Agilent CrossLab
From Insight to Outcome

WAX Column Technology Update and Recent Applications

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Agilent

WAX Columns

- Also referred to as Polyethylene Glycol (PEG) columns
- Used to analyze polar compounds
- Limitations compared to apolar columns
  - Maximum Operating Temperatures 250°C isothermal and 260°C programmed
    - Decreased Thermal Stability
      - Retention Times Shifting
      - Increased Column Bleed
  - Even more vulnerable to oxygen

Most Recent Additions

DB-WAX Ultra Inert

Best in class peak shape for challenging polar compounds

DB-FATWAX Ultra Inert

New WAX-type selectivity column for FAME analysis, including Omega 3 and Omega 6. Good inertness for analysis of free fatty acids

DB-HeavyWAX

New WAX column with increased max. temperature.

WAX column positioning
How to select the right WAX-type column for your application?

Category		Agilent column
Standard WAX Phases	**High Temperature Applications**	**DB-HeavyWAX**
	Best in Class Inertness	**DB-WAX Ultra Inert**
	MS-grade Applications	VF-WAXms
	Industry Standard	DB-WAX
Specialty WAX Phases	**Strong Acidic Analytes**	DB-FFAP
	Strong Basic Analytes	CAM
Application Specific WAX Phases	**Alcohols and Glycols in wine/spirits**	CP-WAX 57 CB
	FAMEs, FAEEs, and fatty acids	**DB-FATWAX Ultra Inert**

WAX column positioning
Agilent's WAX phases by compound type

	Acids	Alcohols	Aldehydes	Amines	Diols	Glycols	Flavors and Fragrances	Free Fatty Acids	FAMEs	FAEEs	Phenols
DB-HeavyWAX	+	++	+++	+	++	++	++		+++	+++	++
DB-WAX Ultra Inert	+++	+++	++	+	+++	+++	+++	+++	+++	+++	+++
VF-WAXms	+	+	+		+	+			+++	+++	+
DB-WAX	+	++	+		++	++			+++	+++	++
DB-FFAP	++							+++			++
CAM				++							
CP-Wax 57 CB		++				++					
DB-FATWAX Ultra Inert								+++	+++	+++	

Legend
+++ Best in class inertness
++ Great inertness
+ Good inertness

How to select the right WAX-type column for your application?

Selecting the Right WAX Column

Agilent J&W WAX GC columns deliver excellent inertness with greater sensitivity than competitive WAX columns. With the increasing number of available Agilent J&W WAX columns, it can be challenging deciding which one is best suited for your needs.

This poster will help you select the right WAX column for optimal performance, based on factors such as application analyte mix and the type of detector being used.

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Pub Number: 5991-9415EN

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DB-WAX Ultra Inert

DE44274.5728240741

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DB-WAX Ultra Inert

- Best in class peak shape for challenging polar compounds
- Excellent inertness from column-to-column
- Withstands repeated temperature cycling to upper limits without sacrificing peak shape performance
- Tolerates aqueous injections
  - For split injections
- Same selectivity compared to DB-WAX

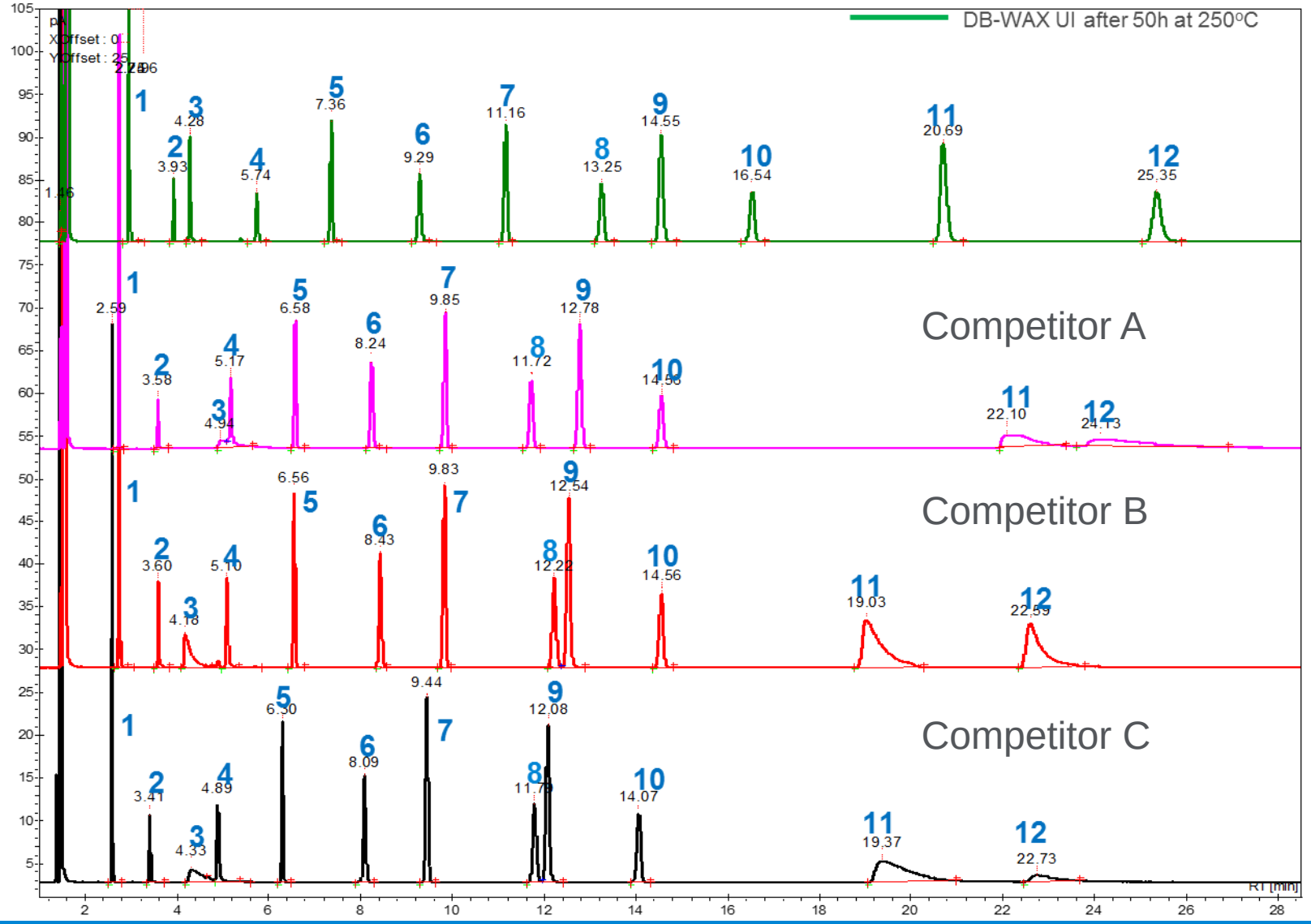
Value:

Improved inertness: *Better data accuracy and less re-run work. This improves overall throughput and turnaround times*

Improved robustness: *Extends product lifetime, lowers cost*

DB-WAX Ultra Inert

Competitor comparison DB-WAX UI test mix after 50 hours at 250°C



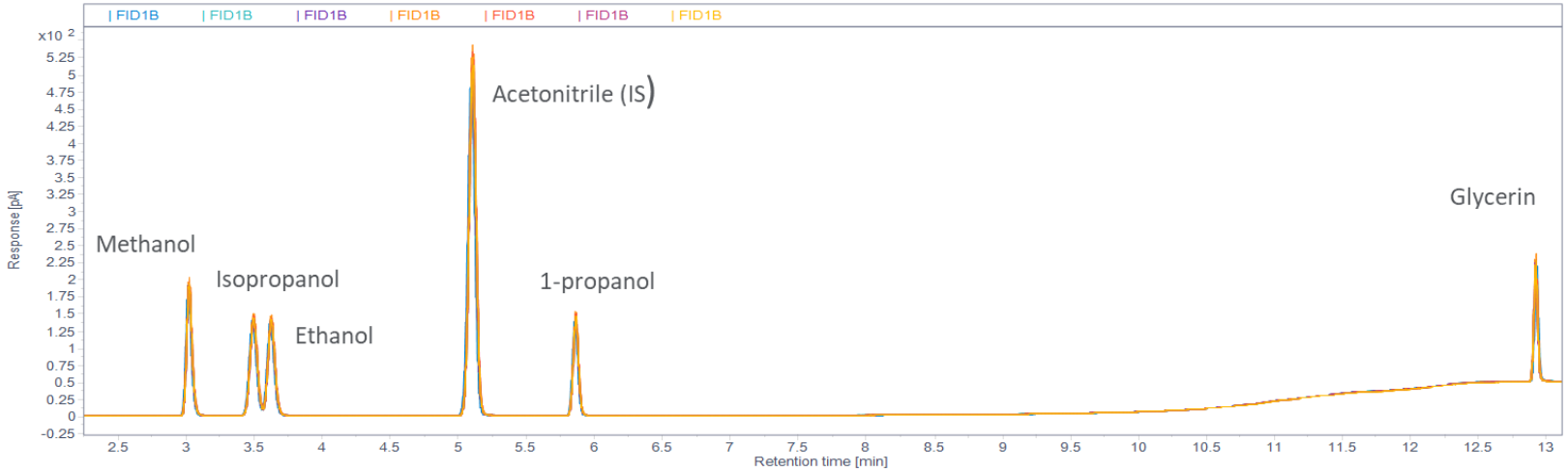
Compound I.D.

*. Methane
1. 5-Nonanone
2. Decanal
3. Propionic Acid
4. Ethylene Glycol
5. Heptadecane
6. Aniline
7. Methyl Dodecanoate
8. 2-Chlorophenol
9. 1-Undecanol
10. Nonadecane
11. 2-Ethylhexanoic Acid
12. Ethyl Maltol

Hand Sanitizer Analysis with a DB-WAX UI and an 8860 GC system

- Resolution: DB-WAX UI column for alcohol analysis
- Good peak shape: optimization of injection volume, split ratio assisted by ultra inert liner and column
- Quantitation precision: 8860 accurate and precise pneumatic and thermal control, stable and sensitive FID.
- Helium conservation module is a recommended option to reduce the operation cost with He as carrier gas.

