



The Inert Flow Path Story: Continuous Improvements in Results Fidelity for Challenging Analytes

Ken Lynam

August 11, 2016



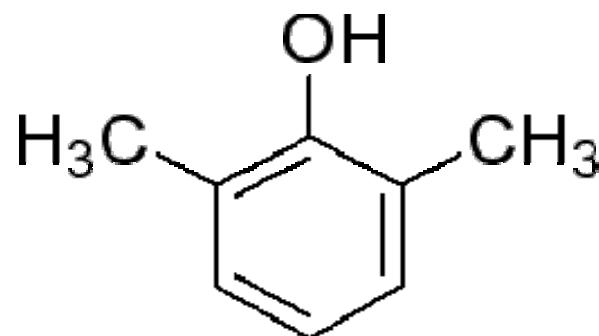
Topics Covered Today

- Inert Flow Path Story Early Efforts
- GC and GC/MS connectors
 - Typical fittings/ferrules used in the gas phase
 - Impact of poor connections
 - Novel solutions for leak free connections
- HPLC and UHPLC connectors
 - Typical fittings from several manufacturers
 - Impact of poor connections
 - Novel solutions for less stress and more success
- Inert polyethylene glycol columns

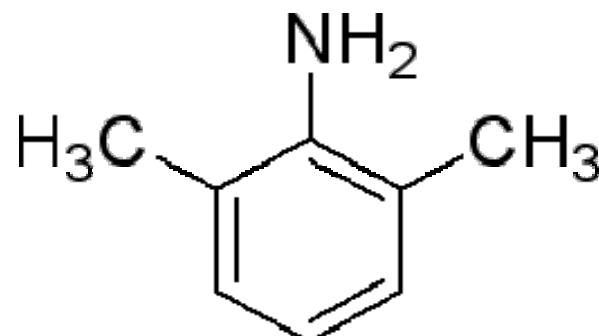


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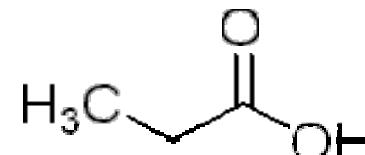
Weak Probes versus Strong Probes



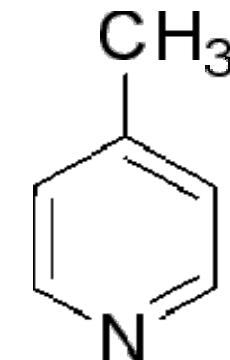
2,6-Dimethylphenol



2,6-Dimethylaniline



1-Propionic acid



4-Picoline

A QC Test for Today's Demanding Applications

Carefully selected probes designed to test inertness effectively.

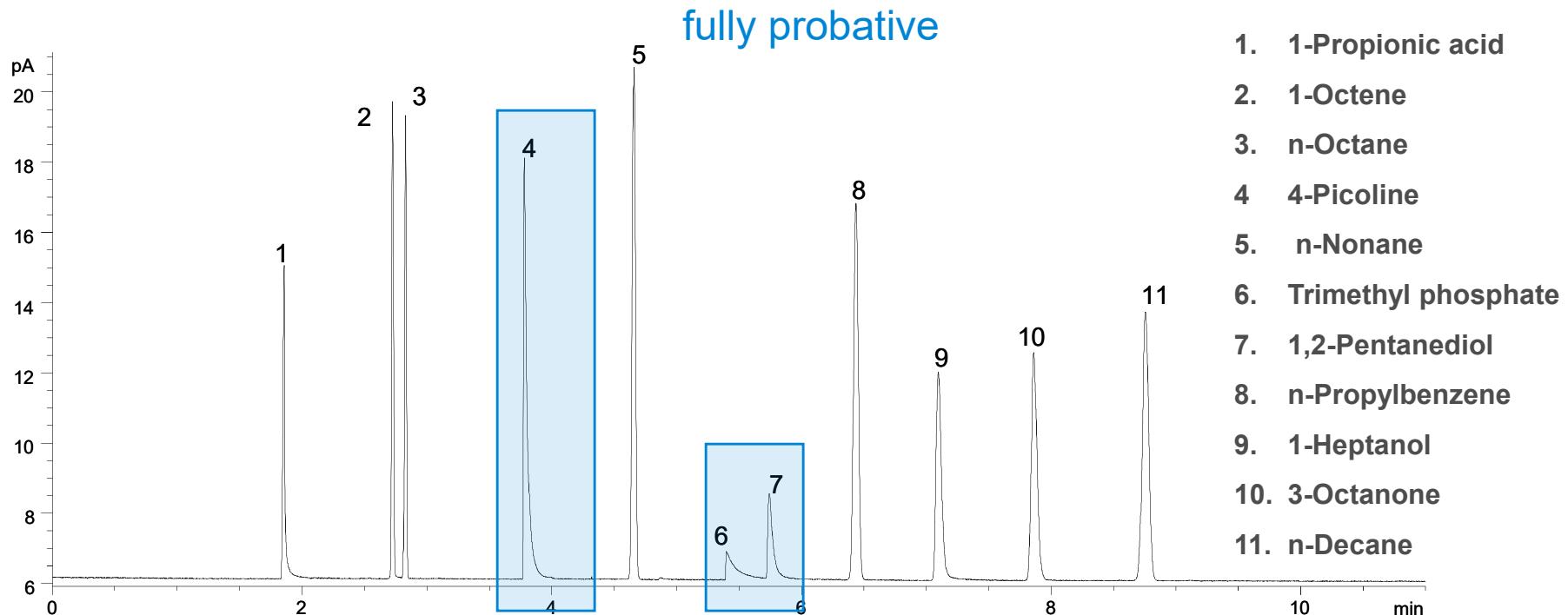
Probe	(ng on column)	Column functional test
1. 1-Propionic acid	1.0	Basicity
2. 1-Octene	0.5	Polarity
3. n-Octane	0.5	Hydrocarbon marker
4. 4-Picoline	1.0	Acidity
5. n-Nonane	1.0	Hydrocarbon marker
6. Trimethyl phosphate	1.0	Acidity
7. 1,2-Pentanediol	1.0	Silanol
8. n-Propylbenzene	1.0	Hydrocarbon marker
9. 1-Heptanol	1.0	Silanol
10. 3-Octanone	1.0	Polarity
11. n-Decane	1.0	Hydrocarbon marker

5989-8665EN



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UI Mix Results on a Brand X “Premium” Column



Sampler: Agilent 7683B, 0.5 μ L syringe (Agilent part # 5188-5246), 0.02 μ L split injection

Carrier: Hydrogen constant pressure, 38 cm/s

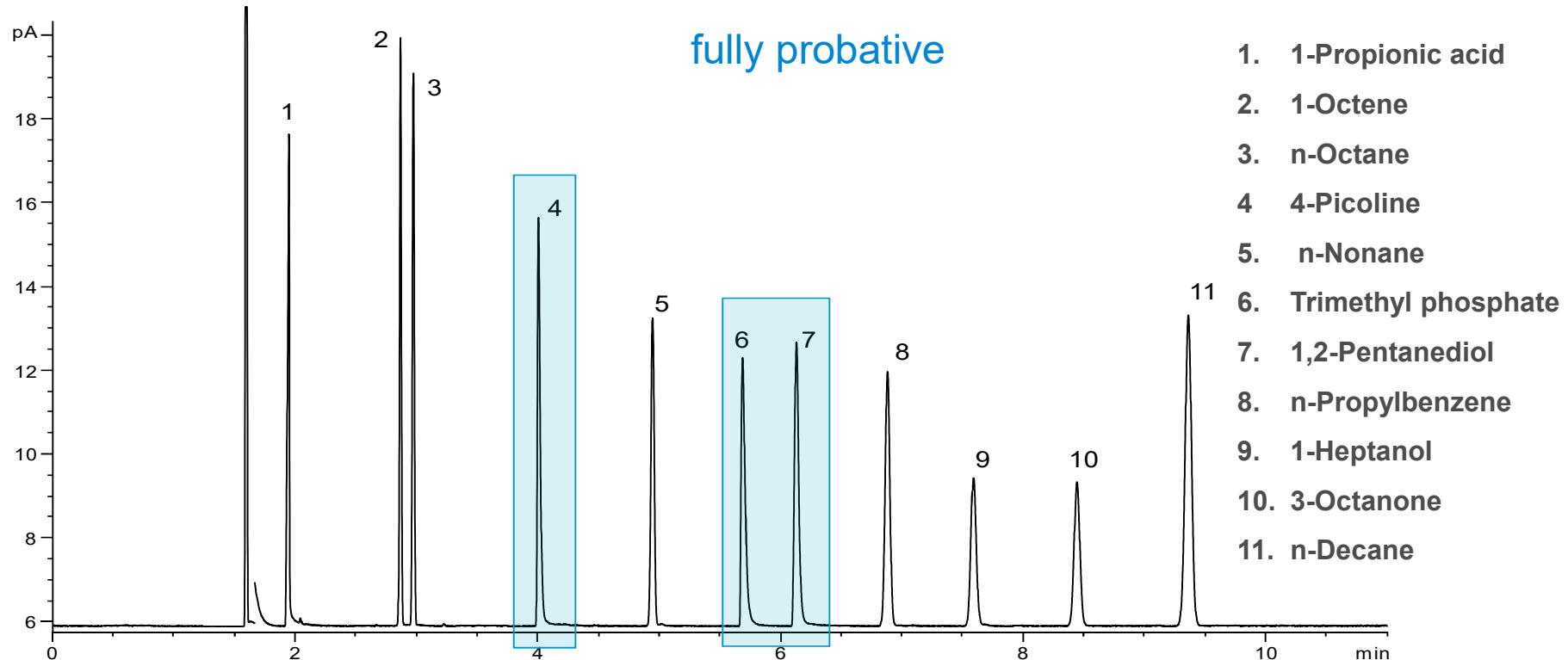
Inlet: Split/splitless; 250 °C, 1.4 ml/min. column flow, split flow 900 ml/min., gas saver flow 75 ml/min. on at 2.0 min.

Liner: Deactivated single taper w/ glass wool (Agilent part # 5183-4647)

Oven: 65 °C isothermal

Detection: FID at 325 °C, 450 ml/min. air, 40 ml/min. hydrogen, 45 ml/min., nitrogen makeup

UI Mix Results on an Agilent J&W DB-5ms Ultra Inert



Sampler: Agilent 7683B, 0.5 μ L syringe (Agilent part # 5188-5246), 0.02 μ L split injection

Carrier: Hydrogen constant pressure, 38 cm/s

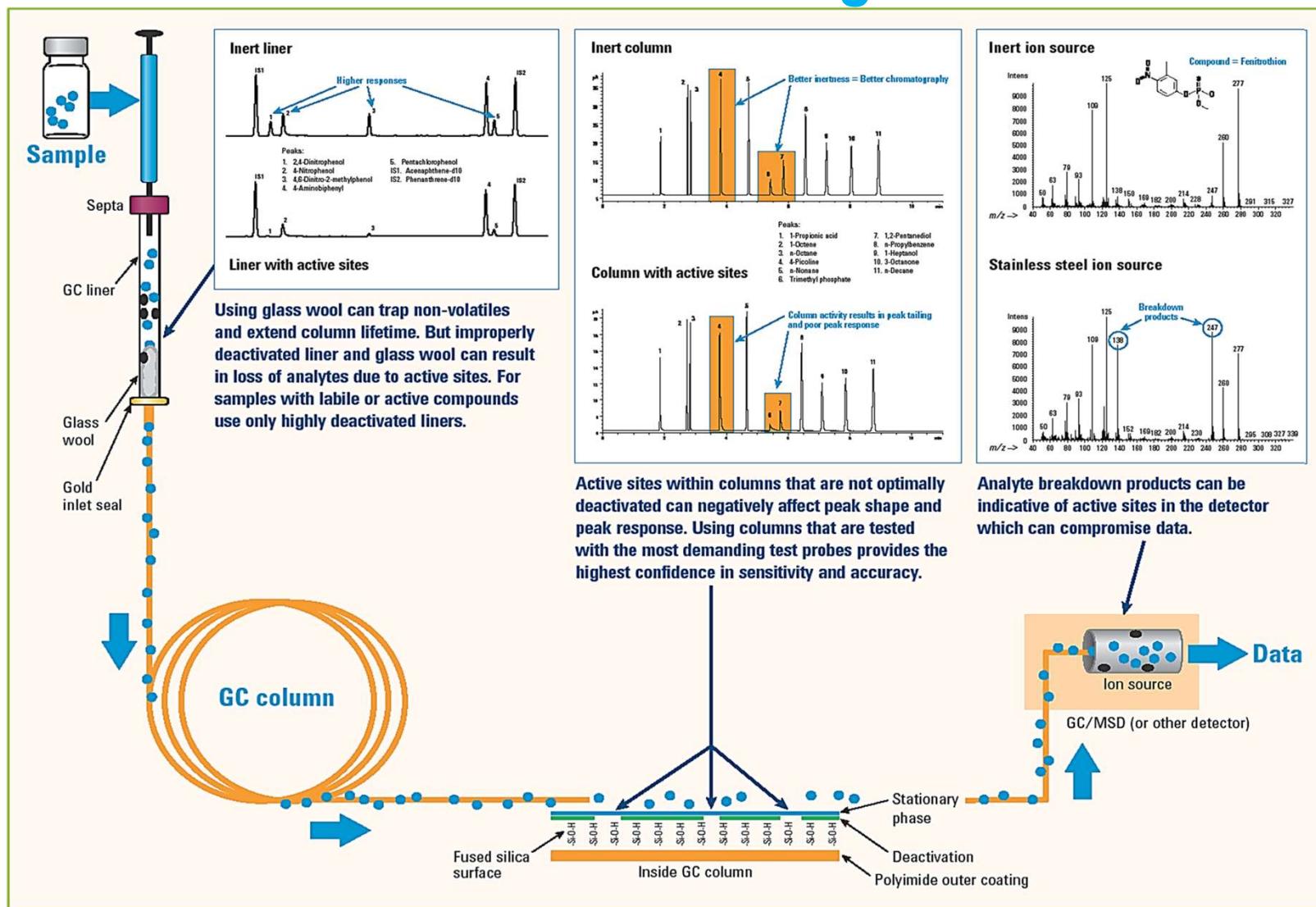
Inlet: Split/splitless; 250 °C, 1.4 ml/min. column flow, split flow 900 ml/min., gas saver flow 75 ml/min. on at 2.0 min.

