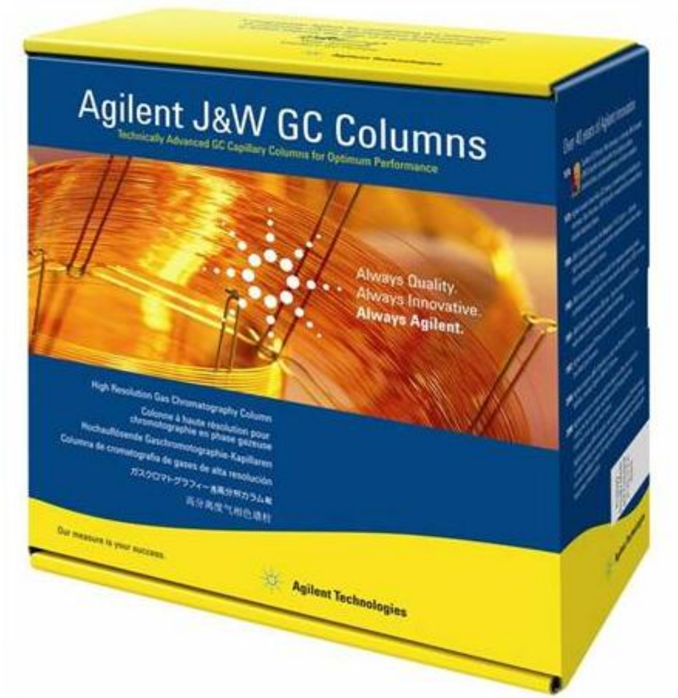
Improving GC Resolution and Dealing with Peak Tailing

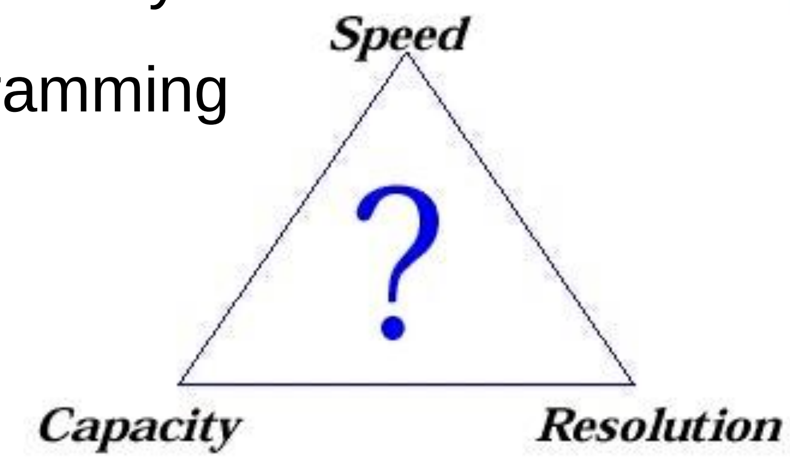


Column Dimensions and Carrier Gas Optimization

Inert Flow Path

Variables for Maximizing Resolution

- Optimized Stationary Phase
- Longer Column Length
- Decrease Internal Diameter
- Carrier Gas: type and linear velocity
- Optimized Temperature Programming



Resolution

$$R_S = \frac{\sqrt{N}}{4}\left(\frac{k}{k+1}\right)\left(\frac{\alpha-1}{\alpha}\right)$$

Efficiency	$N = f\ (\text{gas}, L, r_c)$	L = Length
Retention	$k = f\ (T, d_f, r_c)$	r_c = column radius
Selectivity	$\alpha = f\ (T, \text{phase})$	d_f = film thickness
		T = temperature

WCOT Column Types

Agilent J&W has over 50 different stationary phase offerings

Non Polarity → **Mid**

DB-1	DB-5	DB-XLB	DB-35	HP-Chiral 10β	DB-17	DB-TPH	DB-502.2	DB-VRX	DB-1301
HP-1	HP-5		DB-35ms	HP-Chiral 20β	DB-17ms		HP-VOC		DB-624
DB-1ms	DB-5ms		HP-35		DB-608				HP-Fast Residual Solvent
HP-1ms	HP-5ms				HP-50+				
DB-2887	HP-5ms Semivol				DB-17ht				
DB-Petro	DB-5.625								
DB-PONA	DB-5ht								
DB-HT Sim Dis	Ultra 2								
DB-1ht	HP-PASS								
Ultra 1	DB-EVDX								