Agilent 8860 GC parameters	
S/SL inlet	250 ^{0}C, split ratio 20:1
Injection volume	0.2 µL
Carrier gas	He
Column flow rate	7 mL/min, constant flow mode
Oven	40 ^{0}C (5 min), 30 ^{0}C/min to 230 ^{0}C (3 min)
FID	250 ^{0}C, air:400 mL/min, fuel gas (H_2): 30 mL/min, constant make up (N2): 18 mL/min
Column	Agilent J&W DB-WAX UI, 30 m, 530 µm, 1 µm (p/n 125-7032UI)
Inlet liner	Agilent Ultra Inert, low pressure drop with glass wool (p/n 5190-2295)

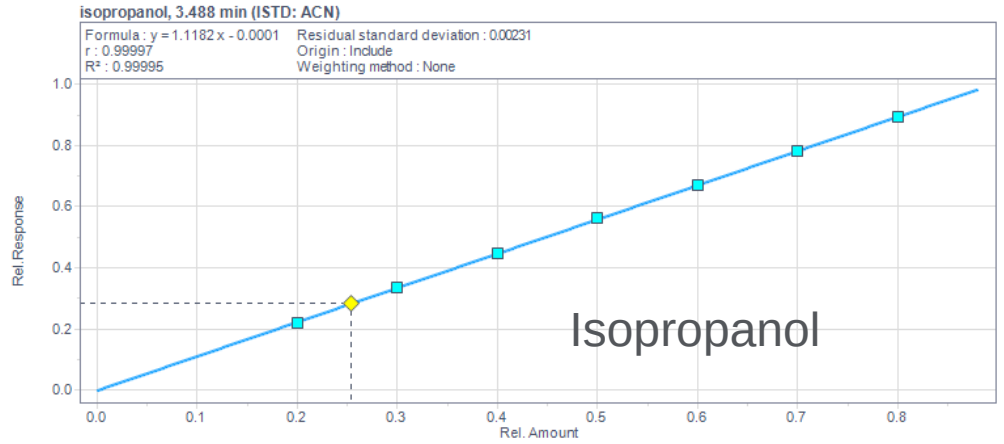


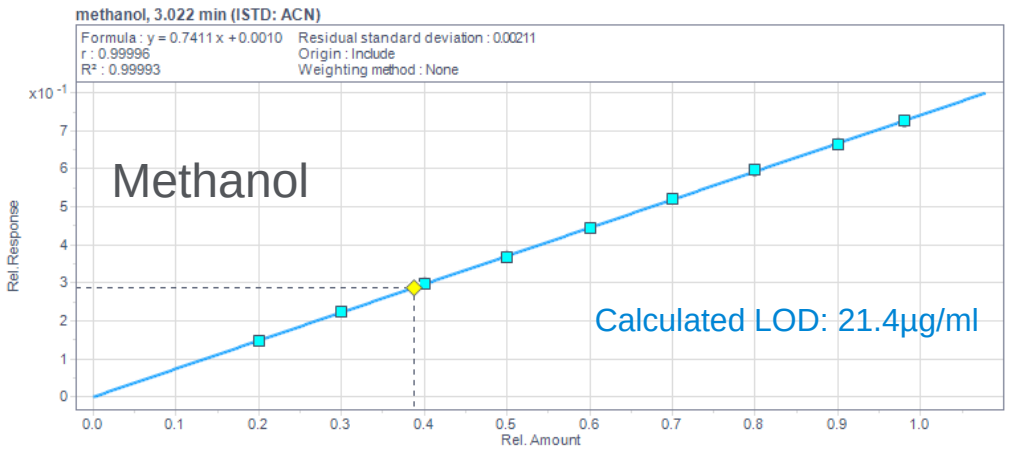
Compound	RT(min)	RSD% of peak response ratio (≤ 4.0%)	Peak Tailing Factor (≤ 2.0)	Resolution with IS (≥ 4)
Methanol	3.015	0.3	1.2	>15
Iso-propanol	3.488	0.41	1.1	>15
Ethanol	3.618	0.16	1.0	15
1-propanol	5.858	0.29	1.1	9
Glycerin	12.917	1.67	1.0	>9

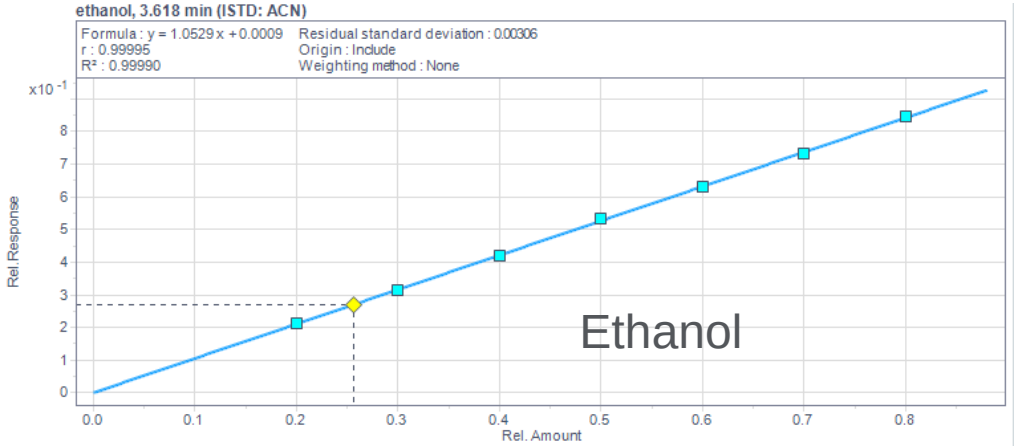
Agilent Publication 5994-2089EN

Quantitation of alcohols on 8860 GC-FID system

Multi-level calibration for analytes quantitation







Level No.	Group One Calibration Standards			Group Two Calibration Standards	
	Ethanol (µL)	IPA (µL)	Glycerin Working Solution (µL)	Methanol (µL)	*n*-Propanol (µL)
1	40 (4.0%)	10 (1.0%)	2 (0.0092%)	49 (4.9%)	1 (0.1%)
2	35 (3.5%)	15 (1.5%)	5 (0.023%)	45 (4.5%)	5 (0.5%)
3	30 (3.0%)	20 (2.0%)	10 (0.046%)	40 (4.0%)	10 (1.0%)
4	25 (2.5%)	25 (2.5%)	20 (0.092%)	35 (3.5%)	15 (1.5%)
5	20 (2.0%)	30 (3.0%)	40 (0.184%)	30 (3.0%)	NA
6	15 (1.5%)	35 (3.5%)	NA	25 (2.5%)	
7	10 (1.0%)	40 (4.0%)		20 (2.0%)	
8	NA	NA		15 (1.5%)	
9				10 (1.0%)	

Calibration Correlation Coefficient ≥0.995

Agilent Publication 5994-2089EN

DB-FATWAX Ultra Inert

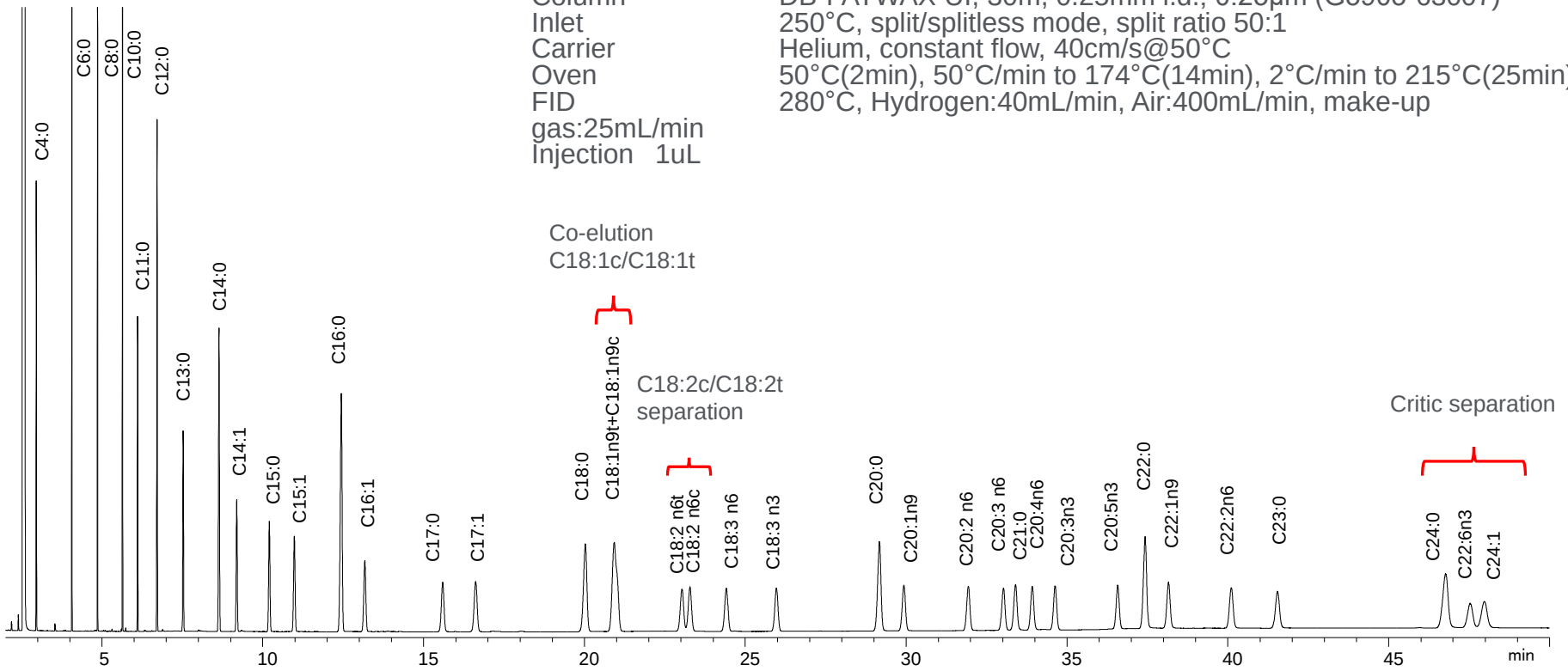
For the analysis of:

- 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

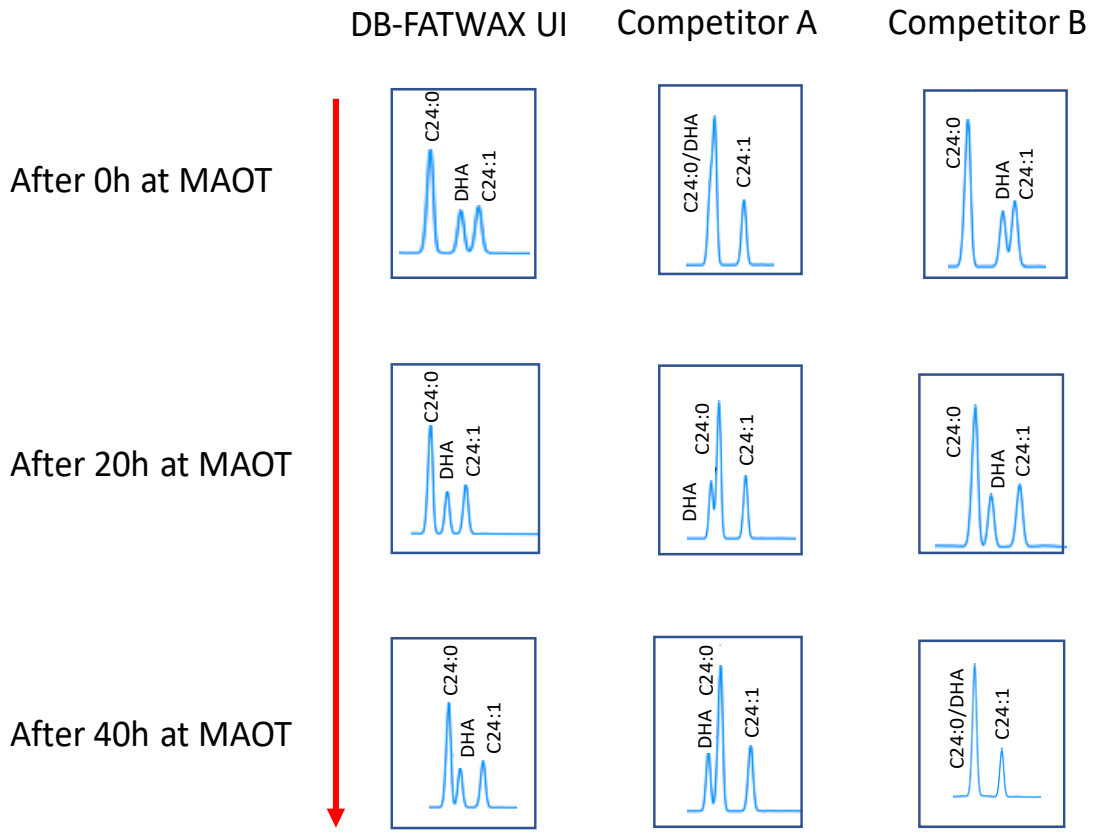
- Best option for saturated and polyunsaturated FAMEs, including Omega 3 and Omega 6
- WAX-type selectivity not ideal for cis/trans separation

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.

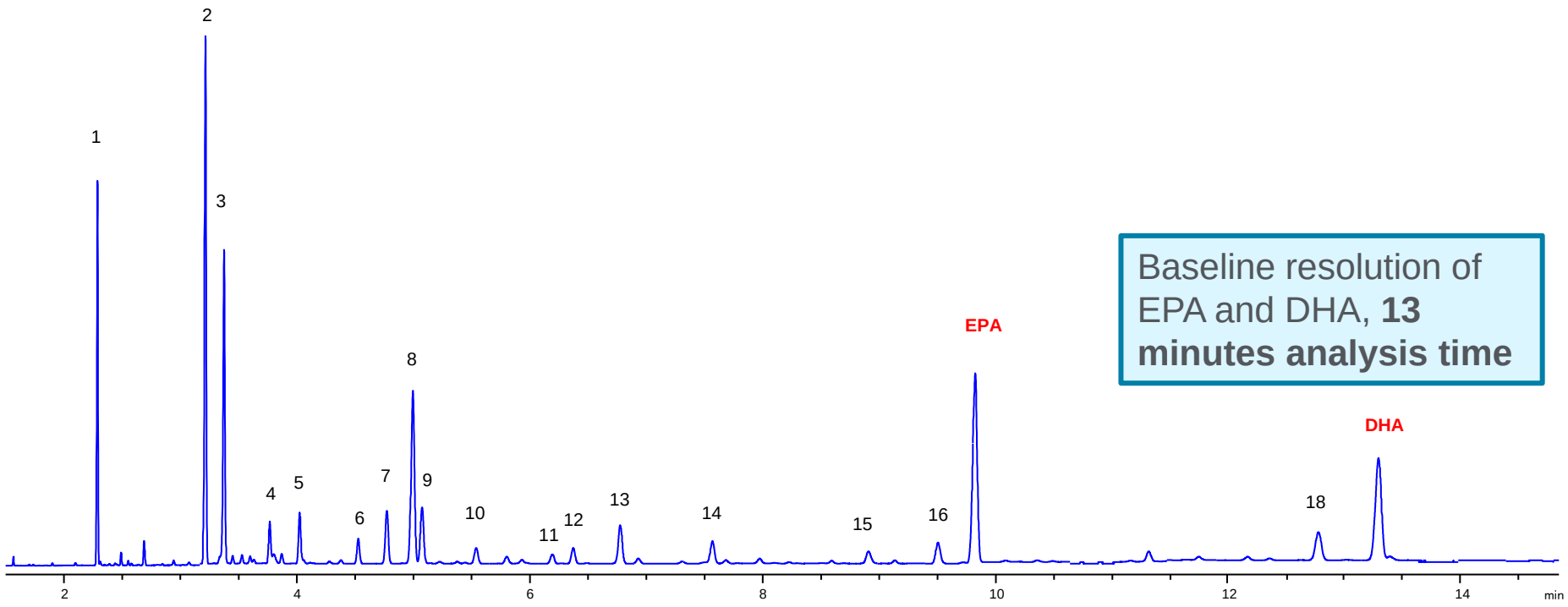


*MAOT: maximum allowable operating temperature

Analysis of real samples

Column	DB-FATWAX UI, **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**

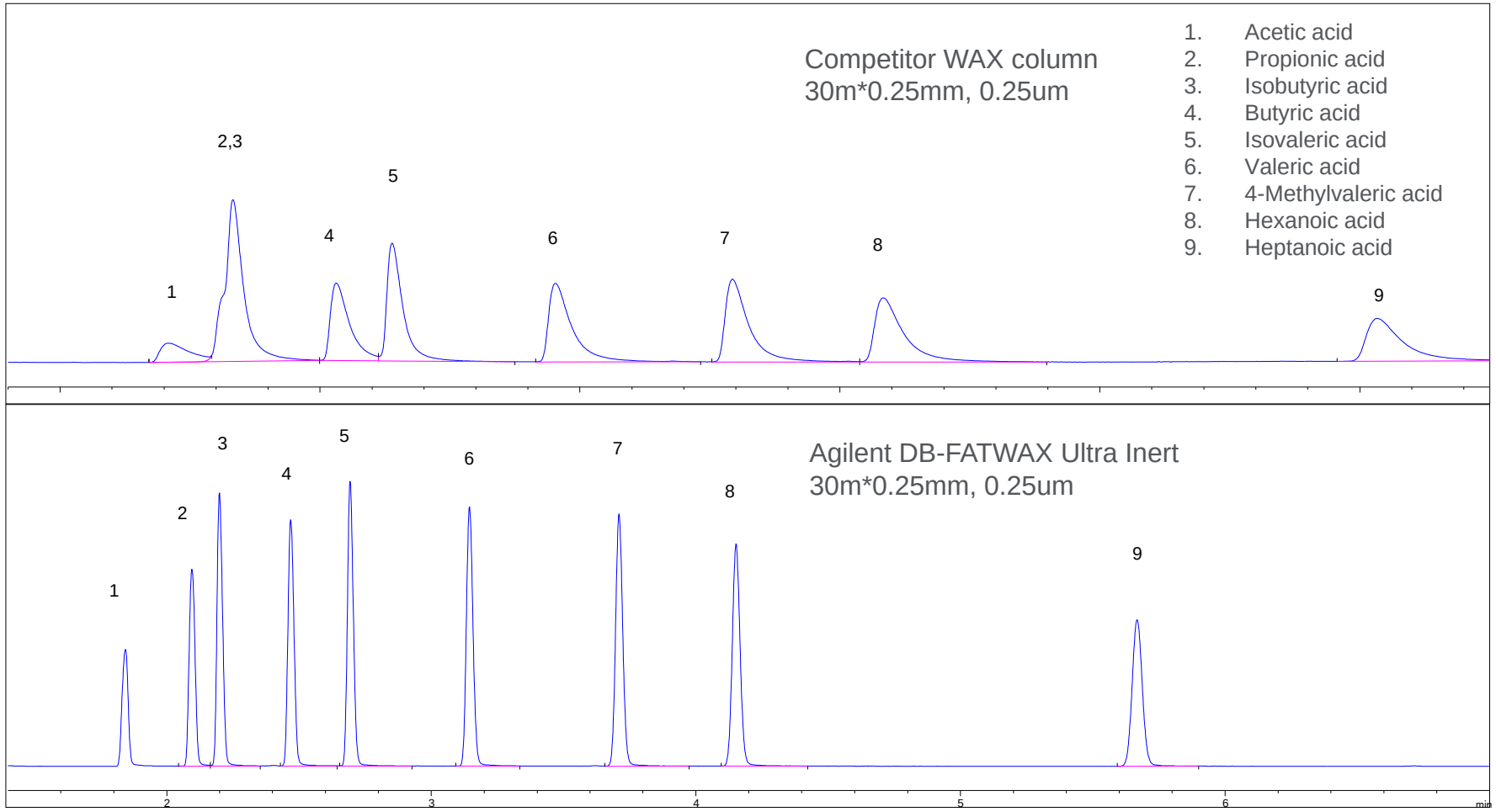
1. C14:0
2. C16:0
3. C16:1n7
4. C16:2n4
5. C16:3n4
6. C16:4n1
7. C18:0
8. C18:1n9
9. C18:1n7
10. C18:2n6
11. C18:3n3
12. C18:3n4
13. C18:4n3
14. C20:1n9
15. C20:4n6
16. C20:4n3
17. C20:5n3
18. C22:5n3
19. C22:6n3