Liner: Deactivated single taper w/ glass wool (Agilent part # 5183-4647)

Oven: 65 °C isothermal

Detection: FID at 325 °C, 450 ml/min. air, 40 ml/min. hydrogen, 45 ml/min., nitrogen makeup

GC Flow Path Diagram



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Tips for Ensuring an Inert Flow Path

- Maintain the inlet
- Prevent sample loss at injection
- Select a highly inert column
- Remember detector inertness
- Use a gas purifier



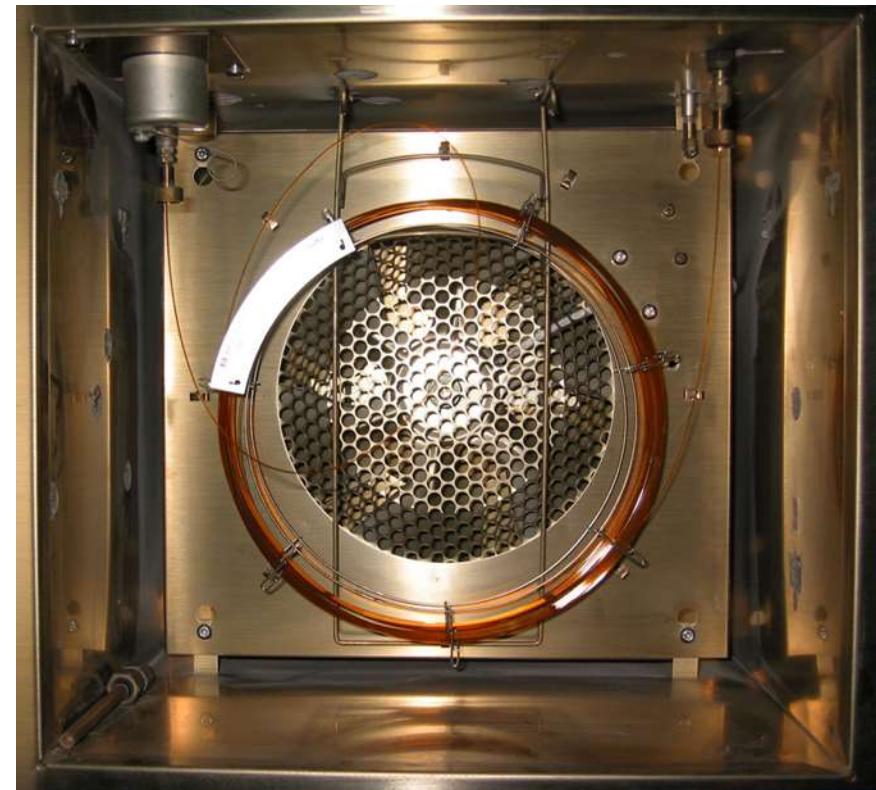
GC Column Installation is a Challenge

A routine, basic procedure, but not easy to do

Plus – a minimum of 2 chances to get it wrong !

- Very small parts
- Wrenches in cramped, dark space

Even when done correctly, fittings are known to be prone to leaks



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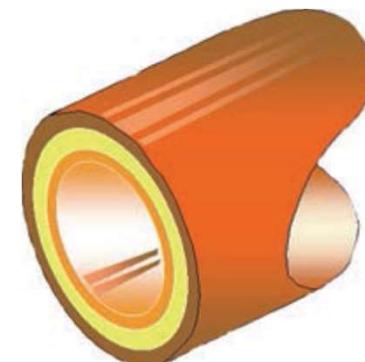
How do you make leak free column connections? Start with the right tools and supplies

Assemble the parts

- Column Nuts
 - Material : stainless – but need brass for MS interface
 - Finger tight available for graphite ferrules
- Choice of right ferrules

Make a good clean cut – every time

- After ferrule is installed
- Fused silica tubing cutters : ceramic
- Magnifier to inspect the cut



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Which Capillary Ferrules?



polyimide



polyimide/graphite



graphite



flexible metal

Composition	Re-Use	Max T	Use	Limitation
Polyimide (Vespel)	yes	280	Easy seal	Shrink after heating which causes leaks after thermal cycle; isothermal only
Graphite	yes	450	Not MS or ECD	Contamination, leakages – not for oxygen sensitive detectors
Polyimide/graphite (85% / 15%)	limited	350	MS	Still shrink after thermal cycles which creates leaks; need to re-tighten regularly
Flexible metal	no	450	CFT ...	May not seal well with damaged, rough surfaces



“Short” ferrules for inlet and detector configurations on Agilent GC’s

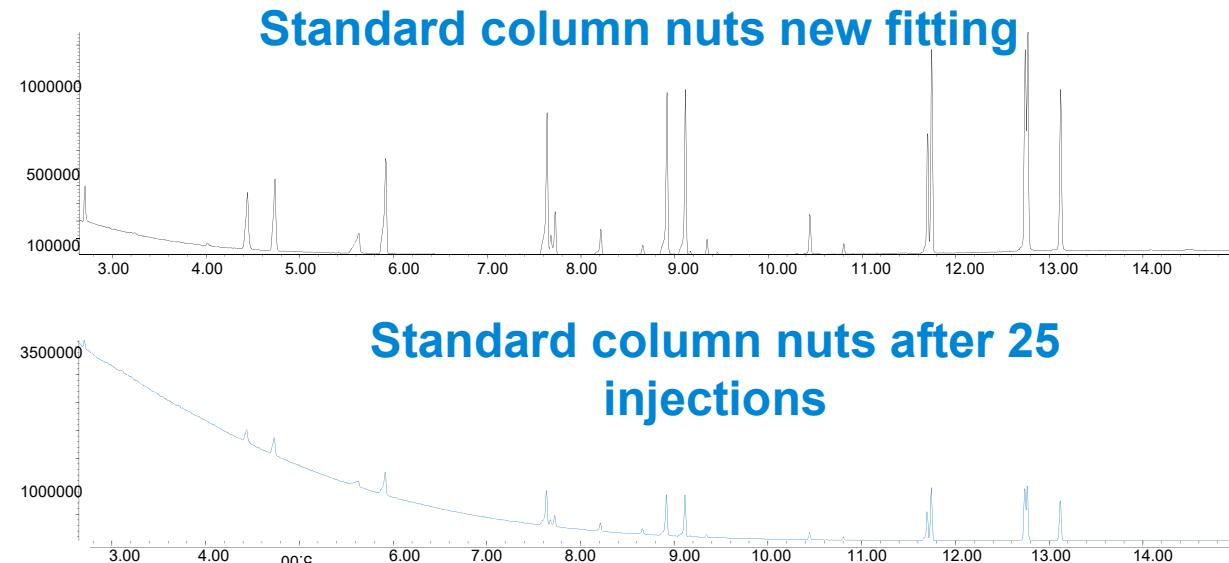


“Long” ferrules for MS transfer lines and MS interface nut



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What Impact Do Leaks Have ?



Leaks at the inlet or before the column

- stationary bleed resulting in shorter column lifetime
- higher baseline and poor integration

At the detector end

- increased detector signal/noise
- oxidation of the ion source in MSD, increasing the need for detector maintenance

Overall, leaks effect system performance and productivity!

Agilent Self Tightening Column Nuts

Ease of use – install in dark, small space in GC oven without wrenches

Wing design for finger tight installation with graphite/polyimide blend ferrules

No tools dramatically reduces force, which prevents over-tightening or damage

Robust stainless steel construction

Plus....

Novel spring driven piston design that continuously presses against the ferrule

to maintain a leak-free fitting

even when the ferrule shrinks during temperature program!



Agilent Self Tightening Column Nuts

Designed for use with *short* graphite/polyimide blend ferrules – both at the inlet and the MS interface – so only one type of ferrule is needed for both ends of the column!



For inlet or detector



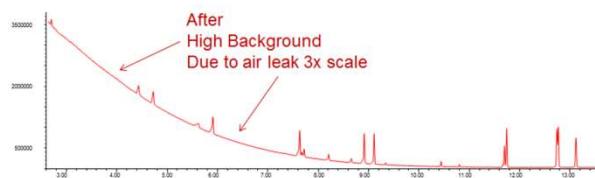
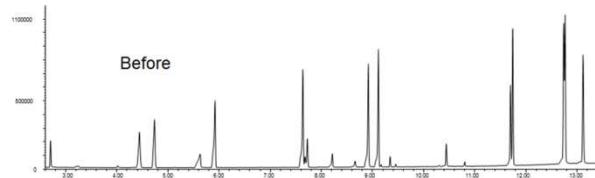
For mass spec transfer line

Short ferrule exposes more thread of the fitting for better sealing



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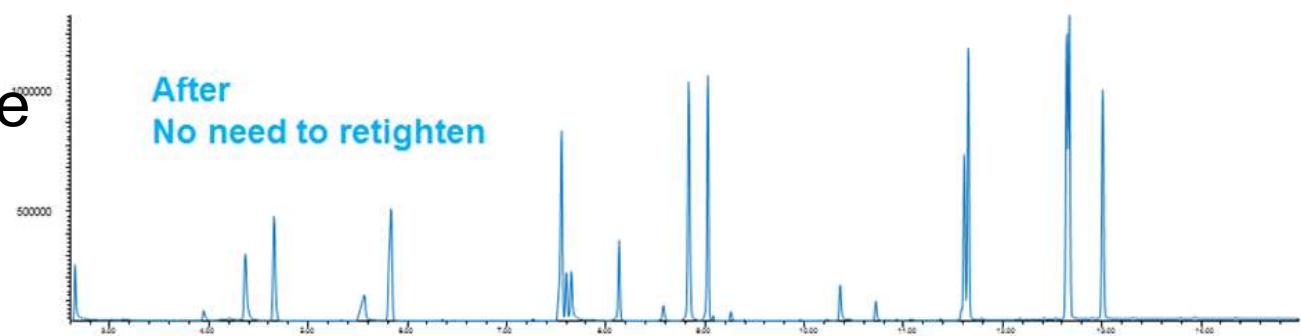
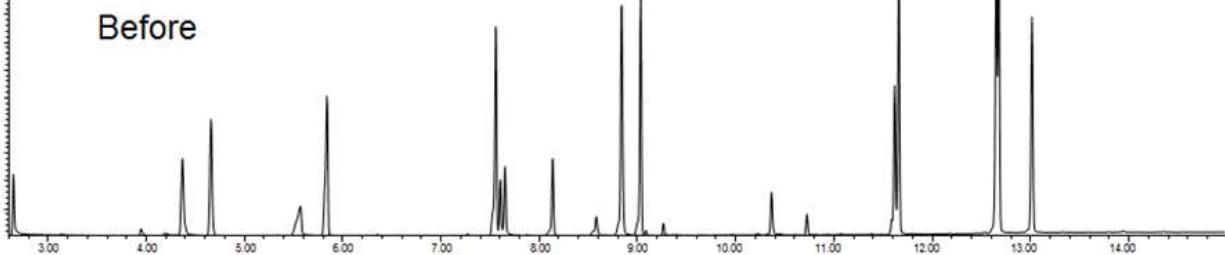
Benefit of Self Tightening Column Nuts



Take you from this....

..... to this!

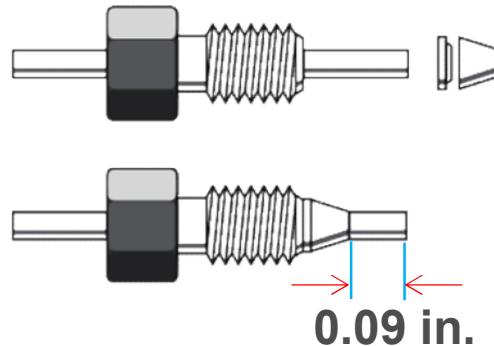
Without retightening, the baseline remains flat after 400 runs with no indication of leaks when using the Self Tightening column nuts



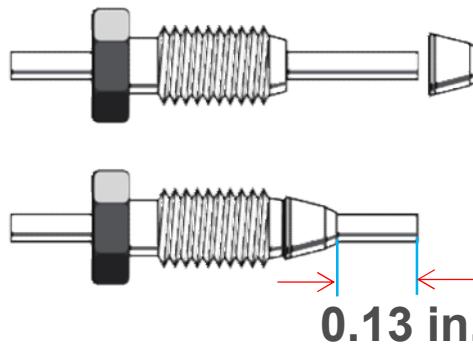
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Common HPLC Connectors from Different Manufacturers

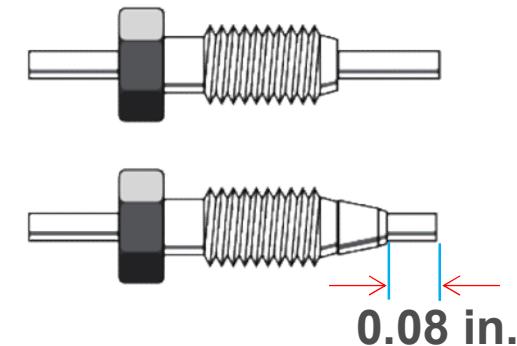
Swagelok



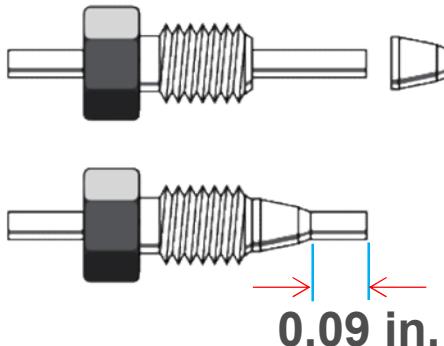
Waters



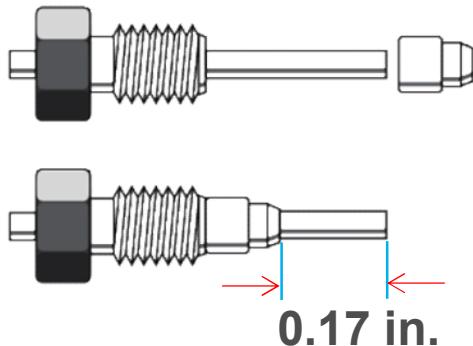
Valco



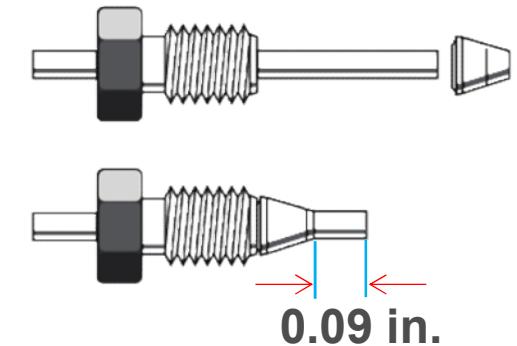
Parker



Rheodyne



Upchurch



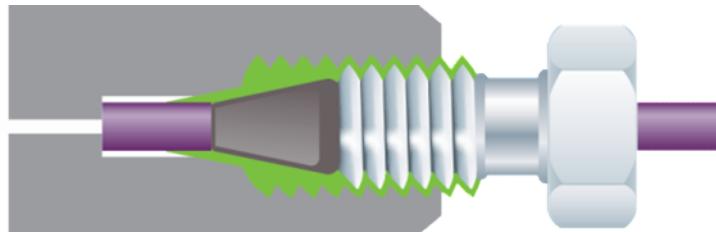
Different brands have different geometric designs for fitting connections.