Polarity → **High Polarity**

DB-1701	DB-ALC2	DB-225	DB-ALC1	DB-Dioxin	DB-200	DB-210	DB-23	HP-88	DB-WAX
DB-1701P		DB-225 ms							DB-WAXetr
CycloSil-β		HP Blood Alcohol							HP-INNOWax
Cyclodex-β									DB-FFAP
									HP-FFAP
									DB-WaxFF

And Now FactorFour™ Phases

VF-1ms, VF-5ms, VF-5ht, VF-5ht UltiMetal™

VF-17ms, VF-17ms for PAH, VF-35ms,

VF-200ms,VF-Xms, VF-23ms, VF-624ms,

VF-DA, VF-1301ms, VF-Pesticides,

VF-1701ms, VF-WAXms

20+ Different "Specialty Phases"

"Specialty phases" are columns that are optimized to perform a specialized GC analysis.

Column	Typical Application
DB-624	EPA and USP volatiles
DB-VRX	volatiles analysis
HP-VOC	volatiles analysis
DB-502.2	EPA Method 502.2
DB-5.625	EPA semi-volatiles analysis
DB-608	EPA Method 608
DB-1701P	EPA pesticides analysis
DB-MTBE	total petroleum hydrocarbon (TPH)
HP-PONA	petroleum hydrocarbon analysis
DB-HT SimDis	hi-temp simulated distillation
DB-ALC1 & ALC2	blood alcohol analysis
HP-88	fatty acid methyl ester (FAME)

And Select ^{TM} Columns

Environmental applications

CP-Sil 88 for dioxins, Select mineral oil, CP-Select 624 CB

Chiral applications

CP-Chirasil Val, CP-Chirasil-DEX CB

Chemical applications

CP-Volamine, CP-Select CB for MTBE, CP-PONA C8, CP-Propox, Select Silanes, CP-SimDist UltiMetal ^{TM}, CP-Lowox ^{TM}

Food and Beverage applications

CB-Carbowax 400, Select FAME, CP-Sil 88 for FAME, CP-FFAP CB

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Optimizing Selectivity

Match analyte polarity to stationary phase polarity

-like dissolves like (oil and water don't mix)

Take advantage of unique interactions between analyte and stationary phase functional groups

Stationary Phase Selection

Existing Information

Critical Separations

Selectivity/Polarity

Temperature Limits

Application Designed

- Examples: DB-CLP1, DB-CLP2, DB-UI 8270D, DB-624UI, DB-Select 624UI<467>, DB-VRX, HP-VOC, DB-MTBE, Lowox, DB-TPH, DB-HTSimDis, DB-2887, DB-HT SimDis, CP-Volamines, DB-ALC1, DB-ALC2, Select Phases, etc.

Choose the column phase that gives the best separation but not at the cost of robustness or ruggedness.

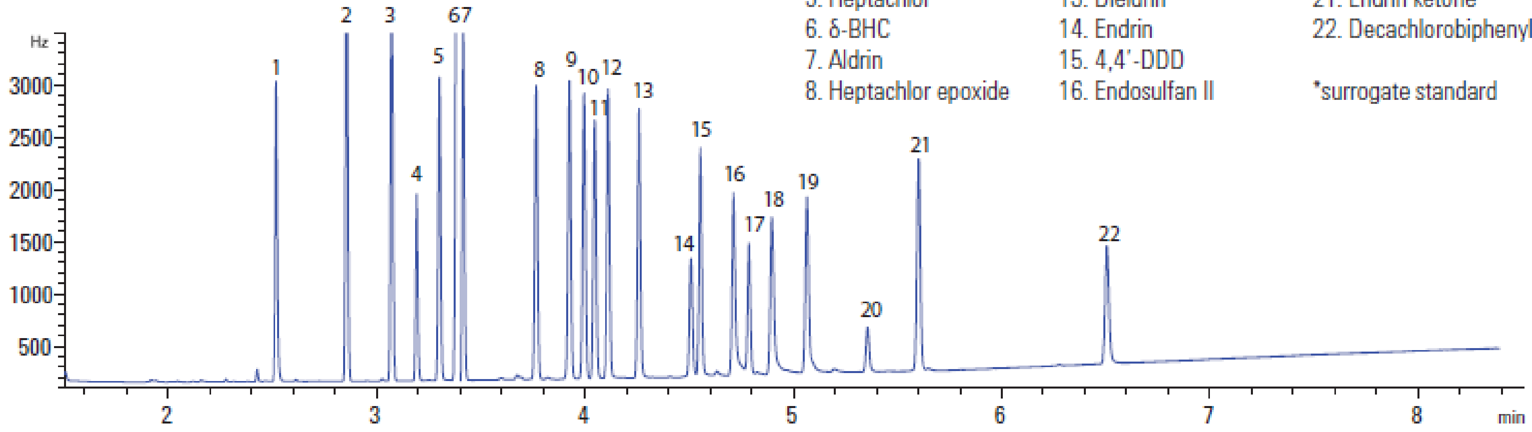
Complete Resolution & Fast CLP Pesticide Analysis