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

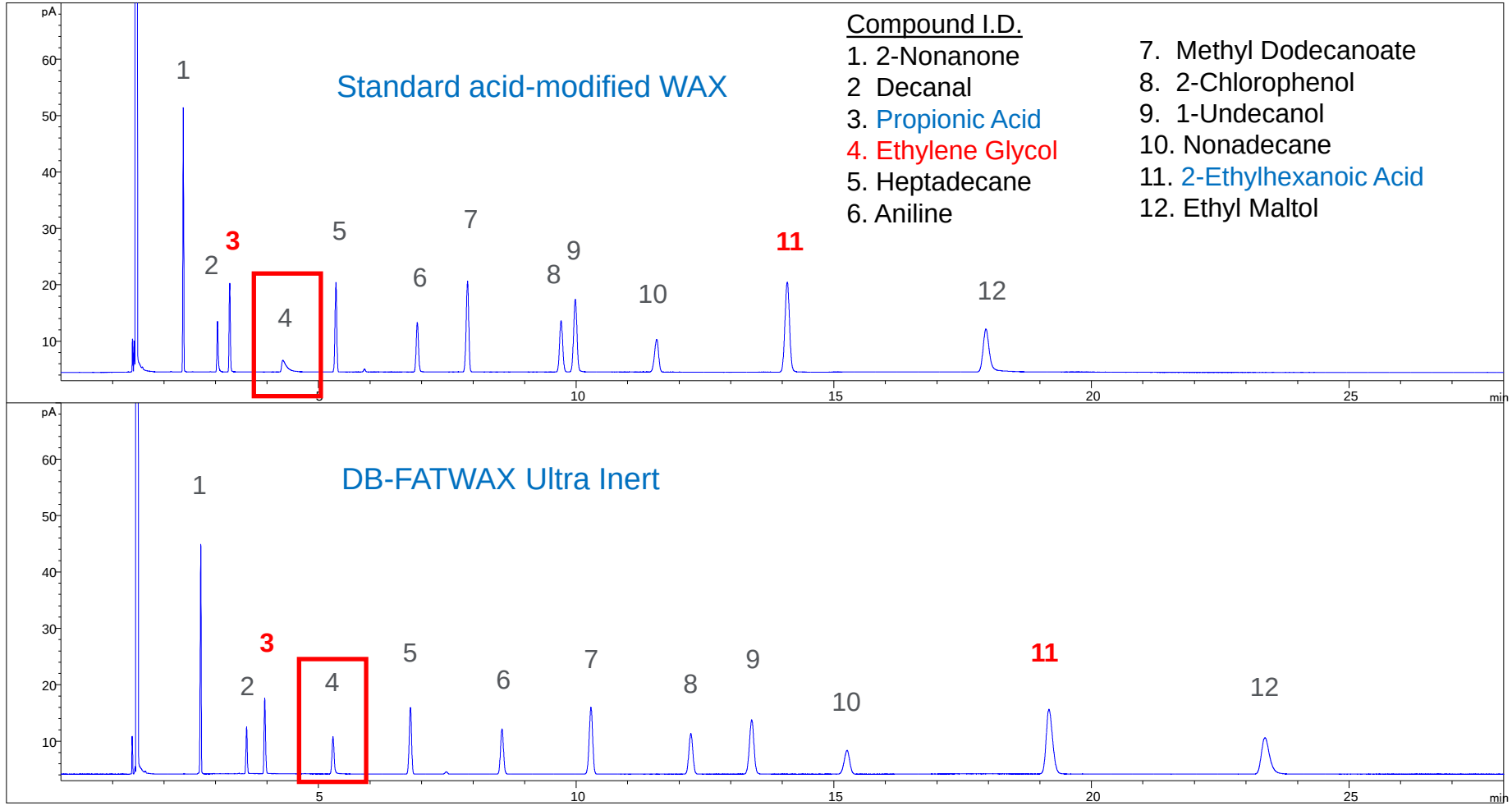
Separation of Short-chain volatile organic acids in water using a competitor WAX column and DB-FATWAX Ultra Inert

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



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

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

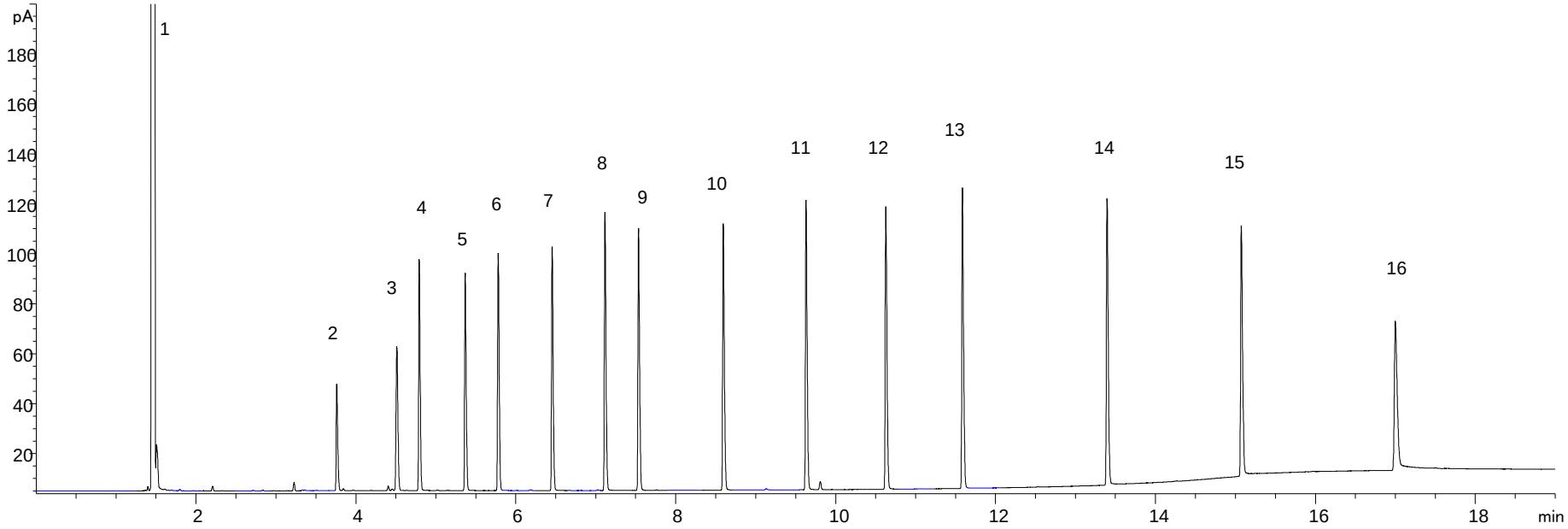


Analysis of Fatty Acids (FA)