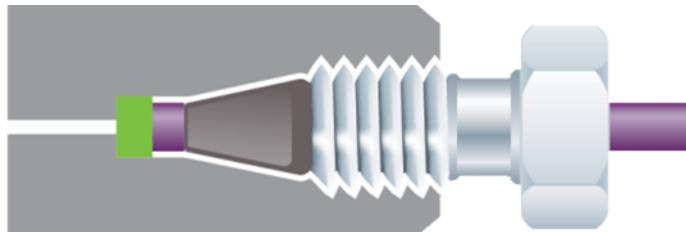
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Comparison of Correct and Incorrect Fitting Connections

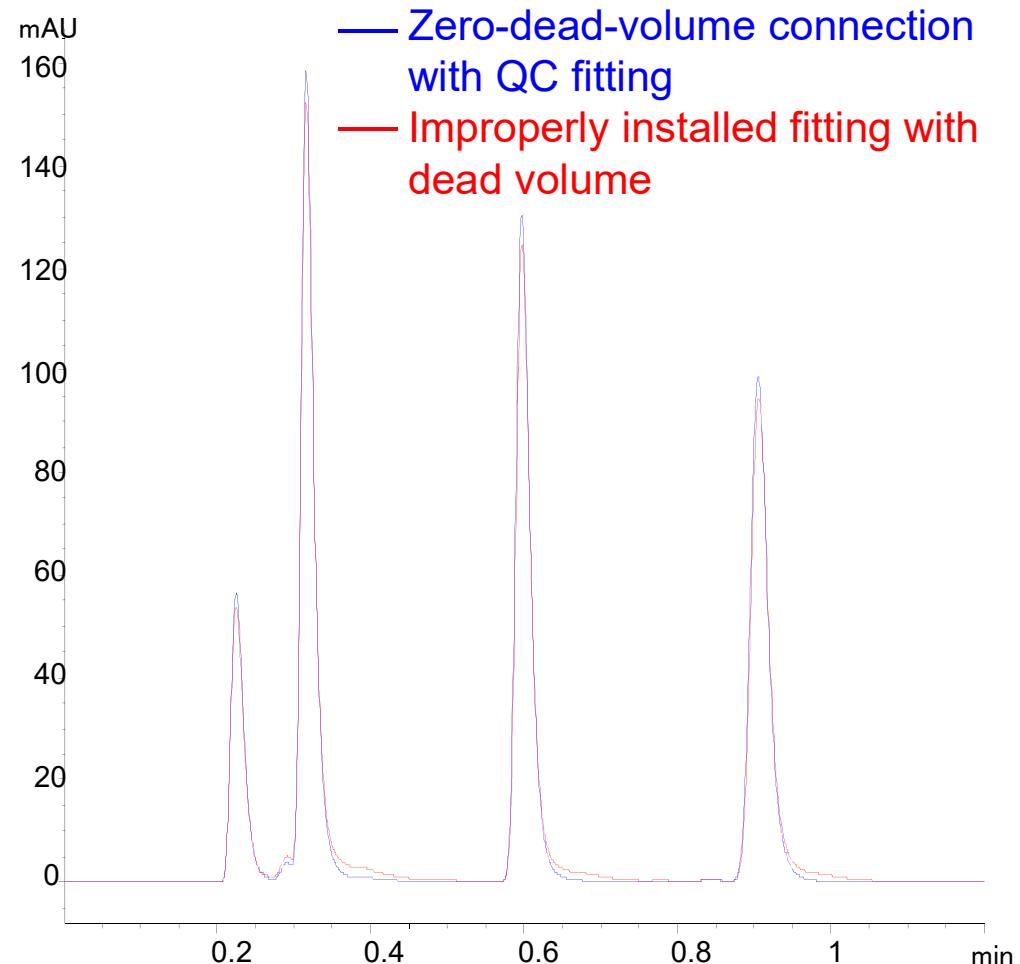
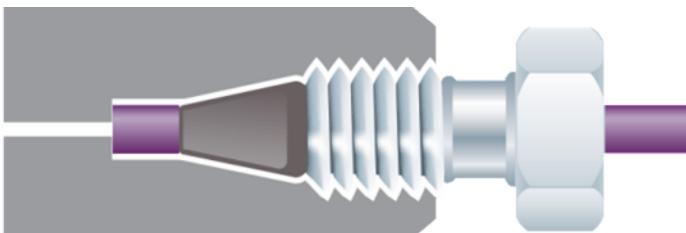
Stem length too long → leak



Stem length too short → dead volume

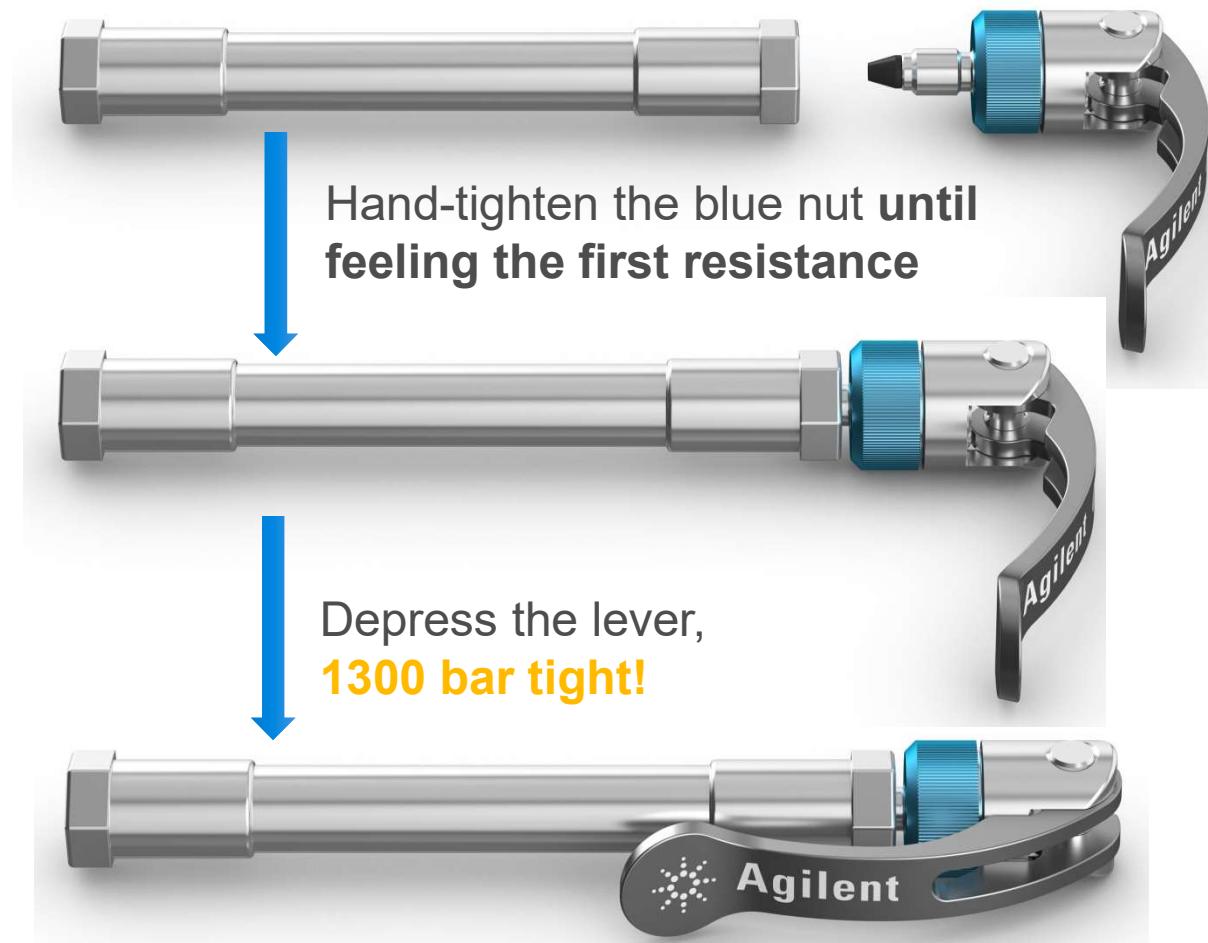


Properly fitted tubing, no dead volume



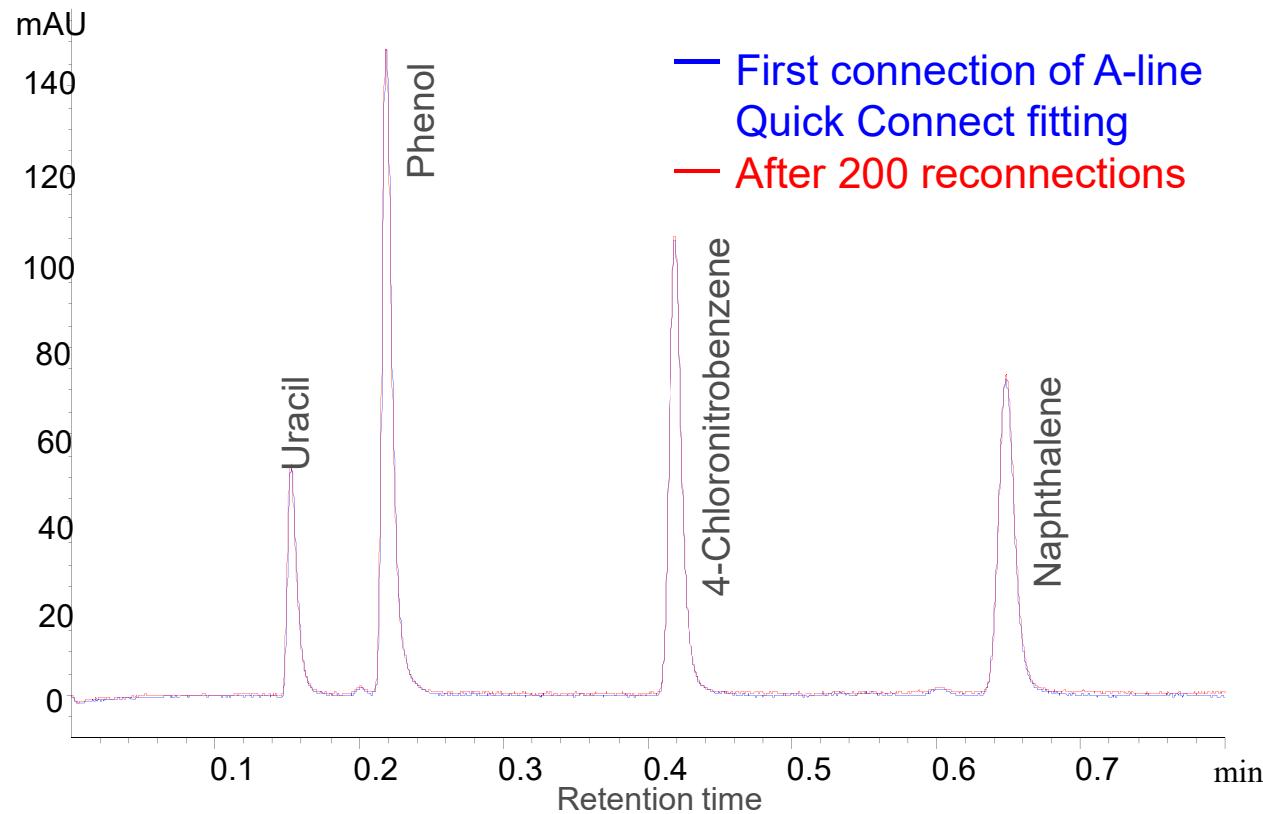
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Installation of A-line Quick Connect Fitting



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Robustness Over 200 Reconnections

