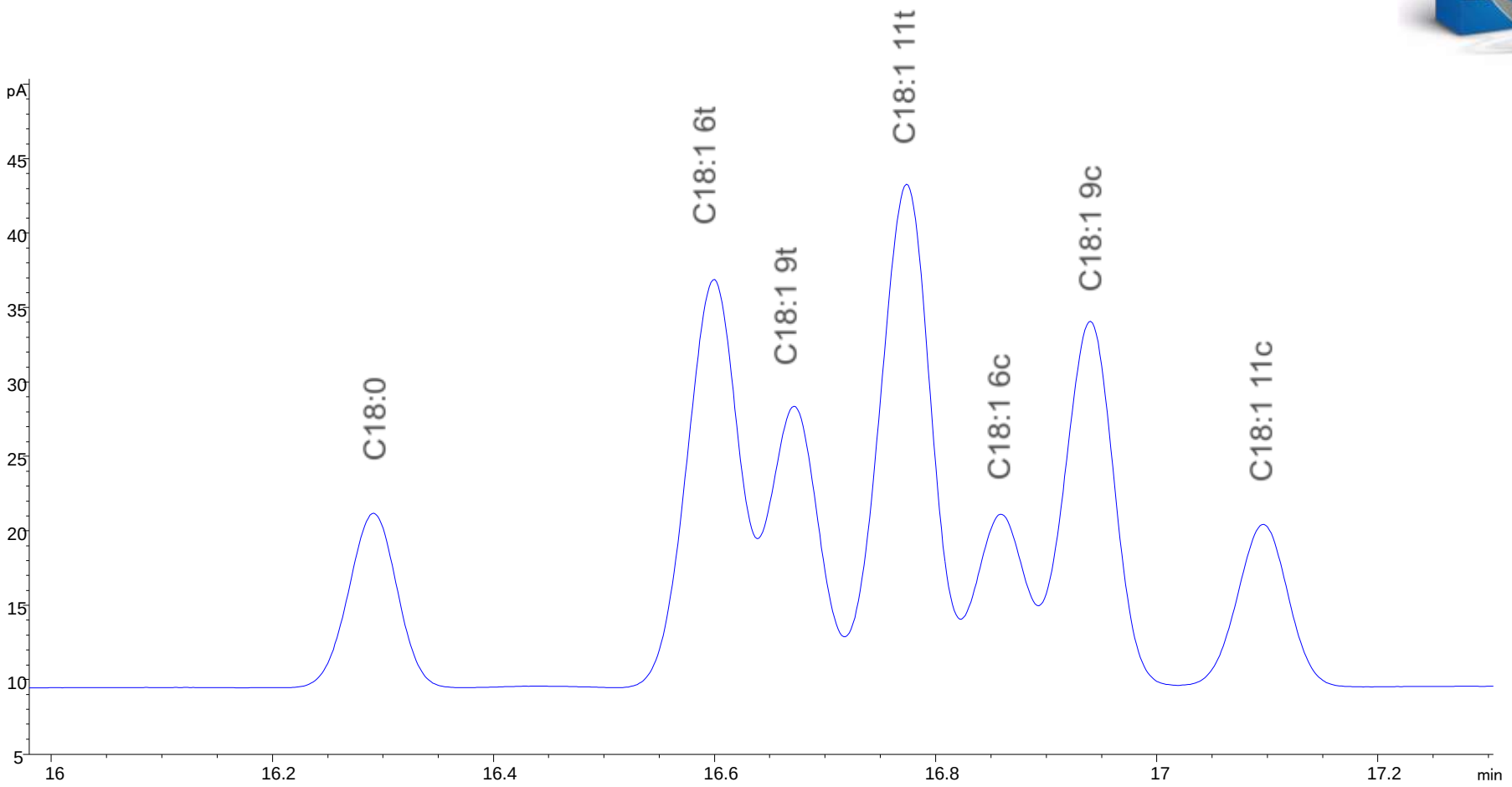
Complex FAME separation < 30 min

List of Trans-FAMEs added to the 37-FAME mix

Trans-FAMEs including

1. C18:3 9t,12t,15t
2. C18:3 9t,12t,15c
3. C18:3 9t,12c,15t
4. C18:3 9c,12c,15t
5. C18:3 9c,12t,15t
6. C18:3 9c,12t, 15c
7. C18:3 9t,12c,15c
8. C18:2 9t,12c
9. C18 : 2 9t,12t
10. C18:2 9c,12t
11. C18:2 10t,12c
12. C18:1 6t
13. C18:1 9t
14. C18:1 11t
15. C22:1 13t
16. C20:1 11t
17. C16:1 9t

Expanded view of C18:1 cis/tran isomers
(from GC/FID Chromatogram of 54-FAMEs)



DB-HeavyWAX

For the analysis of:

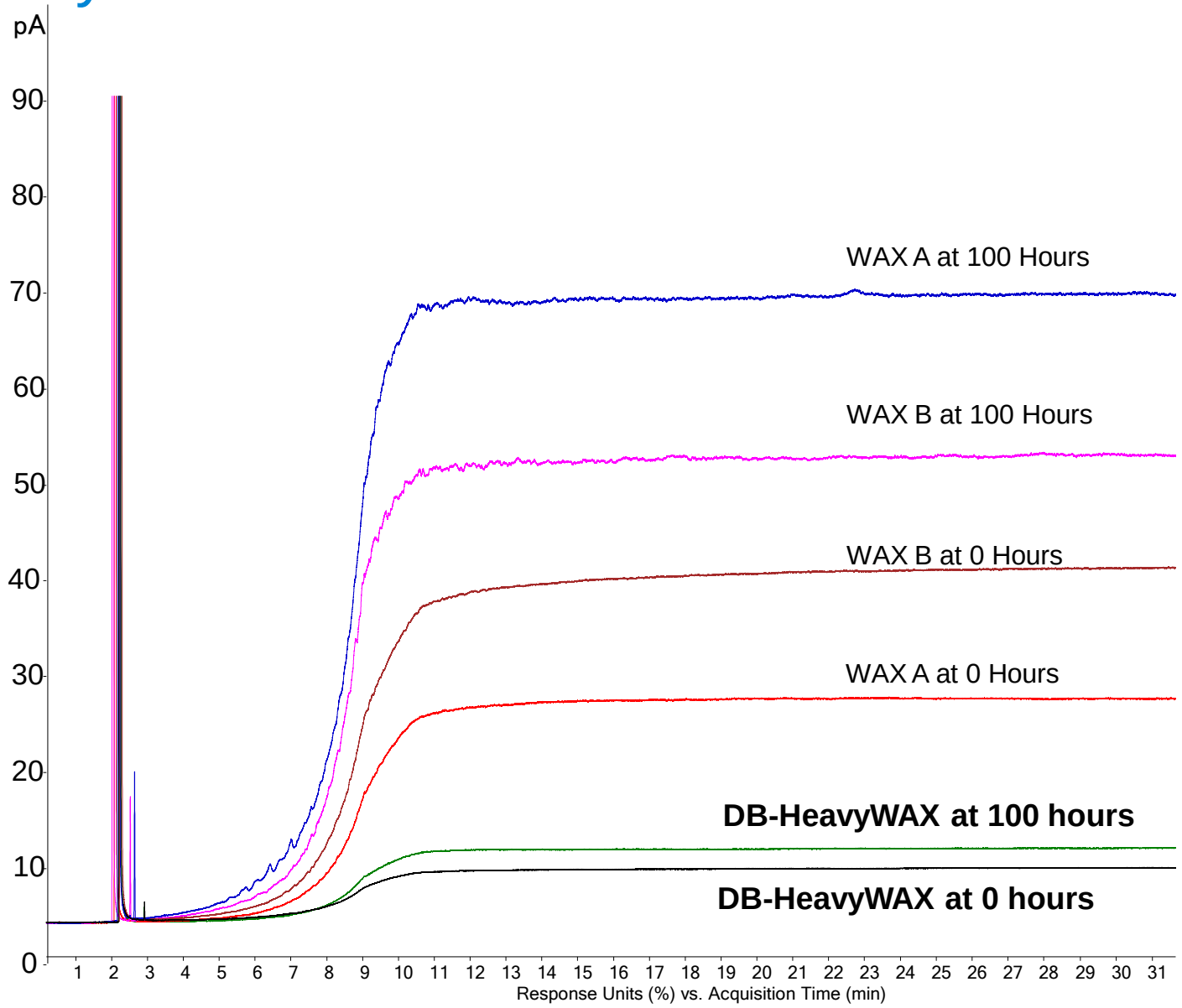
- High temp applications for polar analytes
- Essential oils and flavor & fragrances
- Other high temp. requirements (GCxGC)

Introducing DB-HeavyWAX

- WAX column with increased MAOT compared to existing columns on the market
  - 280°C isothermal and 290°C programmed
- Provides increased thermal stability
- Has a low bleed level
- Advantages