- 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



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DB-HeavyWAX

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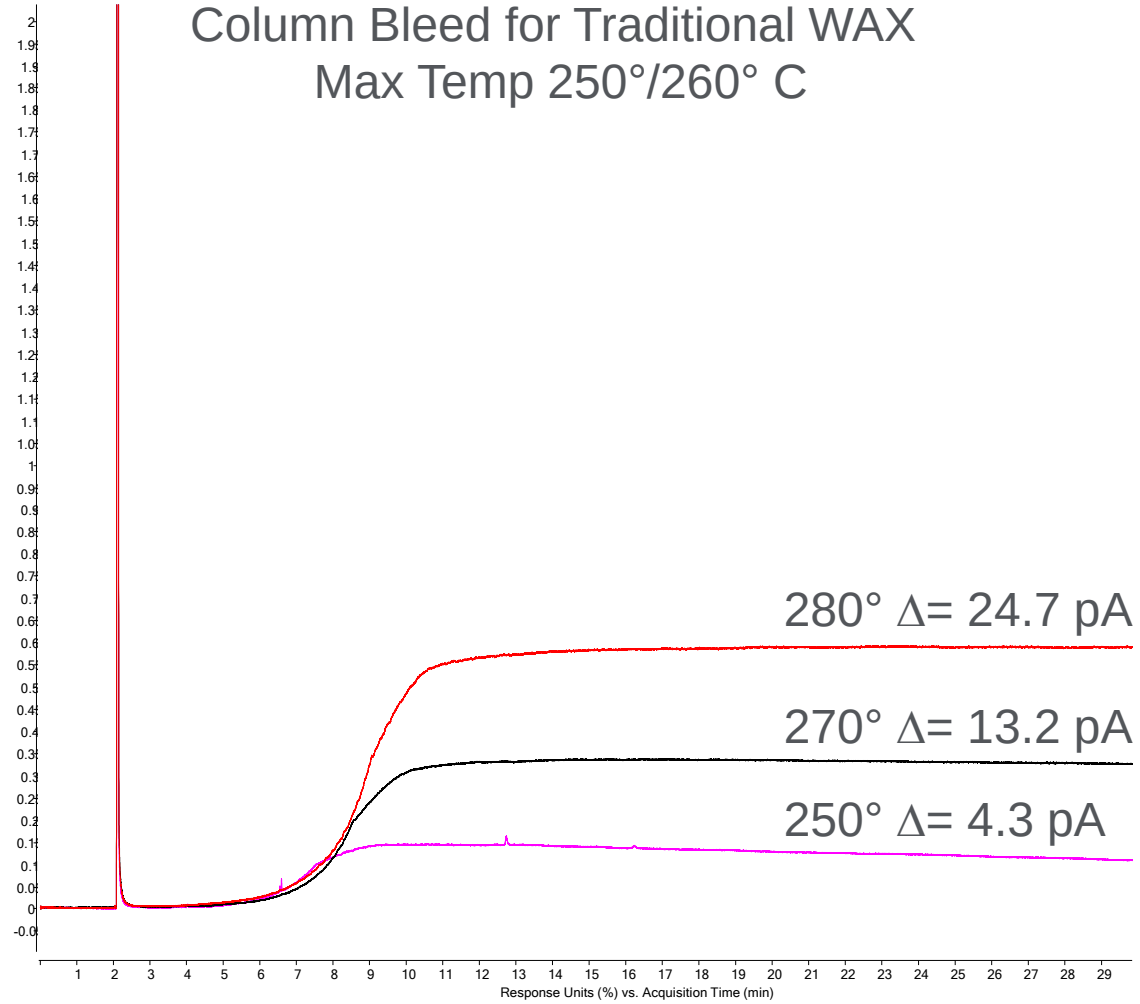
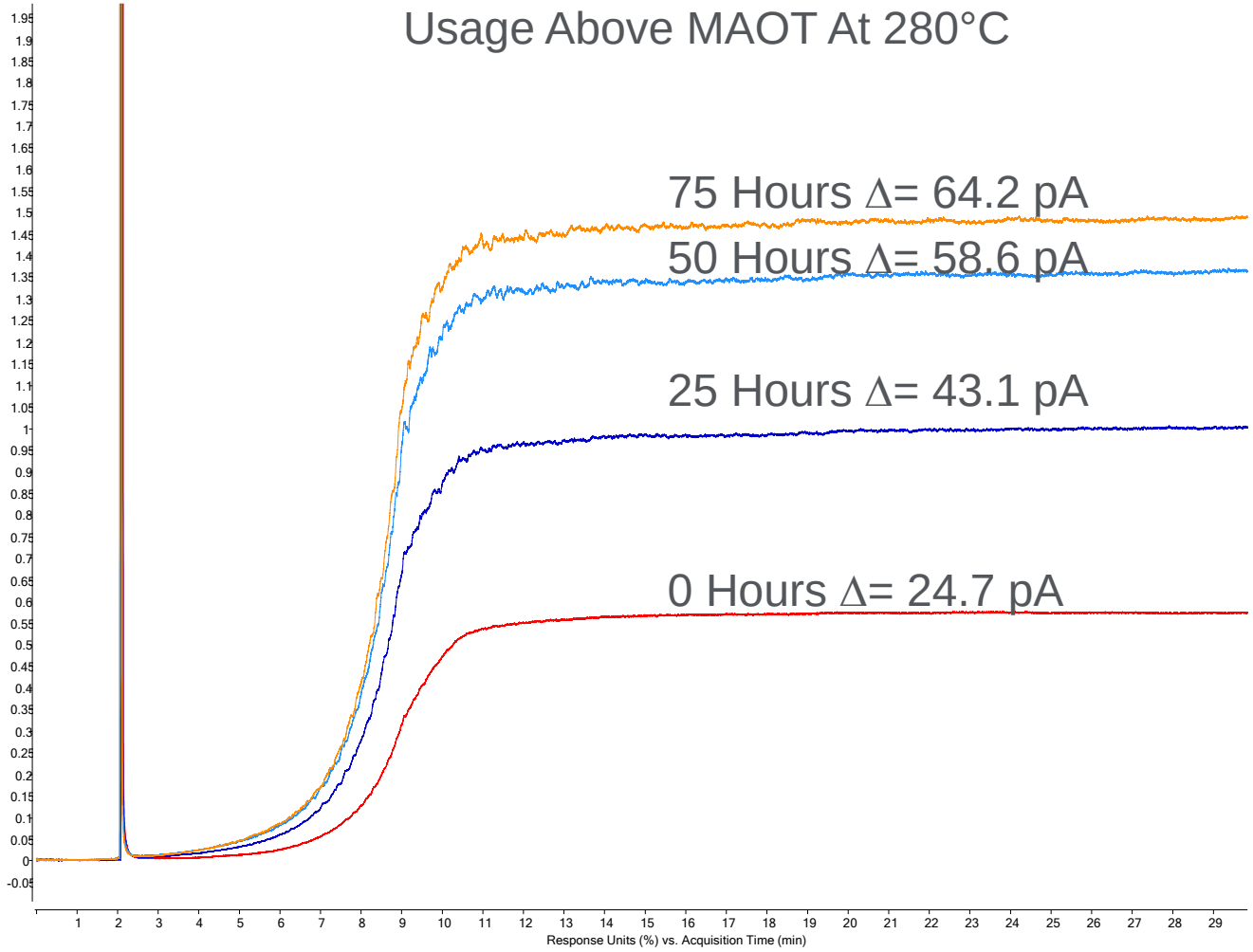
Introducing DB-HeavyWAX

- 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

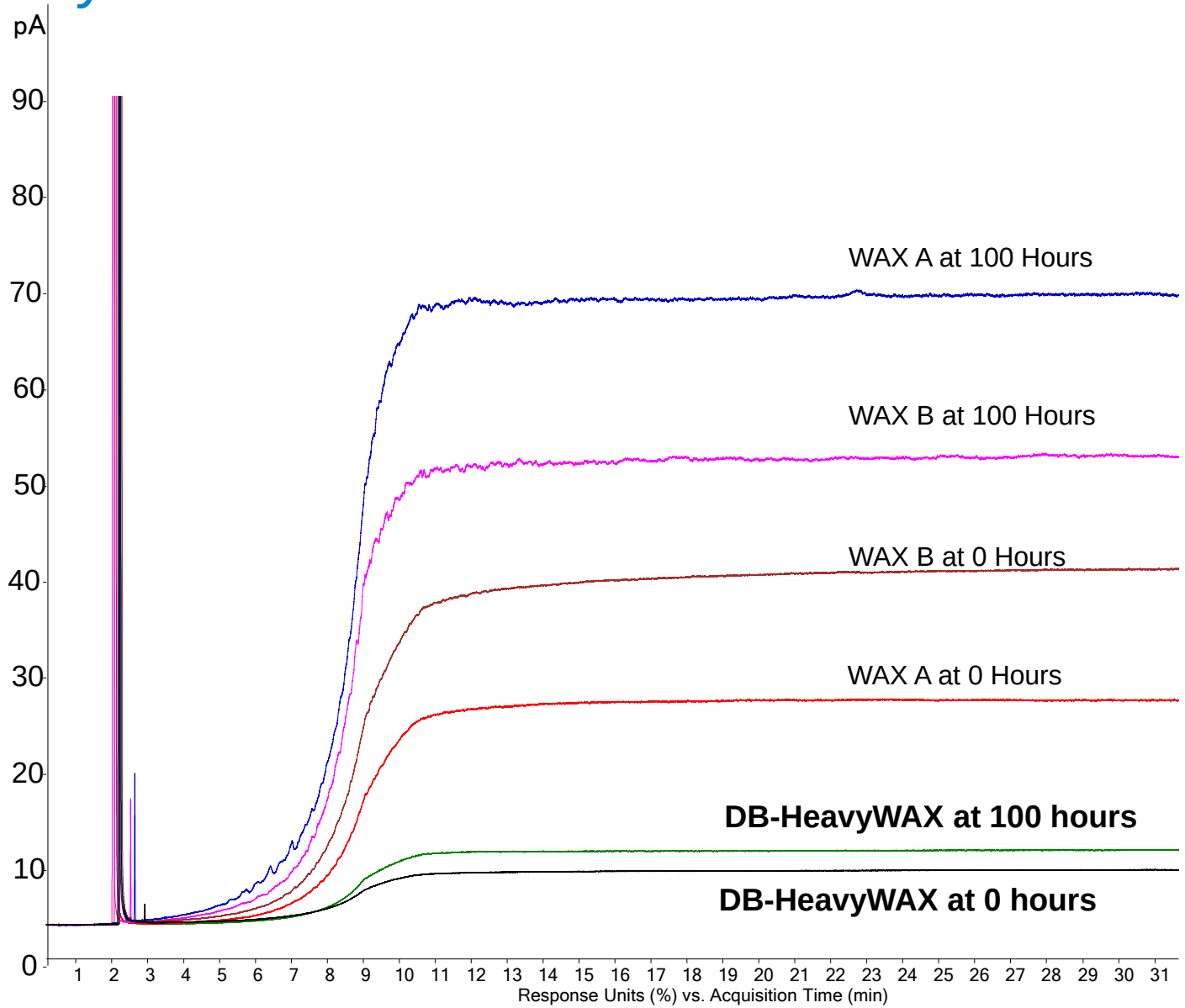
General GC	GC/MS	GC×GC
• Shorter runtimes when late eluters are present • Better S/N ratio, improved detection • Better thermal stability • Faster column bake-out	• Desire for “zero” bleed • Avoid MS contamination by column bleed for longer system uptime and column lifetime • Improve detection limit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