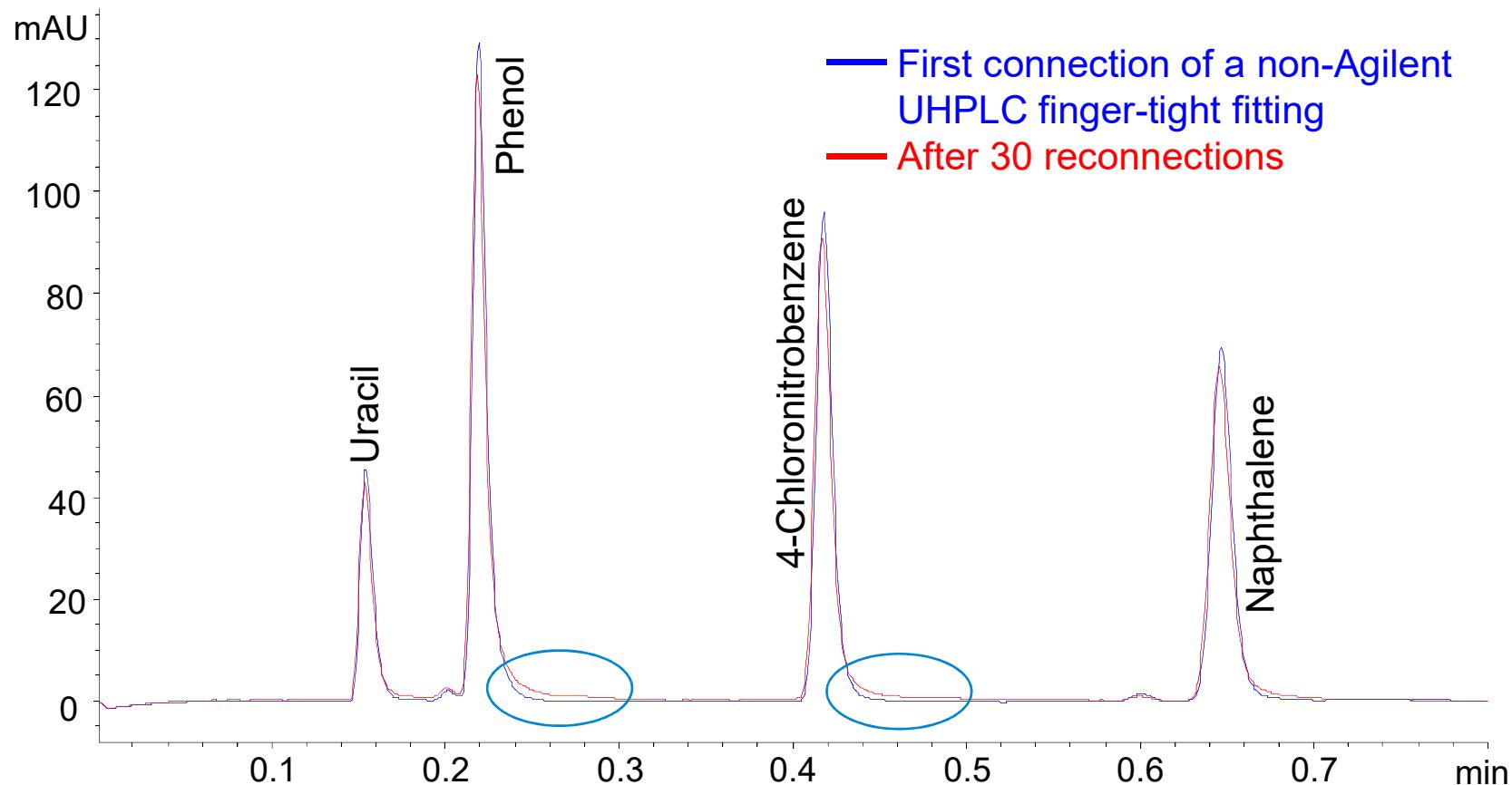


Peak shape stayed stable over 200 reconnections



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UHPLC Finger-Tight Fitting from Other Vendor



Deterioration of peak shape after only 30 reconnections



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Column Connection Summary

- Key attributes to look for in liquid and gas connectors
 - Reliability
 - Leak free
 - Minimal void volume
 - Ease of use
- Agilent has the solution for making the right connections
 - In HPLC and UHPLC
 - **Quick Connect and Quick Turn fittings**
 - In GC and GC/MS
 - Inlet and transfer line **Self Tightening Column Nuts**



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Striving to Improve GC Analyses and Lab Productivity?

Challenges:

- Variable column-to-column peak shape performance
- Limited column lifetime
- Instrument downtime for GC system while troubleshooting
- Re-running samples due to questionable results
- Increased cost of lab operations

For active and complex polar compounds, adsorption caused by GC flow path activity can be easily avoided

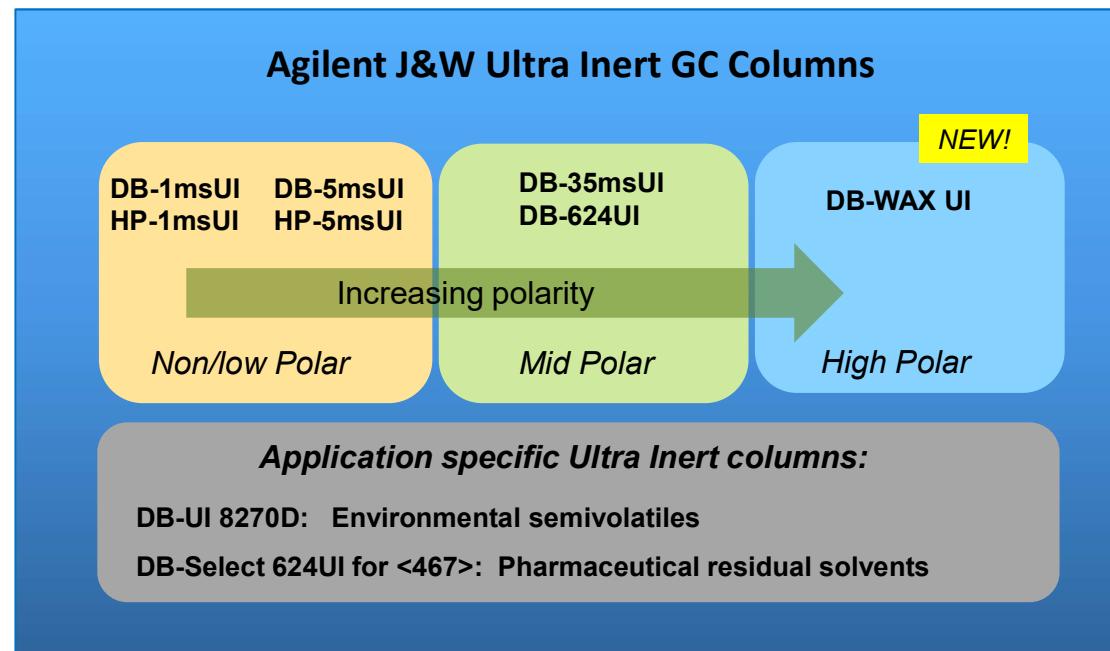


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A New WAX GC Column for Analysis of Challenging Polar Compounds: DB-WAX Ultra Inert

DB-WAX UI GC columns are part of the Agilent J&W Ultra Inert GC column family, which pushes industry standards for consistent column inertness.

Every Ultra Inert GC column is rigorously tested to ensure optimal, active analyte delivery to the GC or MS detector.



The result: lower detection limits and more accurate identification of challenging polar analytes



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Improve Lab Productivity and Lower Operational Cost

- **Spend less time on troubleshooting and re-runs:** With DB-WAX UI GC columns, excellent peak shape and column-to-column inertness reproducibility is obtained, as well as retention time stability.
- **Save money on columns and maintenance:** DB-WAX UI has extended inertness lifetime that withstands repeated temperature cycling to the upper temperature limits of the column.
- **No need for pre-qualifying columns:** Specific inertness testing during quality control guarantees out-of-the-box inertness performance for every DB-WAX UI column.
- **Implement easily, with minimal workflow interruption:** DB-WAX UI GC columns have the same selectivity as standard DB-WAX GC columns. Upgrade to Ultra Inert performance easy with minimal validation. No need to re-create or modify existing compound libraries that are based on DB-WAX selectivity.

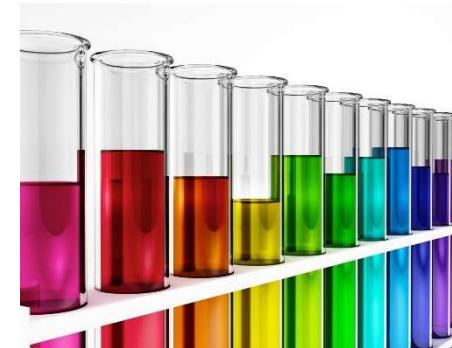
Combine with Agilent Inert Flow Path GC Supplies for optimized performance



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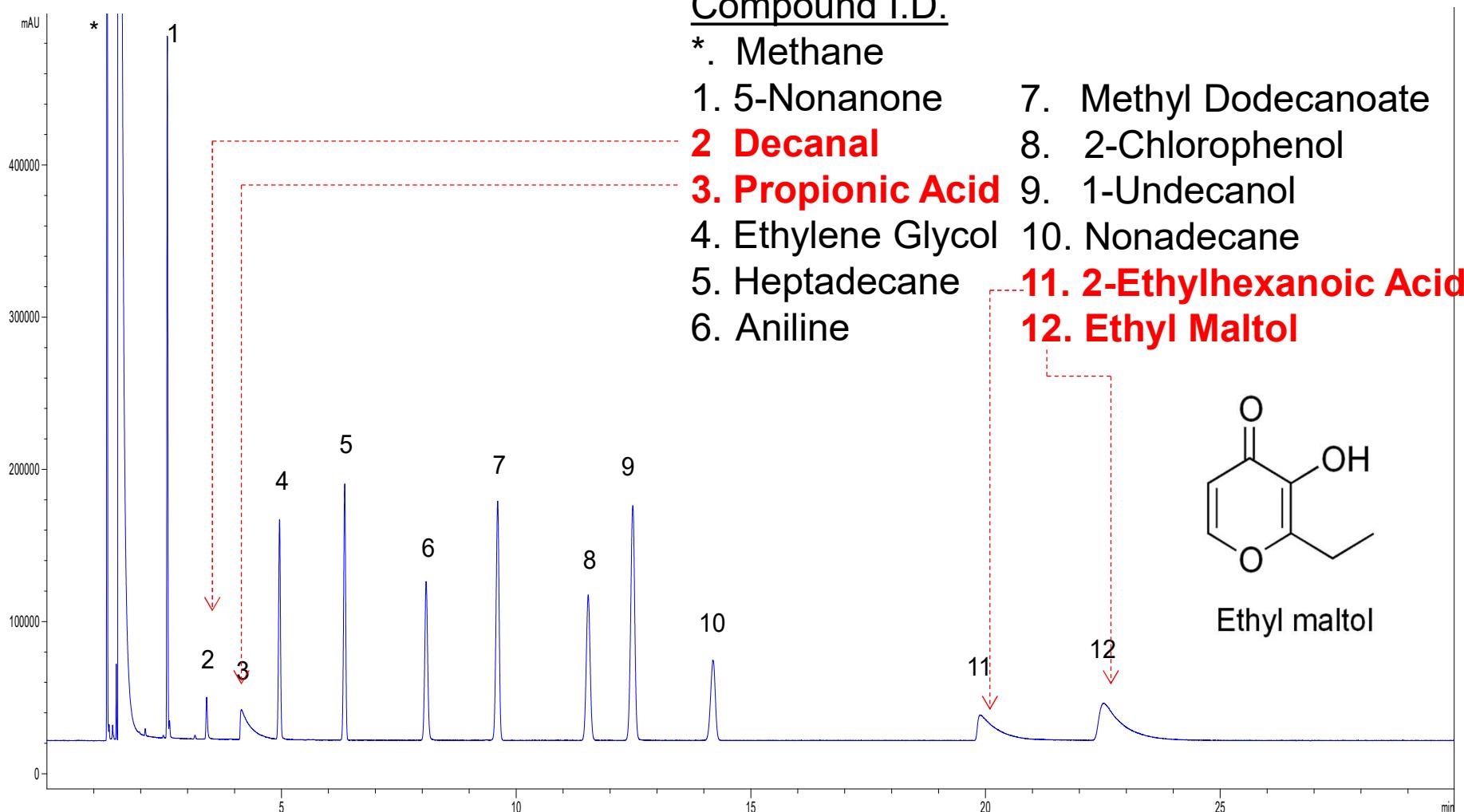
Proof of Inertness: DB-WAX Ultra Inert Testing

- A strong test probe mixture can highlight deficiencies in column activity, while a weak mixture can actually mask such deficiencies.
- Like all of Agilent's Ultra Inert GC columns, DB-WAX UI GC columns are tested with the industry's most demanding test probe mixture based on real-world requirements—and we prove it with a performance summary sheet shipped with each column.
- The DB-WAX Ultra Inert test probe mixture includes decanal, propionic acid, ethyl hexanoic acid, and ethyl maltol to ensure inertness performance for these active polar compounds.



DB-WAX UI Test Mix on Standard WAX column

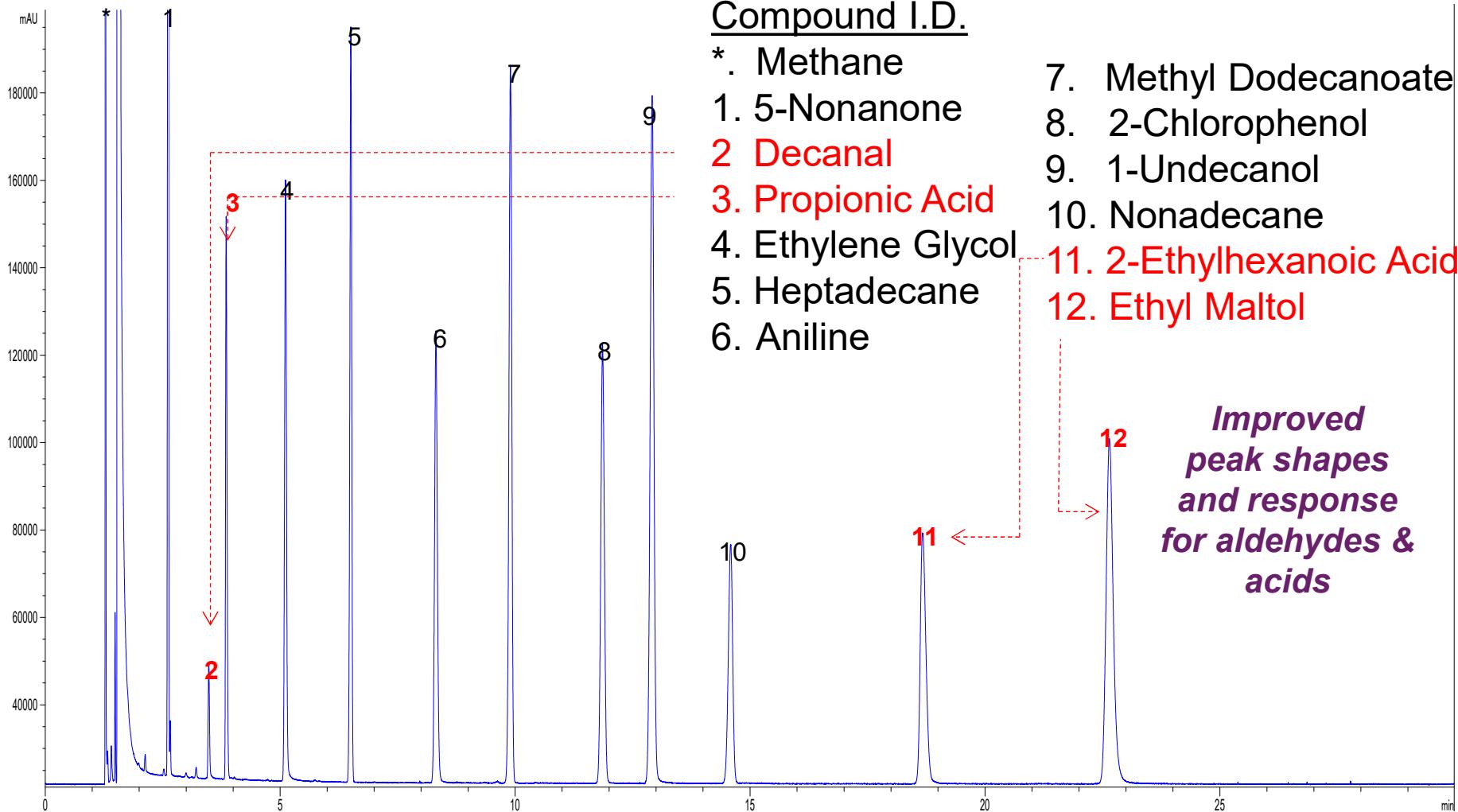
30 m x 0.25 mm x 0.25 µm



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DB-WAX UI Test mix on DB-WAX Ultra Inert