10 ng/mL standard

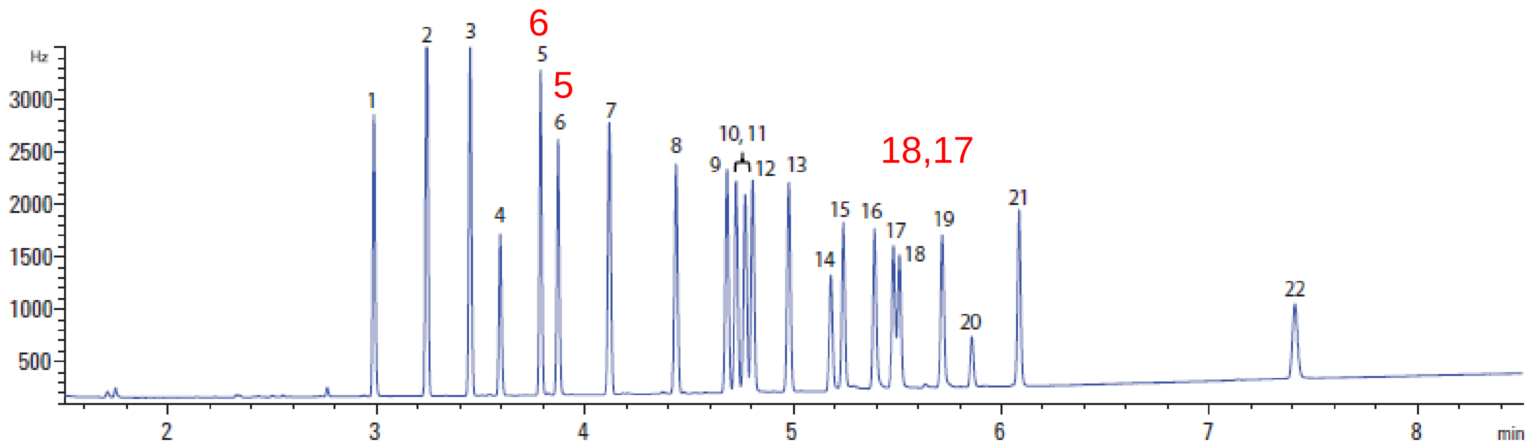
1. Tetrachloro-m-xylene*
2. α-BHC
3. γ-BHC
4. β-BHC
5. Heptachlor
6. δ-BHC
7. Aldrin
8. Heptachlor epoxide
9. γ-Chlordane
10. α-Chlordane
11. Endosulfan I
12. 4,4′-DDE
13. Dieldrin
14. Endrin
15. 4,4′-DDD
16. Endosulfan II
17. 4,4′-DDT
18. Endrin aldehyde
19. Endosulfan sulfate
20. Methoxychlor
21. Endrin ketone
22. Decachlorobiphenyl*