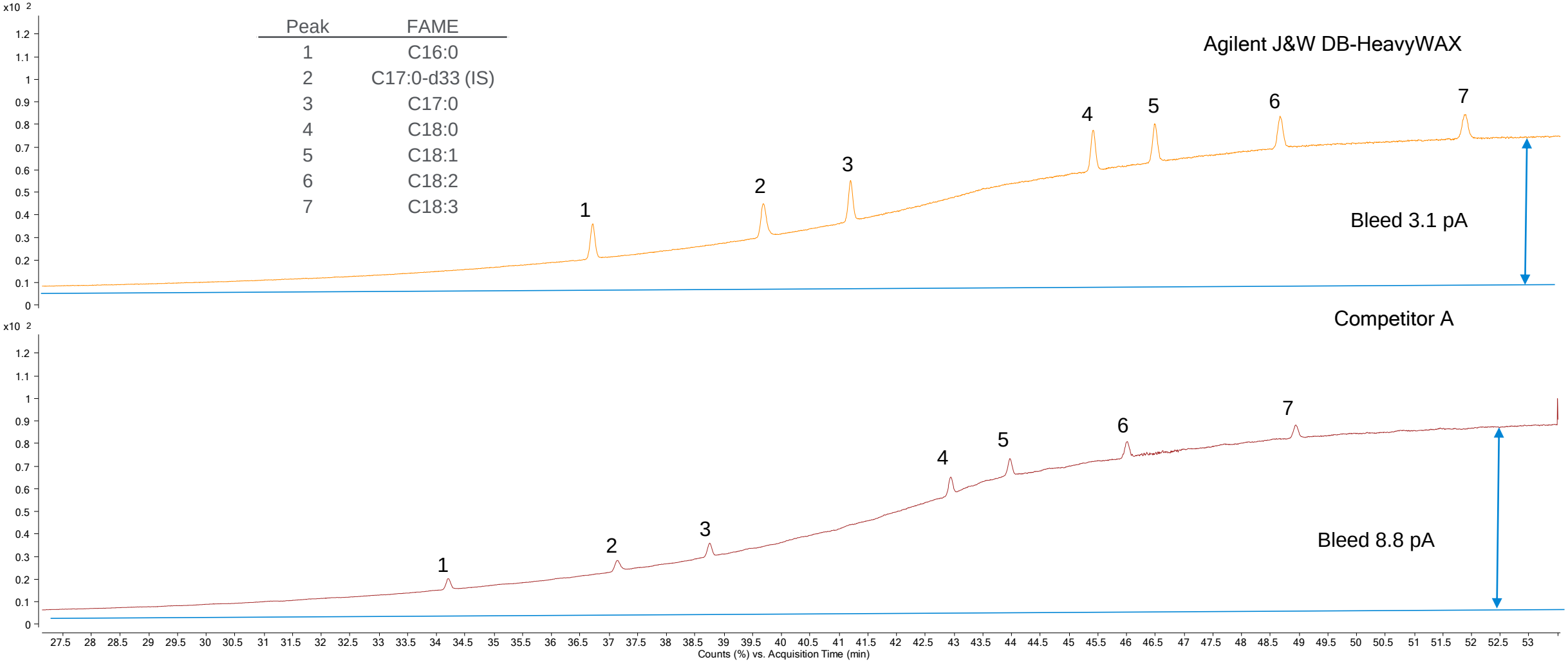
General GC	GC/MS	GC×GC
• Shorter runtimes when late eluters are present • Better S/N ratio, improved detection • Better thermal stability • Faster column bake-out	• Desire for “zero” bleed • Avoid MS contamination by column bleed for longer system uptime and column lifetime • Improve detection limit	• Extended scope of compounds

Bleed Summary at 280°C Over 100 Hours



Bleed reductions for FAMEs in Biodiesel (IP-585) using a DB-HeavyWAX, 60m x 0.25mm x 0.50 µm

Peak	FAME
1	C16:0
2	C17:0-d33 (IS)
3	C17:0
4	C18:0
5	C18:1
6	C18:2
7	C18:3



Retention time stability on a DB-HeavyWAX

IP-585: FAMEs in aviation fuel

- Less bleeds reduces noise level
- Enhance sensitivity without changing the detector
- Increase thermal stability→ Better stability in the SIM window → Less service time

GC Conditions	
Column	60m x 0.25 mm x 0.5 μm
Carrier	Helium, constant flow, 1.2 mL/min
Oven	150°C (5.0 min), Ramp 12°C/min to 200°C (17.0 min), Ramp 3°C/min to 252°C (10 min)

Compound	Operating Hours at 260°C									Average	%RSD
	1	5	7	13	22	39	42	46	49		
C16:0	36.58	36.47	36.45	36.44	36.43	36.43	36.43	36.43	36.42	36.45	0.13%
C17:0 d33 (ISTD)	39.57	39.47	39.45	39.44	39.44	39.44	39.44	39.44	39.44	39.46	0.11%
C17:0	41.09	40.99	40.98	40.96	40.97	40.97	40.97	40.97	40.96	40.98	0.10%
C18:0	45.26	45.16	45.15	45.13	45.14	45.15	45.15	45.15	45.14	45.16	0.08%
C18:1	46.25	46.15	46.14	46.12	46.13	46.13	46.13	46.13	46.13	46.15	0.09%
C18:2	48.20	48.10	48.09	48.07	48.08	48.07	48.08	48.08	48.07	48.09	0.09%
C18:3	51.01	50.89	50.87	50.85	50.84	50.86	50.86	50.87	50.86	50.88	0.10%

Conclusion

- New column technologies for faster & high performance FAME analysis, complements our comprehensive portfolio of GC columns for Fat & Oil analysis
  - DB-FATWAX UI is our new GC Column specially tested for FAMEs in Fish Oil and Animal Fat. Ultra Inert technology makes it possible to analyze fatty acids as well as FAMEs and FAEEs with DB-FATWAX UI, simplifying column selection
  - DB-FastFAME is a new high-content cyanopropyl phase that allows the fast analysis of FAMEs without sacrificing in resolution.
  - DB-HeavyWAX, our new addition to our WAX GC columns portfolio, is a new high temperature WAX phase, ideal for analysis of FAMEs in biodiesel

Thank you!