30 m x 0.25 mm x 0.25 µm



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Inertness Benchmark Testing - Test Mixes

Compounds	DB-WAX UI mix	Amount on column (ng)	Compounds	Modified Grob mix	Amount on column (ng)
1	5-Nonanone	3.3	1	Decane	2.5
2	Decanal	3.3	2	Dodecane	2.5
3	Propionic acid	3.3	3	Decanal	2.5
4	Ethylene glycol	3.3	4	2,3-Butanediol	5
5	Heptadecane	1.65	5	1-Octanol	2.5
6	Aniline	3.3	6	C10 FAME	2.5
7	Methyl dodecanoate	3.3	7	<i>n</i> C11-FAME	2.5
8	2-Chlorophenol	3.3	8	Dicyclohexylamine	5
9	1-Undecanol	3.3	9	<i>n</i> C12-FAME	2.5
10	Nonadecane	1.65	10	2,6-Dimethylaniline	2.5
11	2-Ethylhexanoic acid	6.6	11	2,6-Dimethylphenol	2.5
12	Ethyl maltol	6.6	12	2-Ethylhexanoic acid	5
			13	Ethyl maltol	5

Running conditions:

Injector temp: 250°C, split 1:75, injection volume 1µl, Flow rate 1.1 ml/min, H₂ gas. Oven temp: 130°C isothermal, FID detector: temp 260°C.

Running conditions:

Injector temp: 250°C, split 1:100, injection volume 1µl; Flow rate 1.35 ml/min, H₂ gas. Oven temp: initial temp (60°C), ramp 3°C/min, final temp 200°C; FID detector: temp 260°C.

Durability?
Columns evaluated after 1 and 50 hours at 250°C

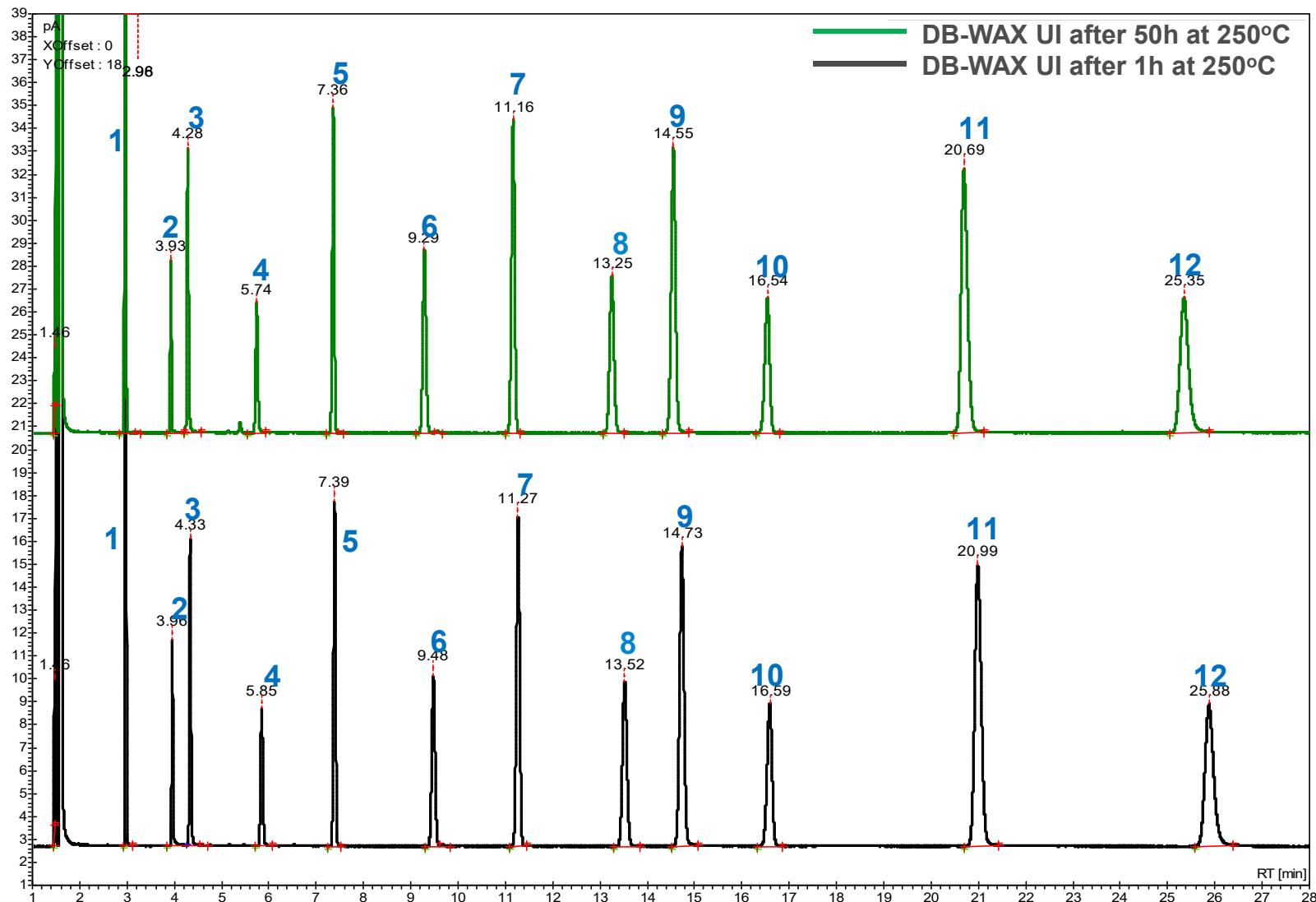


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DB-WAX UI Test Mix on DB-WAX UI Column

1h @ 250°C and 50h @ 250°C conditioning

Excellent stability and peak shape after extended exposure at the temperature limit



DB-WAX UI Applications

Food, Flavor and Fragrance Labs

Applications:

- Essential and flavor oils
- Personal care products
- Fragrance standards/allergens
- Free fatty acids, Alcohols, Glycols
- Beverages and aroma analysis
- Solvents

- ✓ Production quality control
- ✓ Analysis of feed stocks and raw materials
- ✓ Support for production operations troubleshooting
- ✓ Method development
- ✓ R&D

Industrial Chemical Labs

Applications:

- Solvent purity – alcohols, ketones, aldehydes etc.
- Mixed solvent/chemical formulations (cleaners, paint additives etc.)

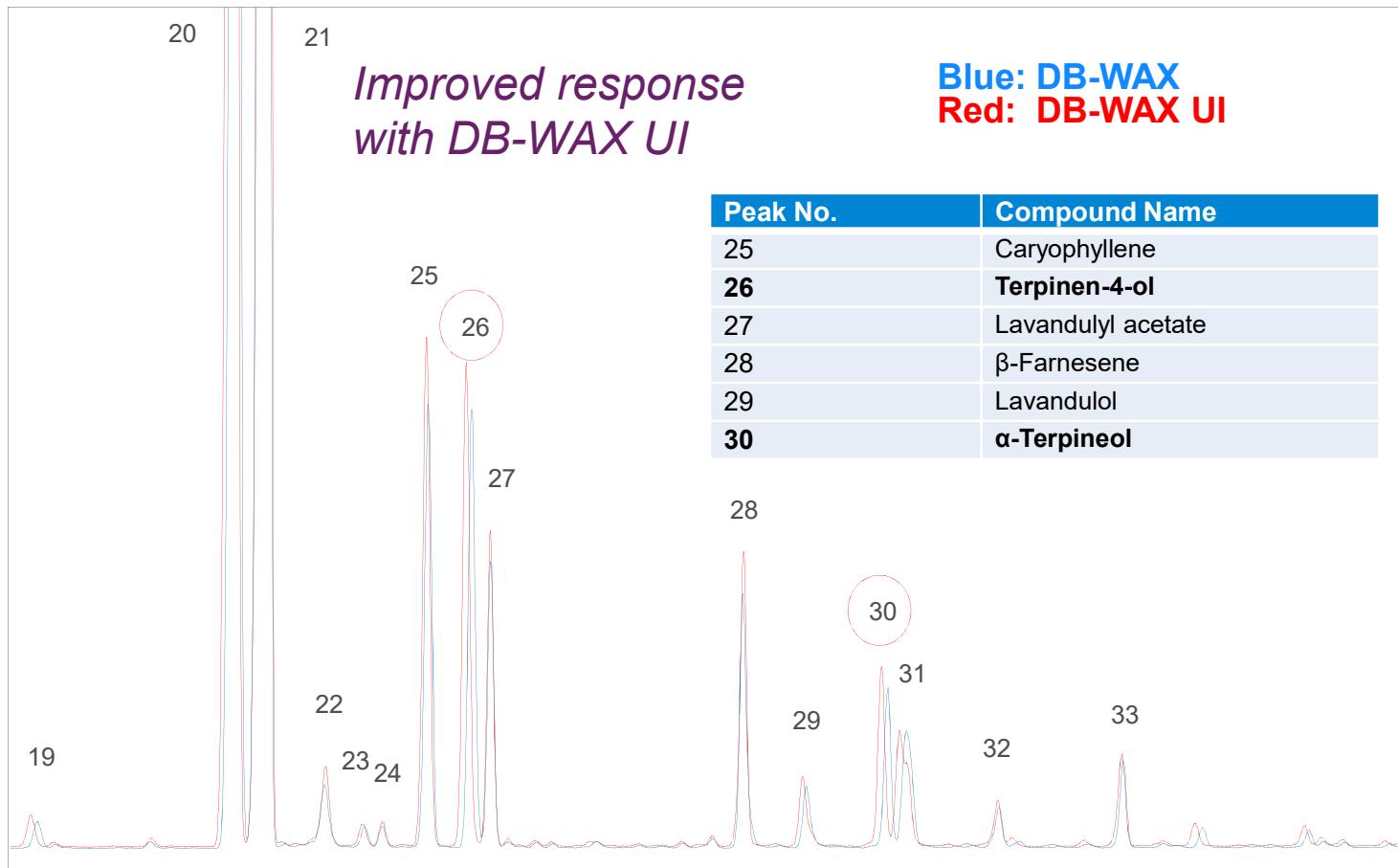
Pharmaceutical Labs

Application: USP regulated Residual Solvents confirmation column



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Lavender Oil: Enlarged Section GC/MS Chromatogram

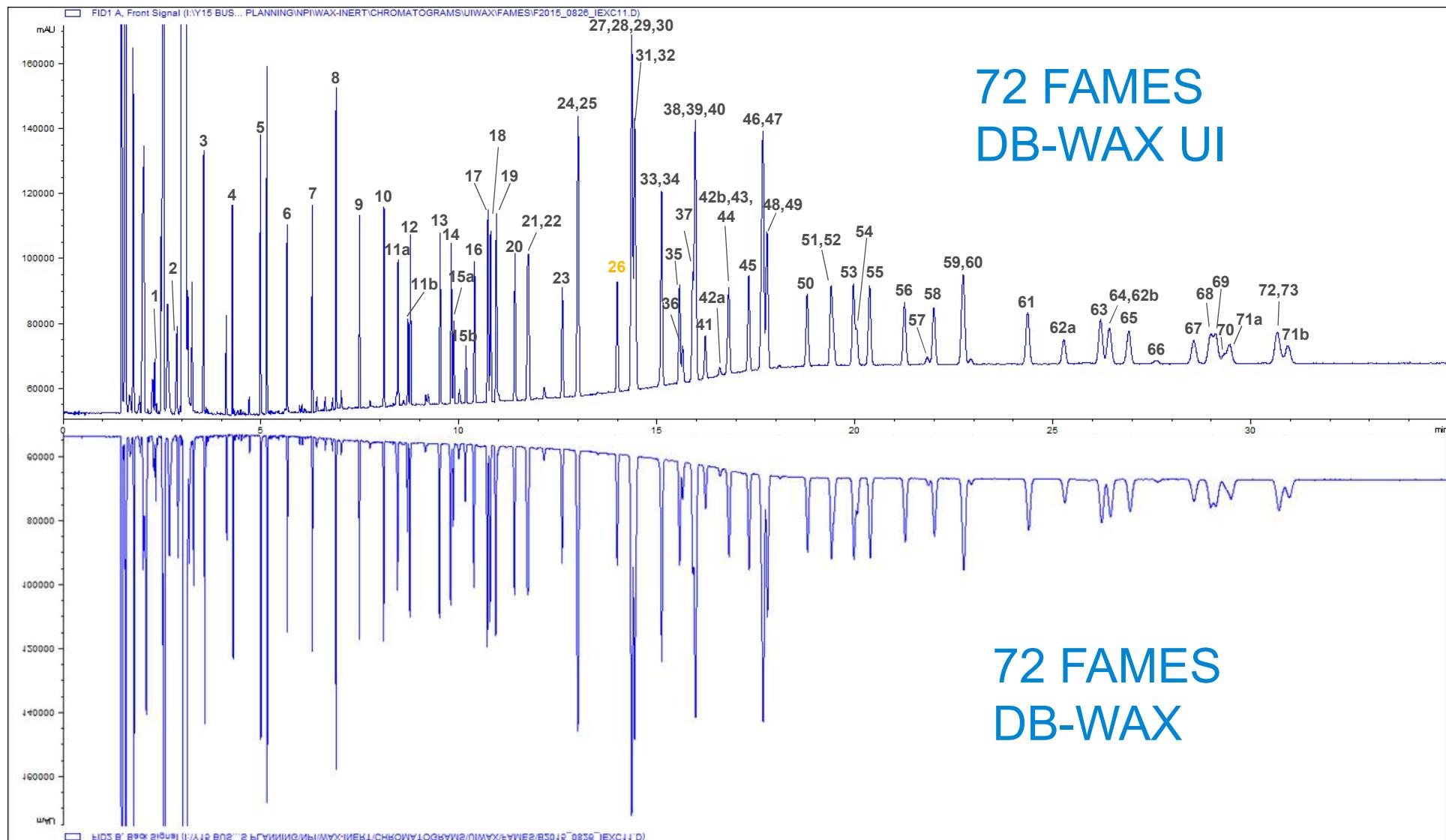


Identical column selectivity is crucial for customers in flavor / fragrance industry who have built extensive compound libraries using DB-WAX