Traditional WAX and Going Above the MAOT





Bleed Summary at 280°C Over 100 Hours



Thermal Stability and Retention Time Shifting for a traditional WAX phase

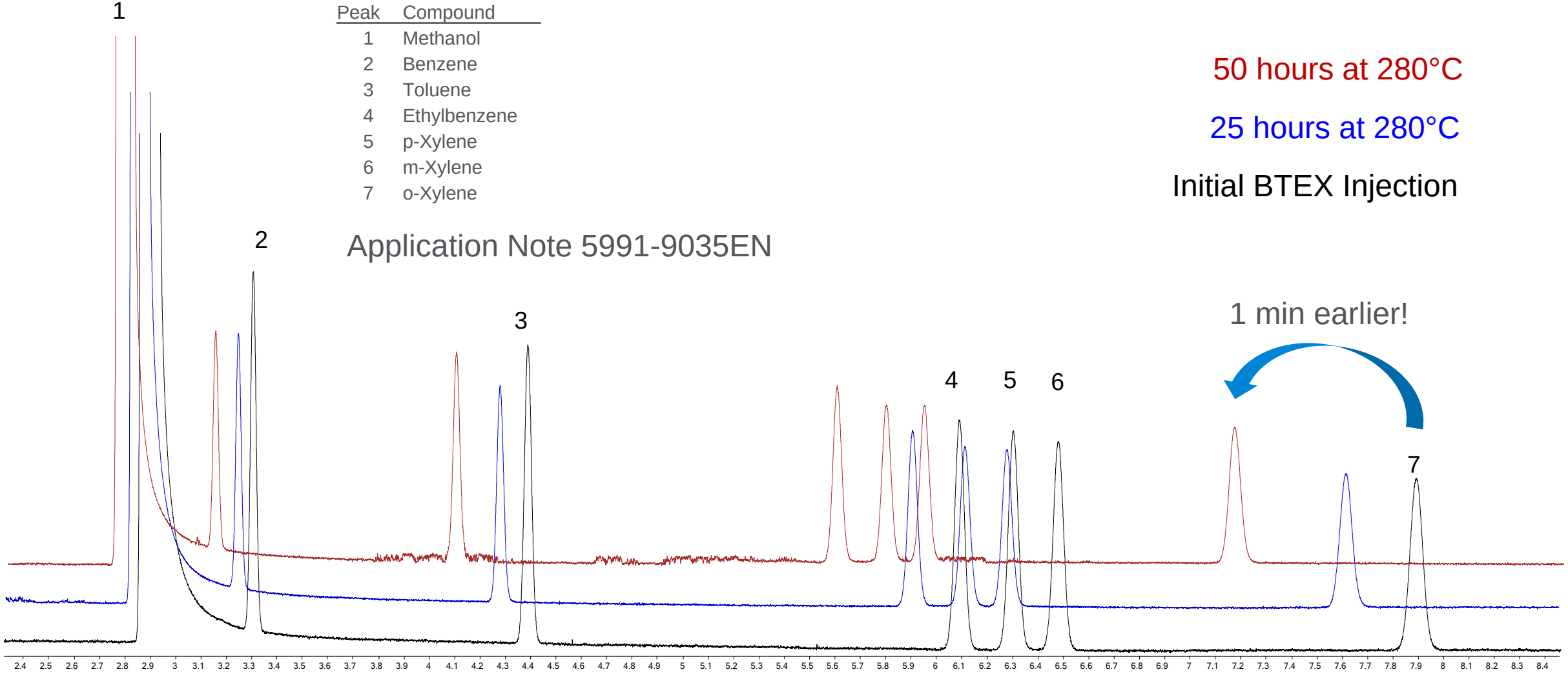
Peak	Compound
1	Methanol
2	Benzene
3	Toluene
4	Ethylbenzene
5	p-Xylene
6	m-Xylene
7	o-Xylene

Application Note 5991-9035EN

50 hours at 280°C

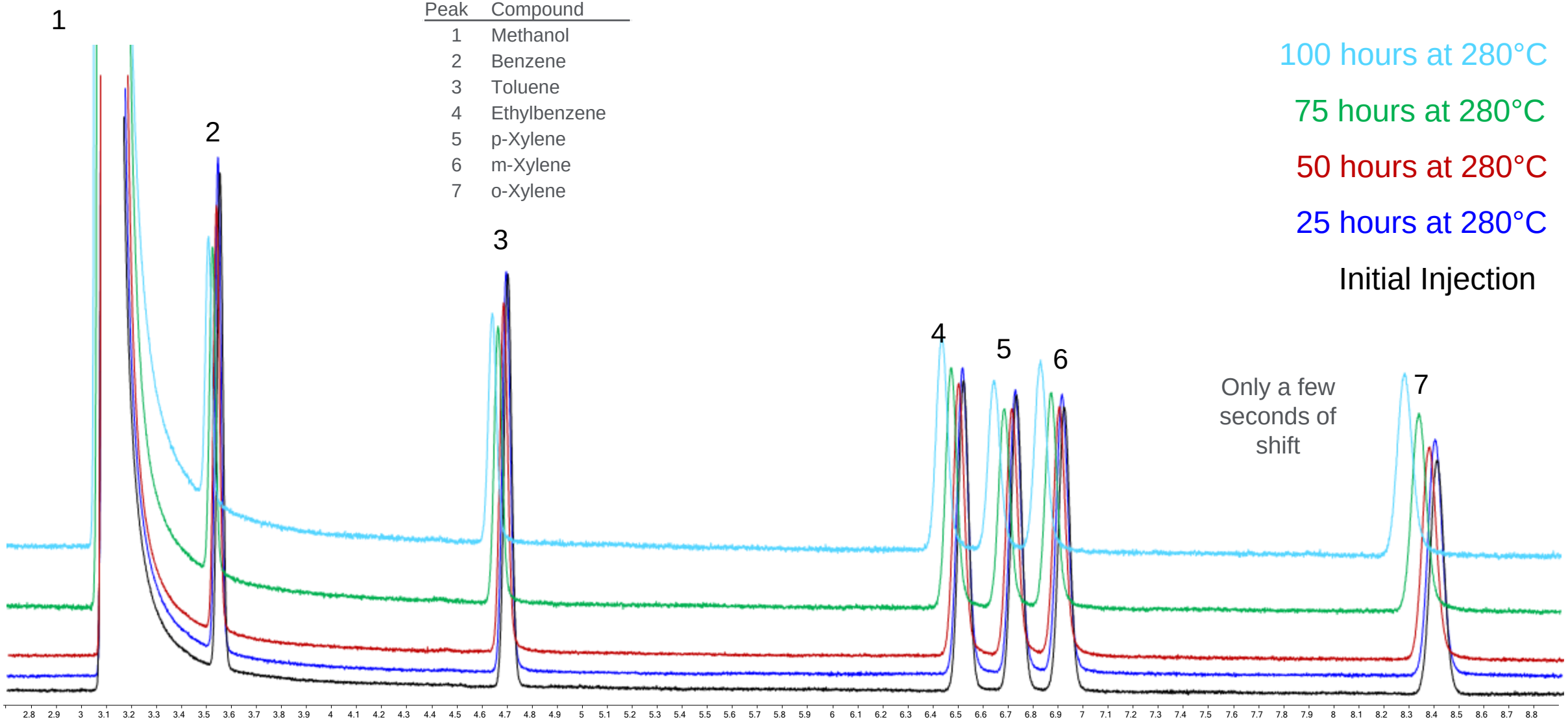
25 hours at 280°C

Initial BTEX Injection



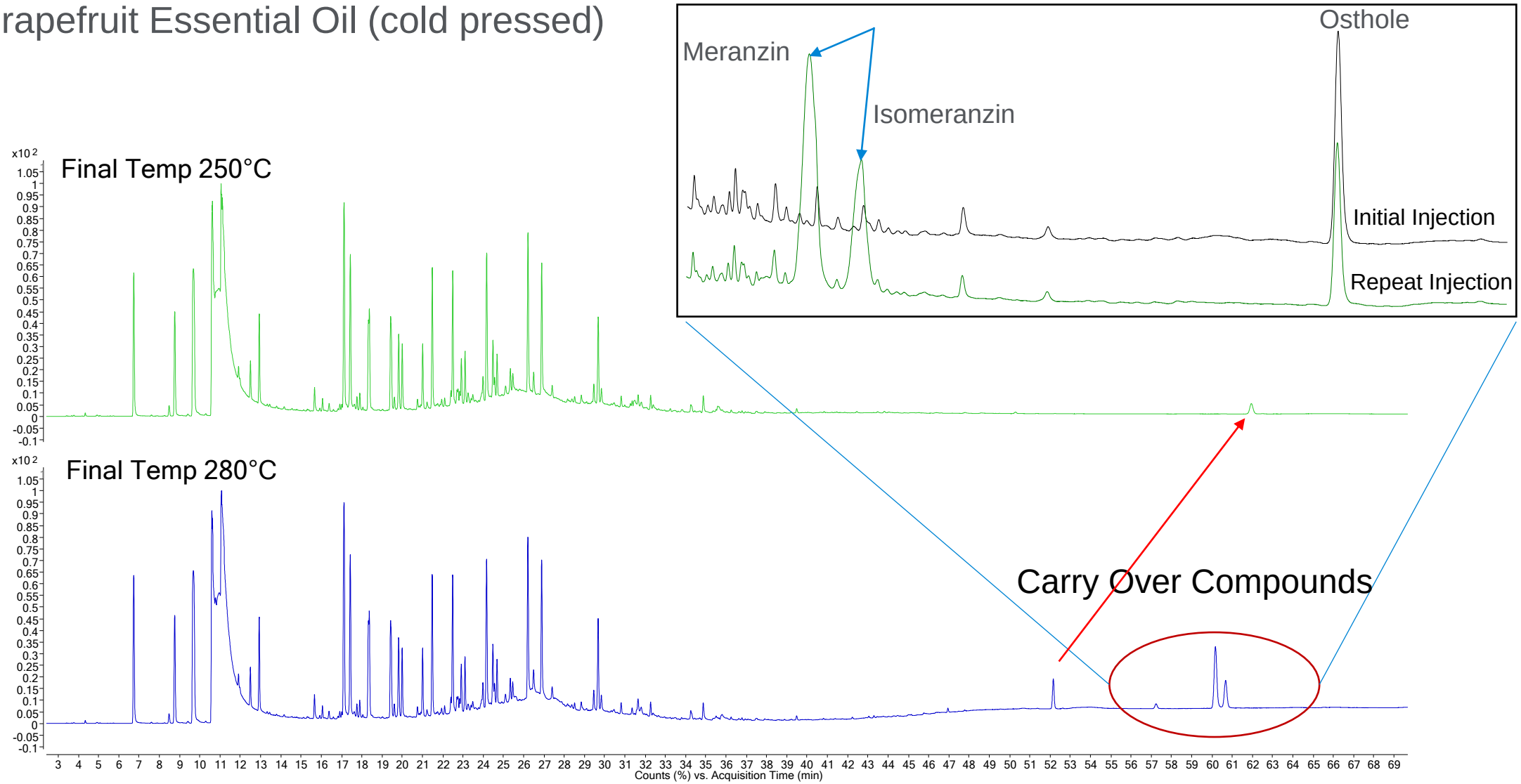
DB-HeavyWAX

Peak	Compound
1	Methanol
2	Benzene
3	Toluene
4	Ethylbenzene
5	p-Xylene
6	m-Xylene
7	o-Xylene