*surrogate standard

Agilent J&W DB-CLP1



Agilent J&W DB-CLP2



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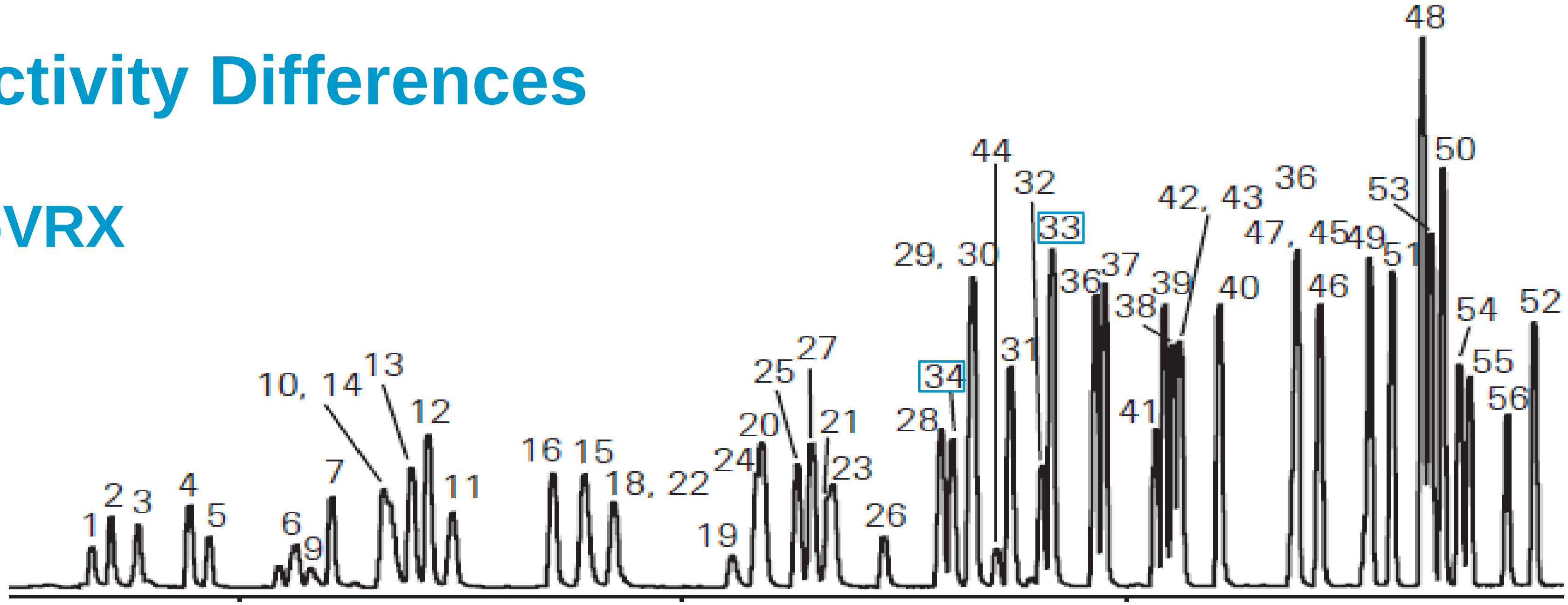
Agilent J&W DB-CLP1 and DB-CLP2
For 9 EPA Methods+ (More than any other CLP column pair!)

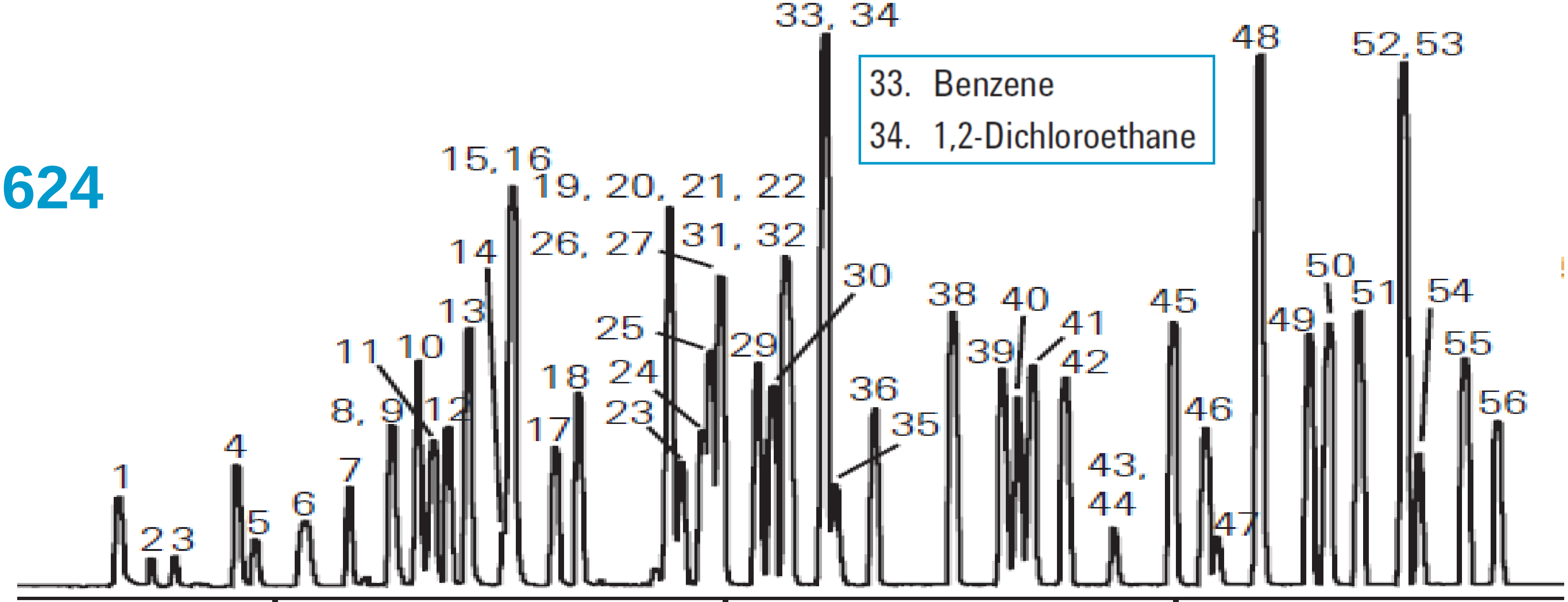
EPA Contract Lab Program Pesticides	Organochlorine pesticides
EPA Method 504.1	Halogenated pesticides
EPA Method 505	Organohalide pesticides
EPA Method 508.1	Organochlorine pesticides and herbicides
EPA Method 551	Chlorinated solvents, trihalomethanes & disinfectant by-products
EPA Method 552.3	Haloacetic acids and dalapon
EPA Method 8081B	Organochlorine pesticides
EPA Method 8082A	PCBs and arachlors
EPA Method 8151A	Chlorophenoxy acid herbicides