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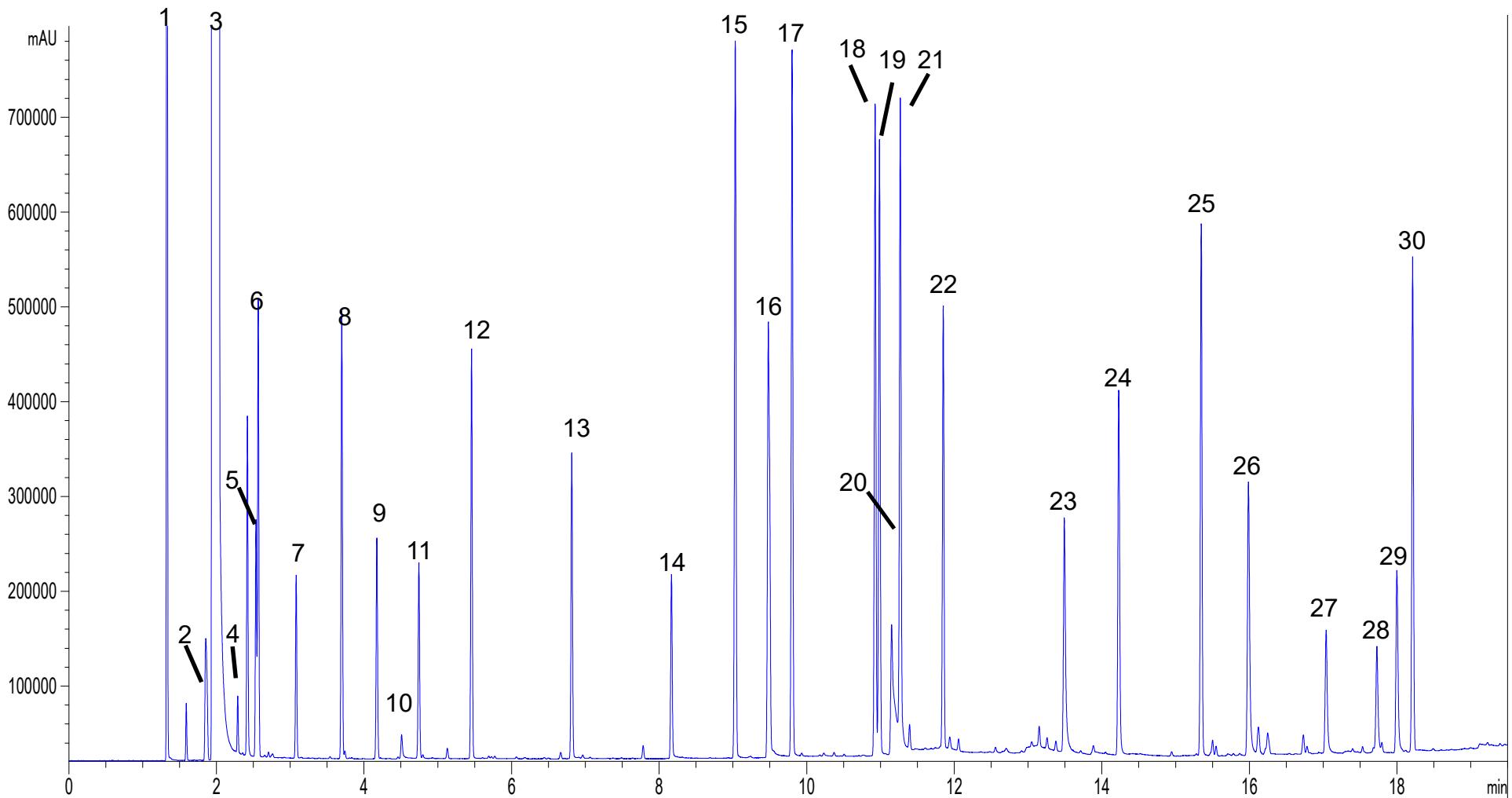
DB-WAX Selectivity Maintained on DB-WAX UI



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Aldehydes Mix on DB-WAX, 122-7032

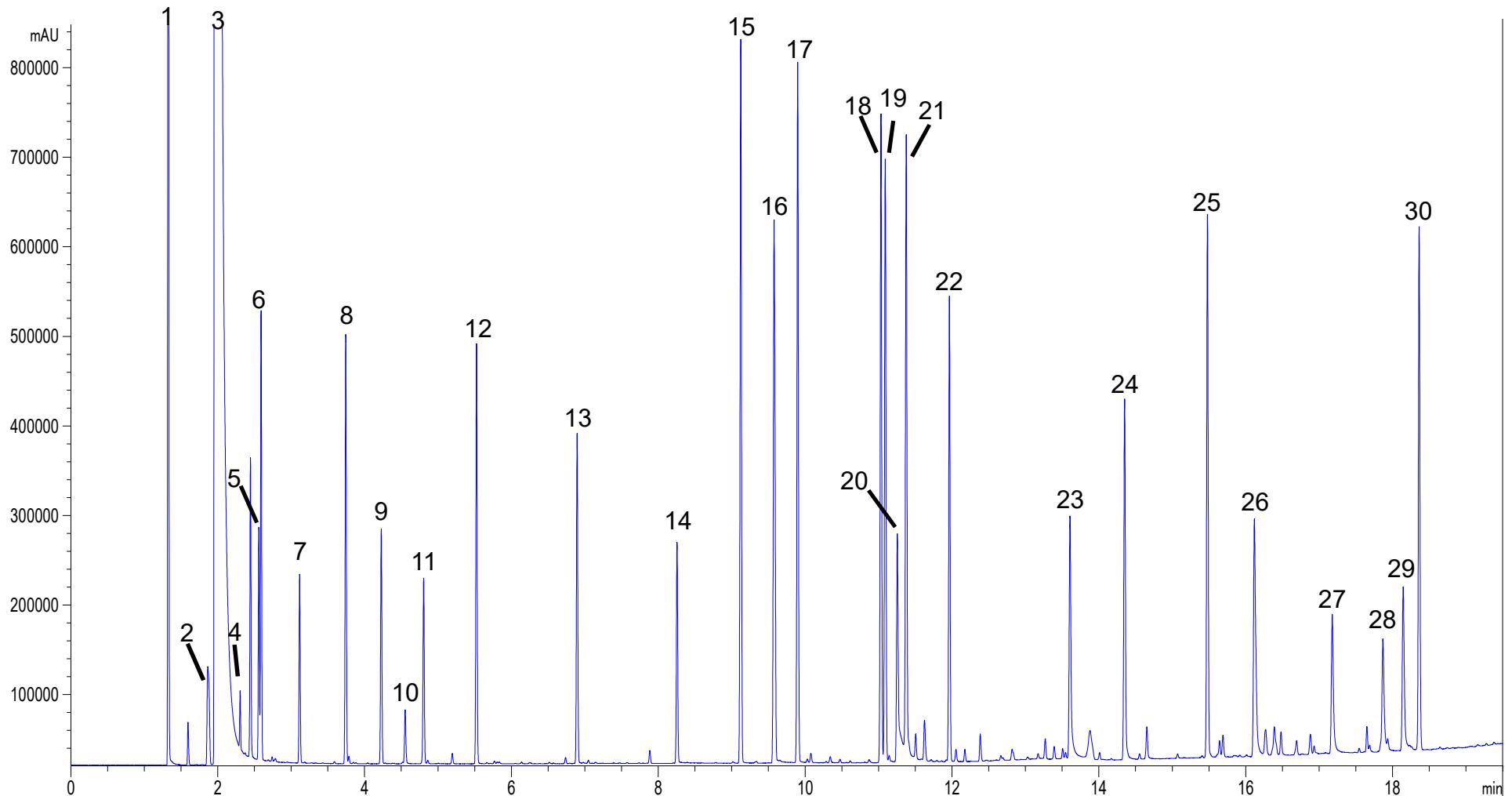
30 m X 0.25 mm I.D., 0.25 um film



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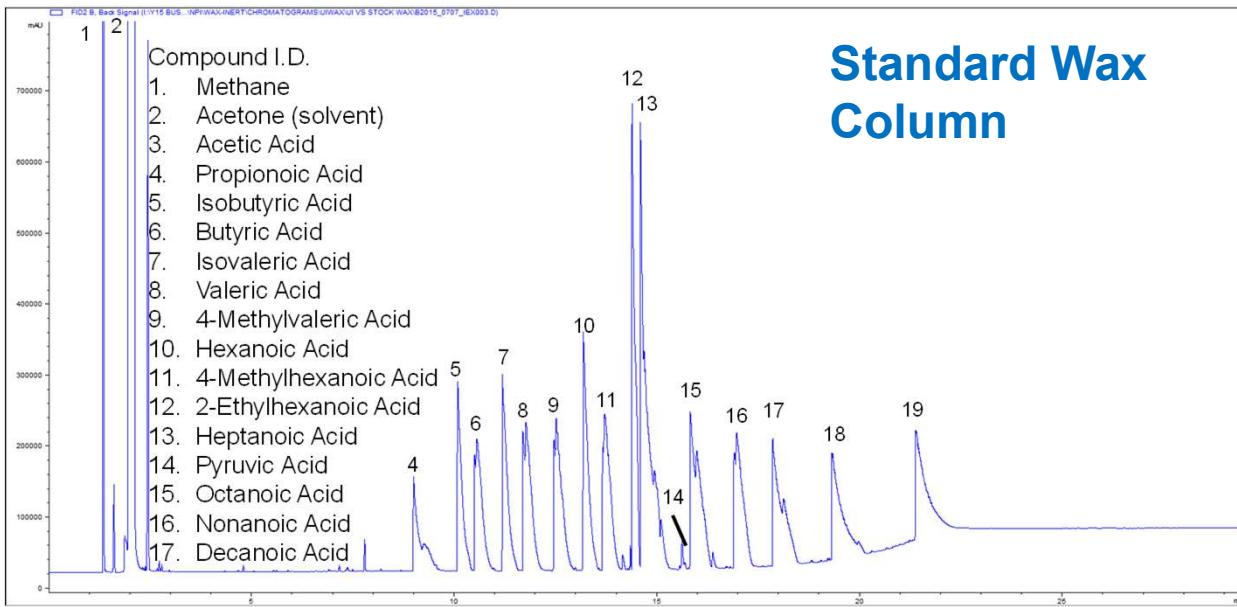
Aldehydes Mix on DB-WAX UI