Higher Final Temperatures = Earlier Elution of Heavy Compounds, Sharper Late Eluting Peaks and Reduced Carry Over

Pink Grapefruit Essential Oil (cold pressed)

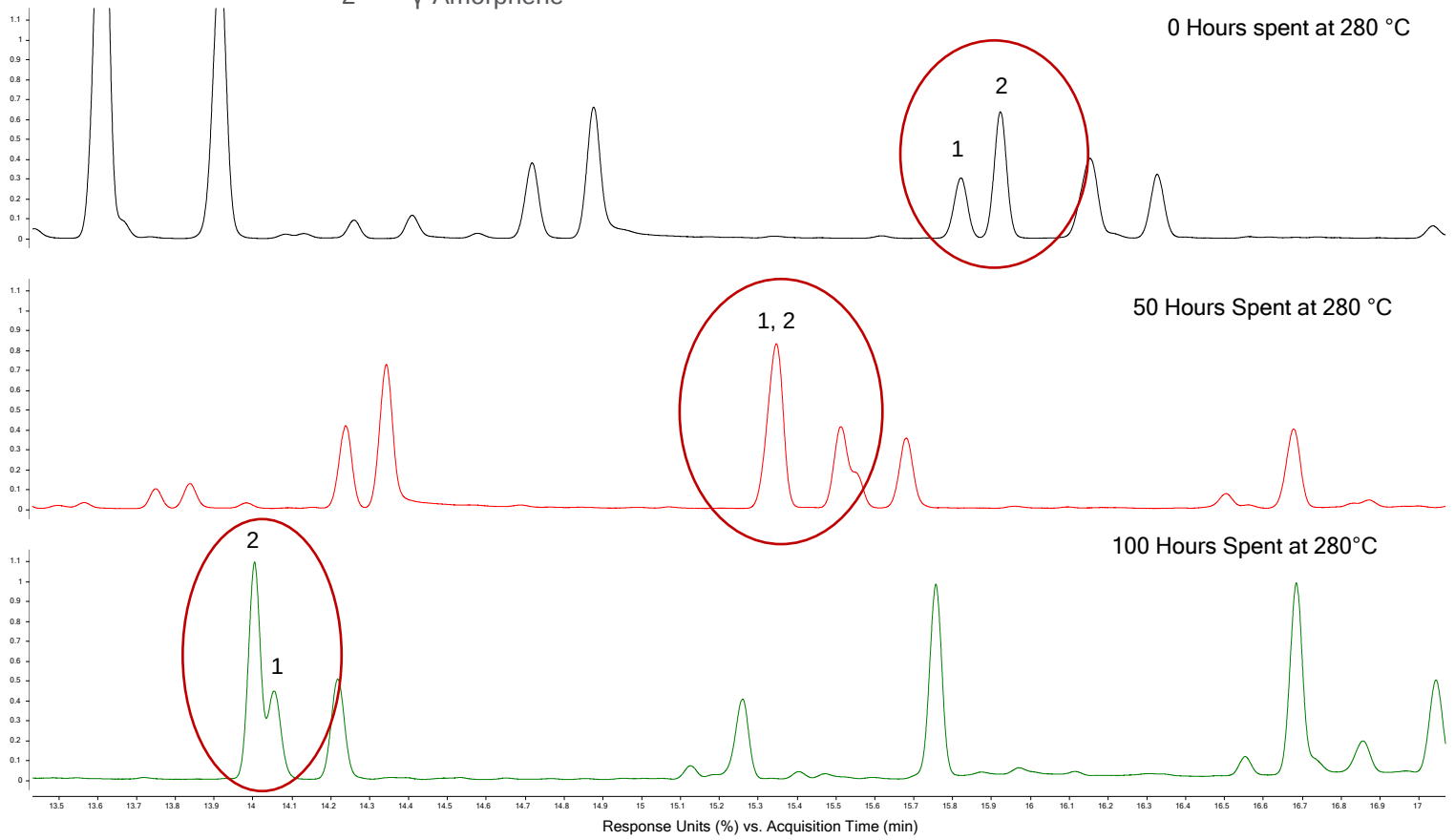


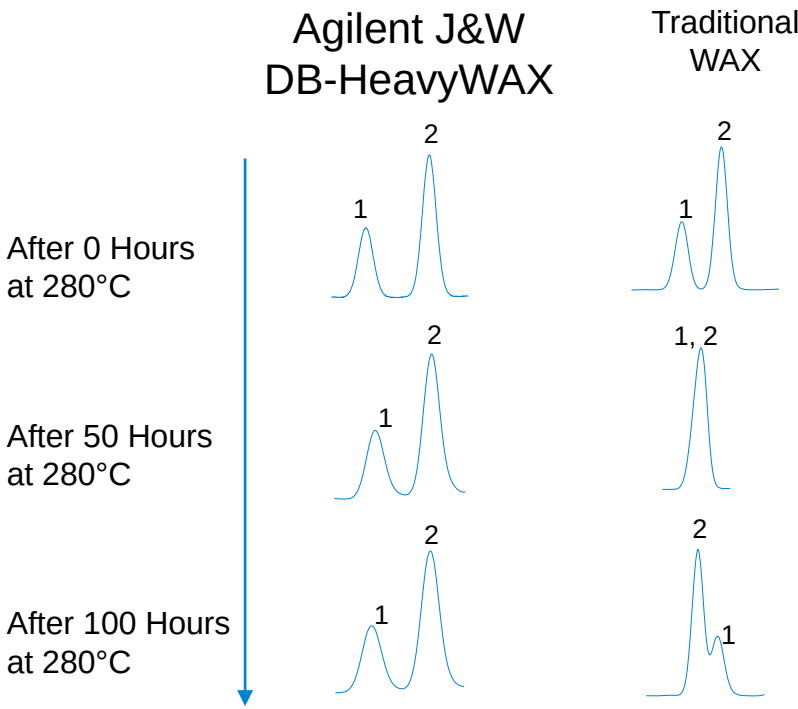
Application Note 5991-9078EN

Thermal Stability and Selectivity: In Action

Pink Grapefruit Essential Oil (cold pressed)