30 m X 0.25 mm I.D., 0.25 um film



Analysis of Organic Acids

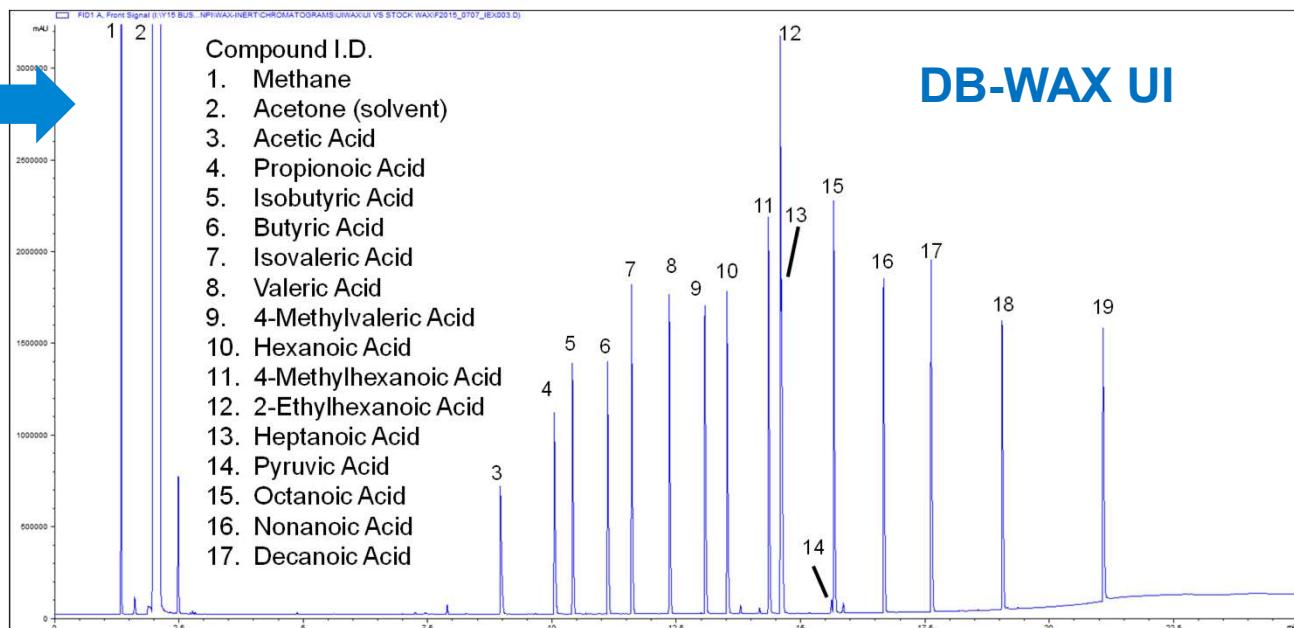
Standard Wax Column



Excellent peak shape
with DB-WAX Ultra Inert

No need to run a
separate FFAP column
for organic acids

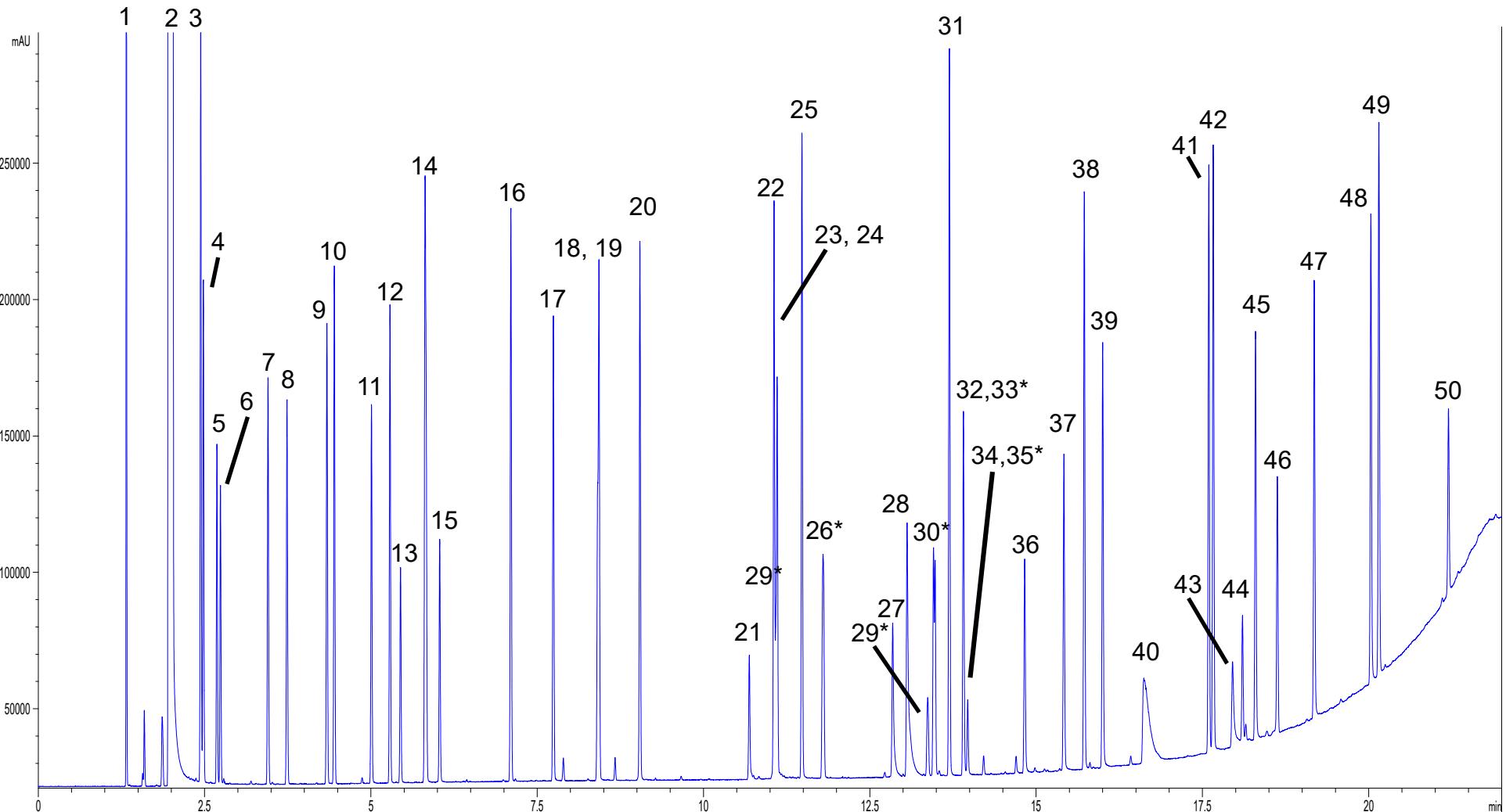
Standardize on DB-WAX
UI to broaden application
range



DB-WAX UI

50 Alcohol/Diol Mix on DB-WAX

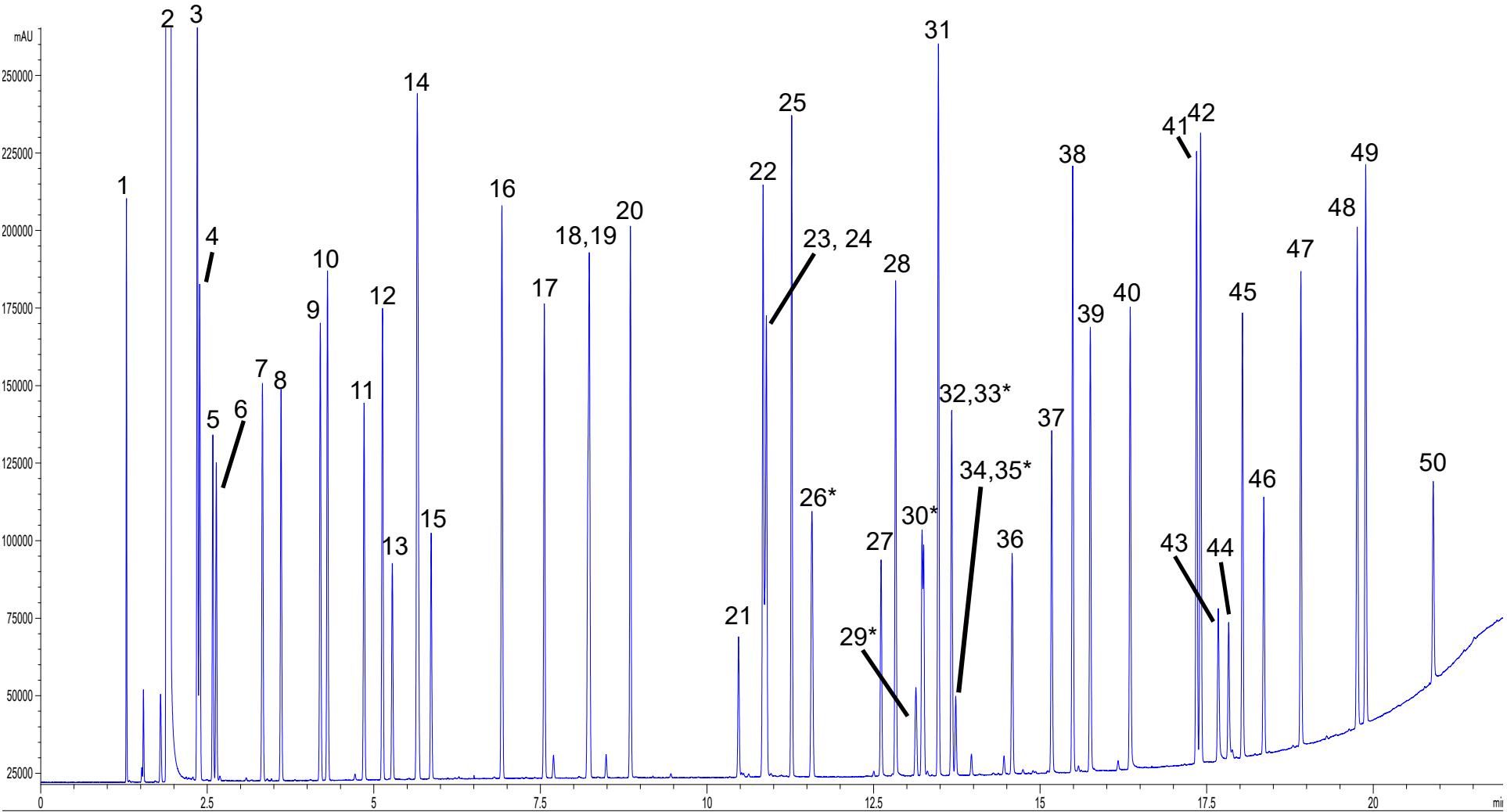
30 m X 0.25 mm I.D., 0.25 μ m film



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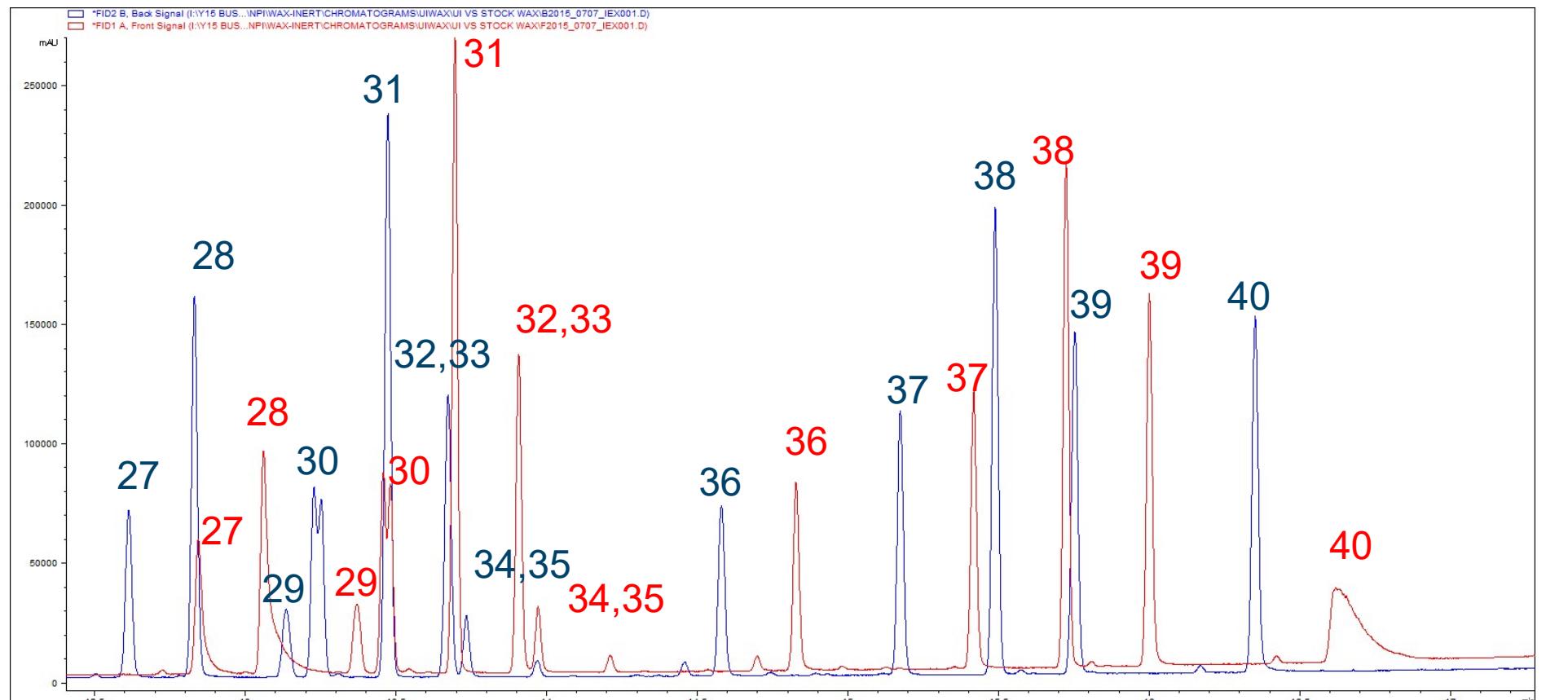
50 Alcohol/Diol Mix on DB-WAX UI

30 m X 0.25 mm I.D., 0.25 μ m film



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Overlay Showing Select Alcohols and Diols on DB-WAX (red) and DB-WAX UI (blue)



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Chromatographic Conditions

GC system	Agilent 7890B/FID
Column	Agilent J&W DB-WAX UI, 30 m x 0.25 mm, 0.25 µm (p/n 122-7032UI)
Autosampler	Agilent 7683B autosampler and sample tray, 5 µL syringe (p/n G4513-80213), 0.5 µL injection volume
Carrier gas	Helium, constant flow mode, 0.7ml/min
Inlet	Split/splitless, 250°C, split ratio 30:1
Oven	For sample run 40°C (4 min), 5°C/min to 100°C, 10°C/min to 200°C (10 min) (run time = 36min) For solvent run 40°C (4 min), 5°C/min to 56°C, 100°C/min to 200°C (10 min) (run time = 18.64min)
FID	250°C

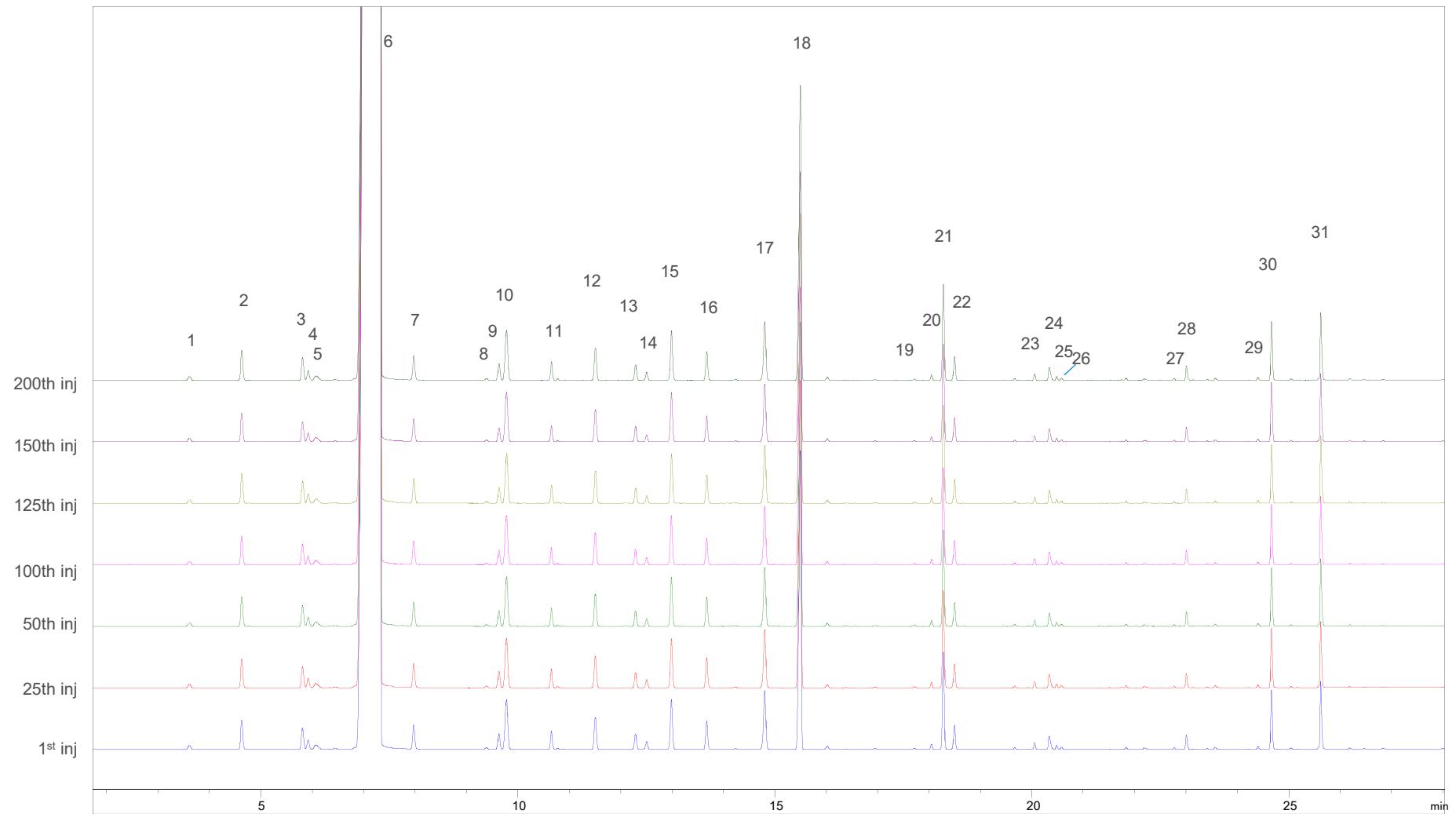
SAMPLES:

1. Test sample: 38% (v/v) Chinese liquor
2. solvent : Ethanol : water = 38:62



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GC/FID Chinese Liquor Sample on an Agilent J&W DB-WAX Ultra Inert 30 m x 0.25 mm x 0.25 µm GC column



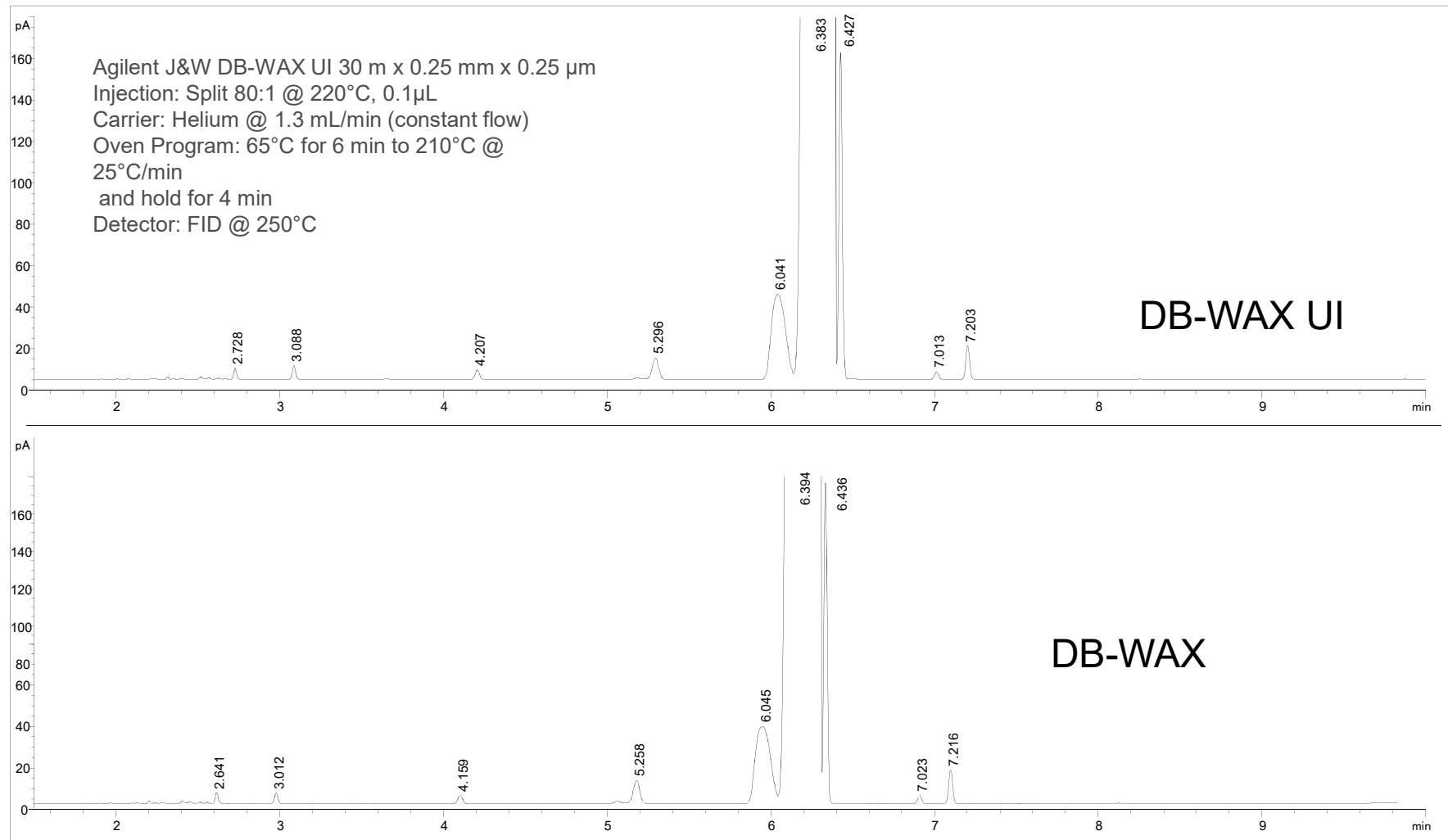
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Identified Compounds

Peak #	Compounds	Peak #	Compounds
1.	Acetaldehyde	17.	Isoamyl alcohol
2.	Acetone	18.	Ethyl hexanoate
3.	Ethyl acetate	19.	2-Heptanol
4.	Acetal (acetaldehyde diethyl acetal)	20.	Ethyl heptanoate
5.	Methanol	21.	Ethyl lactate
6.	Ethanol	22.	1-Hexanol
7.	2-Pentanone	23.	Ethyl octanoate
8.	2-Butanol	24.	Acetic acid
9.	Ethyl butyrate	25.	Isoamyl caproate
10.	n-propanol	26.	Furfural
11.	Butyl acetate (IS)	27.	hexyl hexanoate
12.	iso-Butanol	28.	Butyric acid
13.	2-Pentanol	29.	Valeric acid
14.	Ethyl valerate	30.	2-Ethylbutyric acid
15.	1-Butanol	31.	Hexanoic acid
16.	Amyl acetate		



Chromatogram of BTEX - Resolution of Xylene Isomers



Summary UI Wax Columns

- Improved inertness of a DB-WAX UI means better analytical outcomes for:
 - Ethyl maltol
 - Aldehydes
 - Alcohols
 - Organic acids
- Tight RI control specification for excellent batch-to-batch consistency
- Economic Value
 - Faster column qualification for proprietary library matching
 - Less re-work, higher laboratory throughput
 - No need for separate analyses for organic acids with an FFAP column
- Saving time and delivering better laboratory outcomes for our customers



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Customer Feedback

“With DB-WAX UI, we are seeing better reproducibility of total aldehyde content and less tailing because of the improved inertness. No selectivity change compared to the standard DB-Wax is essential for complex citrus oil analysis. In addition, time spent for pre-testing new columns is eliminated.”

Dr. Andreas Böker

MCI Miritz Citrus
Research & Development
Director QC



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Thank You

- Collaborators and beta testers
 - Dr. Frank David, Research Institute for Chromatography, Kortrijk Belgium
 - Dr. Andreas Böker, MCI Miritz Citrus, Warwick, NY
 - Early adopters who choose to remain anonymous

International Agilent colleagues

- Mark Furer, Waldbronn, Germany
 - Tiantian Lee Waldbronn, Germany
 - Roland Webber, Waldbronn, Germany
 - Ngoc A Dang, Middelburg, the Netherlands
 - Yun Zou, Shanghai, China
 - Johan Kuipers, Middelburg, the Netherlands
 - Laura Provost, Middelburg, the Netherlands
 - Allen Vickers, Folsom, California
 - Gary Lee, Folsom, California
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- You for your kind attention



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For More Information:

DB-WAX UI Application notes:

A New PEG GC Column with Improved Inertness reliability and Column Lifetime 5991-6683EN

Analysis of Distilled spirits Using Agilent J&W DB-WAX Ultra Inert Capillary GC Column 5991-6638EN

Analysis of Lavender Essential Oil by Agilent J&W DB-WAX Ultra Inert Capillary GC Column 5991-6635EN

Analysis of Glycols in Toothpaste Using Agilent J&W DB-WAX Ultra Inert Capillary GC Column 5991-6637EN

Flow Path Solutions 5990-8532EN

Increase Your Analytical Confidence for Polar Compounds 5991-6709EN

Link to the latest Inert Flow Path Poster and Inert Flow Path Web Page

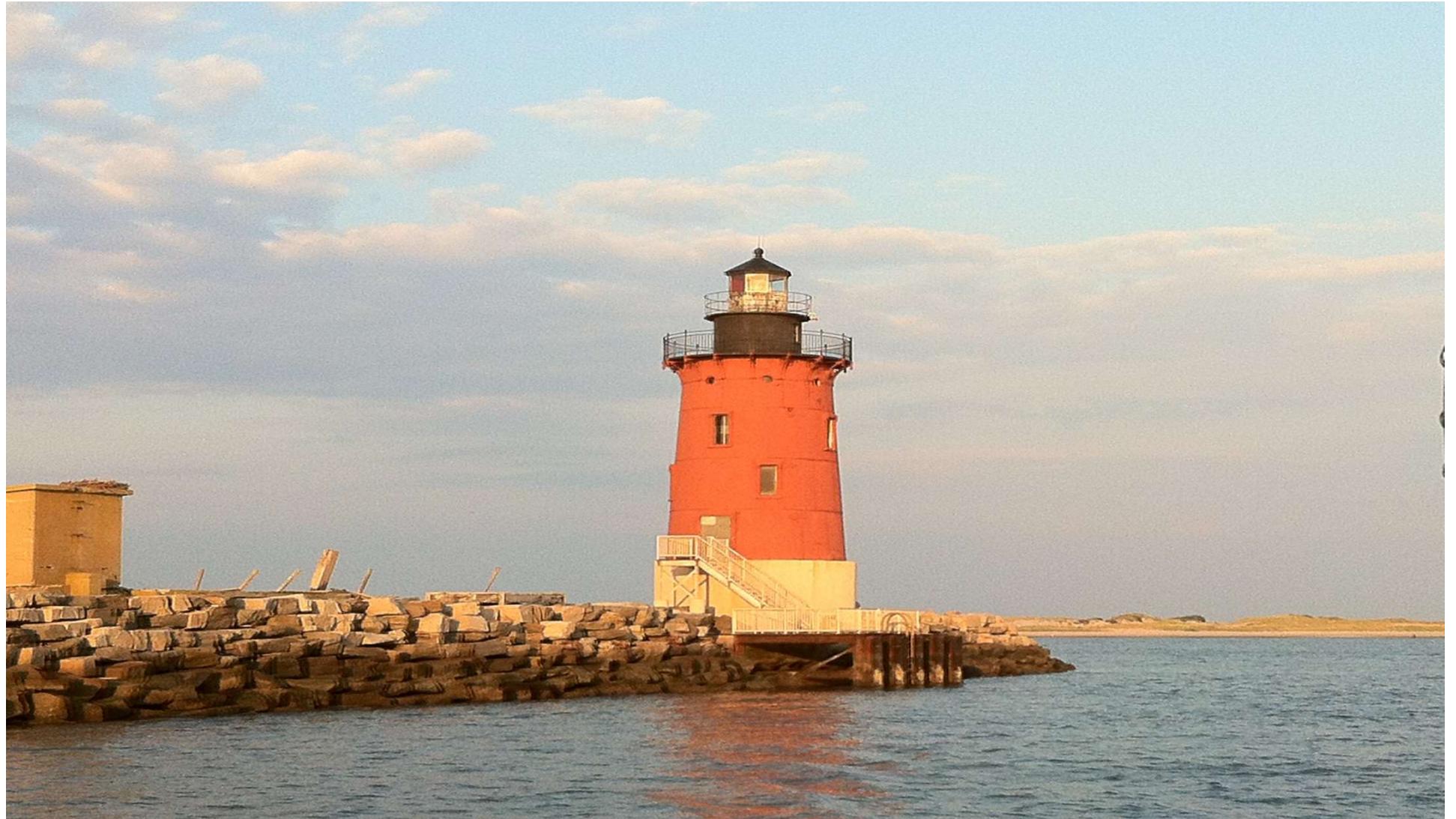
<http://www.agilent.com/cs/library/posters/public/5990-8902EN%20UI%20Poster.pdf>

<https://www.agilent.com/en-us/promotions/inertflowpath>



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Questions ?



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