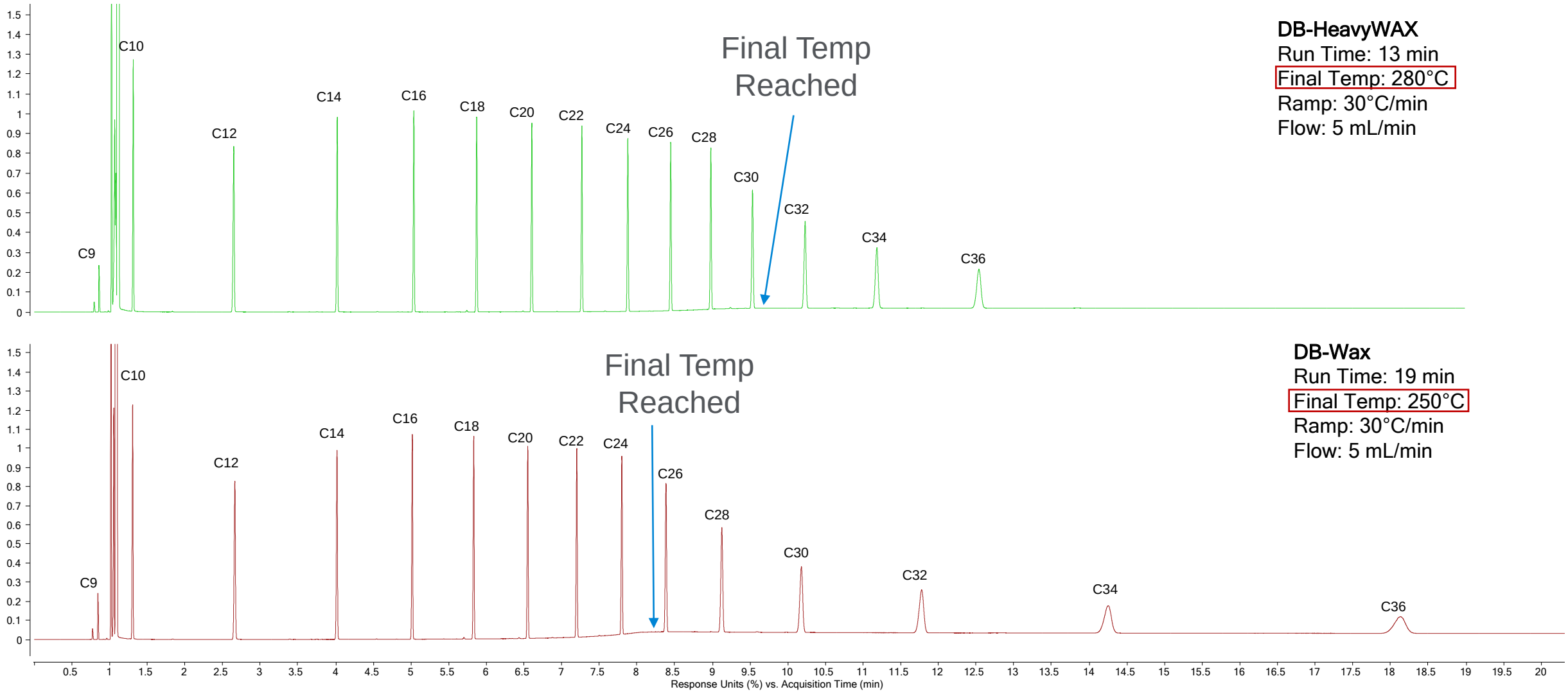
Peak	Compound
1	Linalool
2	γ-Amorphene





Application Note 5991-9078EN

Better Peak Shape and Decrease Runtime



Benefits of Low Bleed

Pyrolysis Gasoline

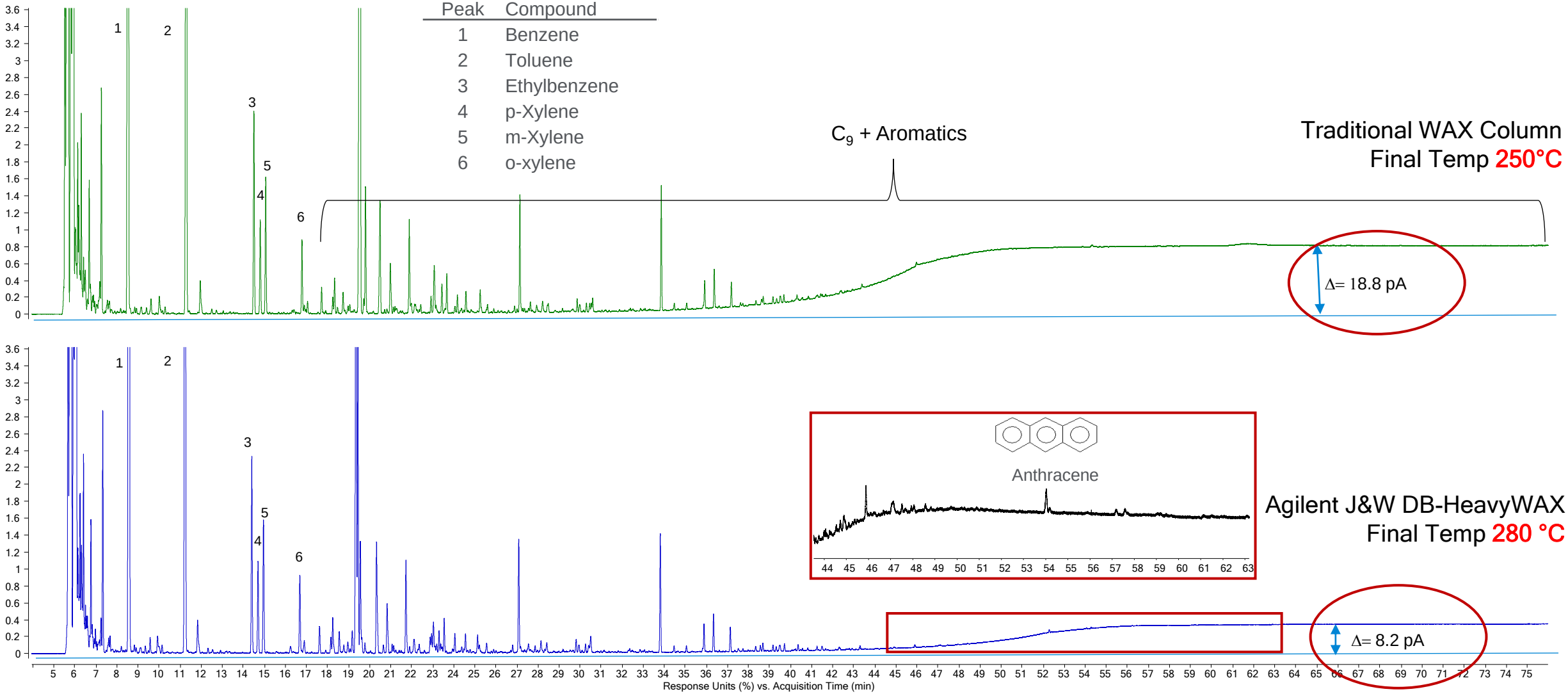
- ASTM D6563
- Heavier Aromatic Compounds
- Lower bleed at 280°C than traditional WAX at 250°C

GC Conditions	
Column	60m x 0.25 mm x 0.25 µm
Carrier	Helium, constant flow, 1.2 mL/min
Oven	70°C (10.0 min),Ramp 5°C/min to 280°C (30 min)

- ✓ Increased Sensitivity for Later Eluting Compounds
- ✓ Increased Column Lifetime

5991-9115EN

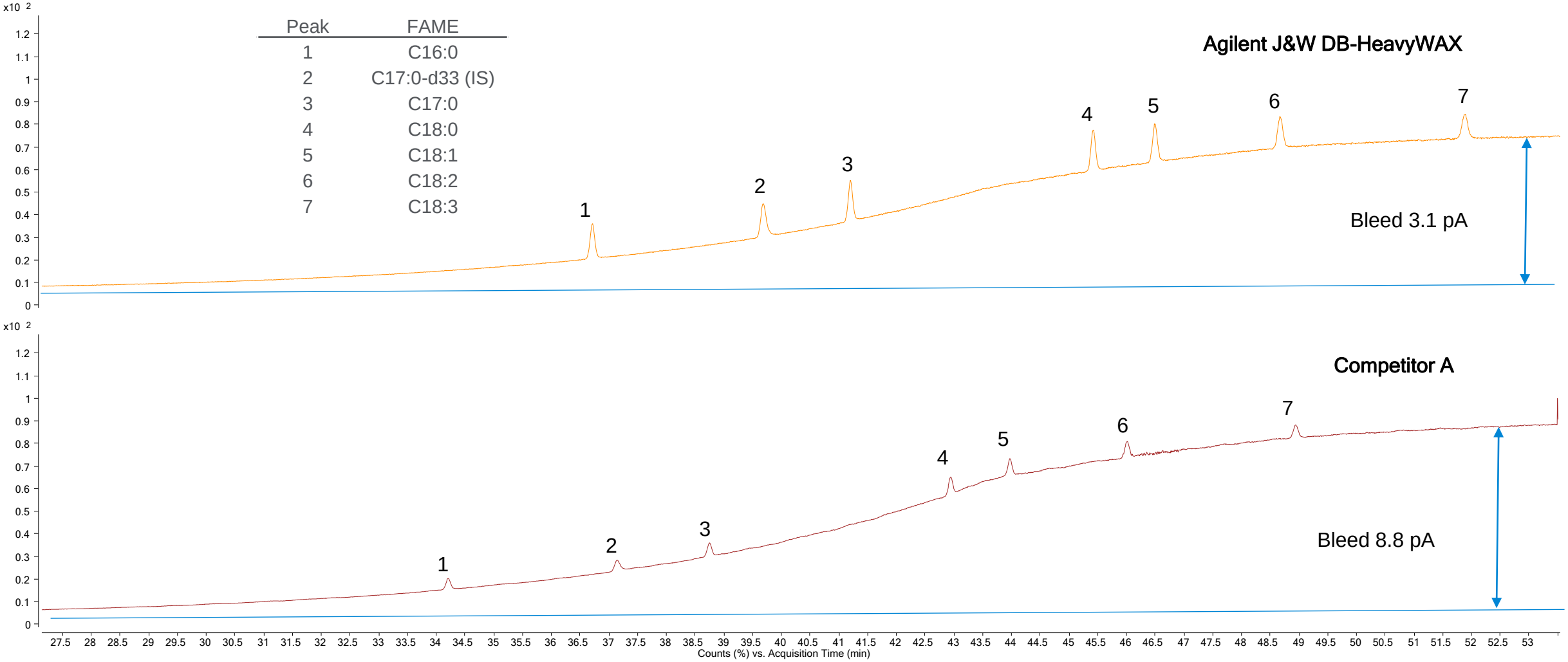
Pyrolysis Gasoline



Application Note 5991-9115EN

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)

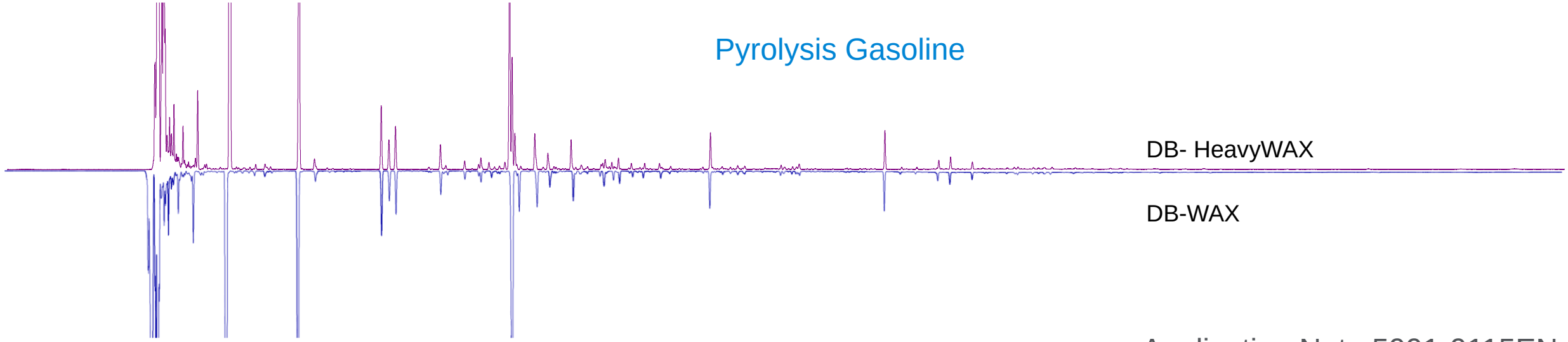
Compound	Operating Hours at 260°C									Average	%RSD
	1	5	7	13	22	39	42	46	49		
C16:0	36.58	36.47	36.45	36.44	36.43	36.43	36.43	36.43	36.42	36.45	0.13%
C17:0 d33 (ISTD)	39.57	39.47	39.45	39.44	39.44	39.44	39.44	39.44	39.44	39.46	0.11%
C17:0	41.09	40.99	40.98	40.96	40.97	40.97	40.97	40.97	40.96	40.98	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%

It Is A WAX!

Application Note 5991-9078EN

Pink Grapefruit Essential Oil

Pyrolysis Gasoline



Application Note 5991-9115EN

Conclusions for the J&W DB-HeavyWAX

- ✓ Increased Thermal Stability (280°C Isothermal, 290 °C Programmed)
  - ✓ Stable Retention Times
  - ✓ Consistent Peak Order
- ✓ Deceased Column Bleed
  - ✓ Lower noise at higher temps for greater sensitivity for “heavier” compounds
  - ✓ Increased analyte range
  - ✓ Decreased analysis time
  - ✓ Safely bake out column up to 290
- ✓ DB-WAX Selectivity
  - ✓ Simpler method translation

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

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