Selectivity Differences

DB-VRX



DB-624



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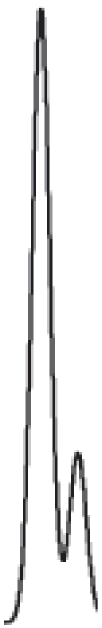
Benzene and 1,2-Dichloroethane Separation

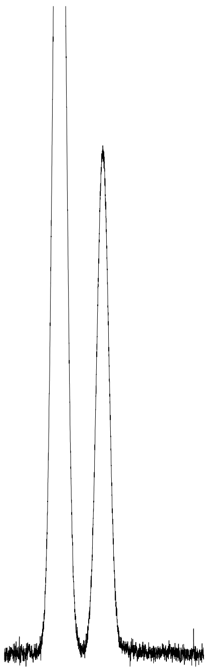
DB-624

Co-elution

DB-Select 624UI<467>



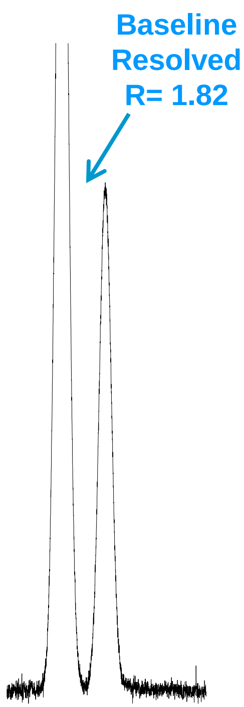
30m x 0.53mm



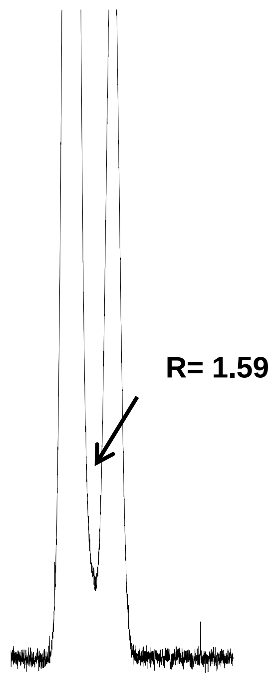
30m x 0.32mm

Benzene and 1,2-Dichloroethane Separation

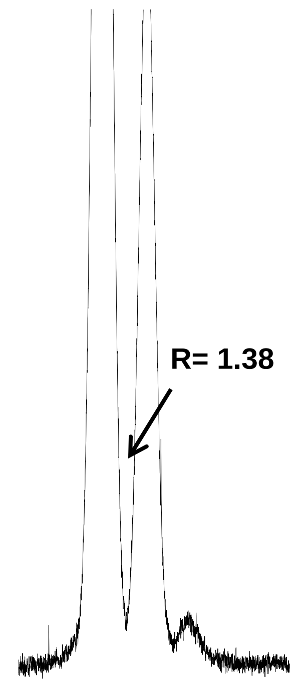
DB-Select 624UI<467>



Vendor R G43



Vendor P G43



All 30m x 0.32mm

Resolution

$$R_S = \frac{\sqrt{N}}{4}\left(\frac{k}{k+1}\right)\left(\frac{\alpha-1}{\alpha}\right)$$

Efficiency $N = f\ (\text{gas}, L, r_c)$

Retention $k = f\ (T, d_f, r_c)$

Selectivity $\alpha = f\ (T, \text{phase})$

L = Length

r_c = column radius

d_f = film thickness

T = temperature

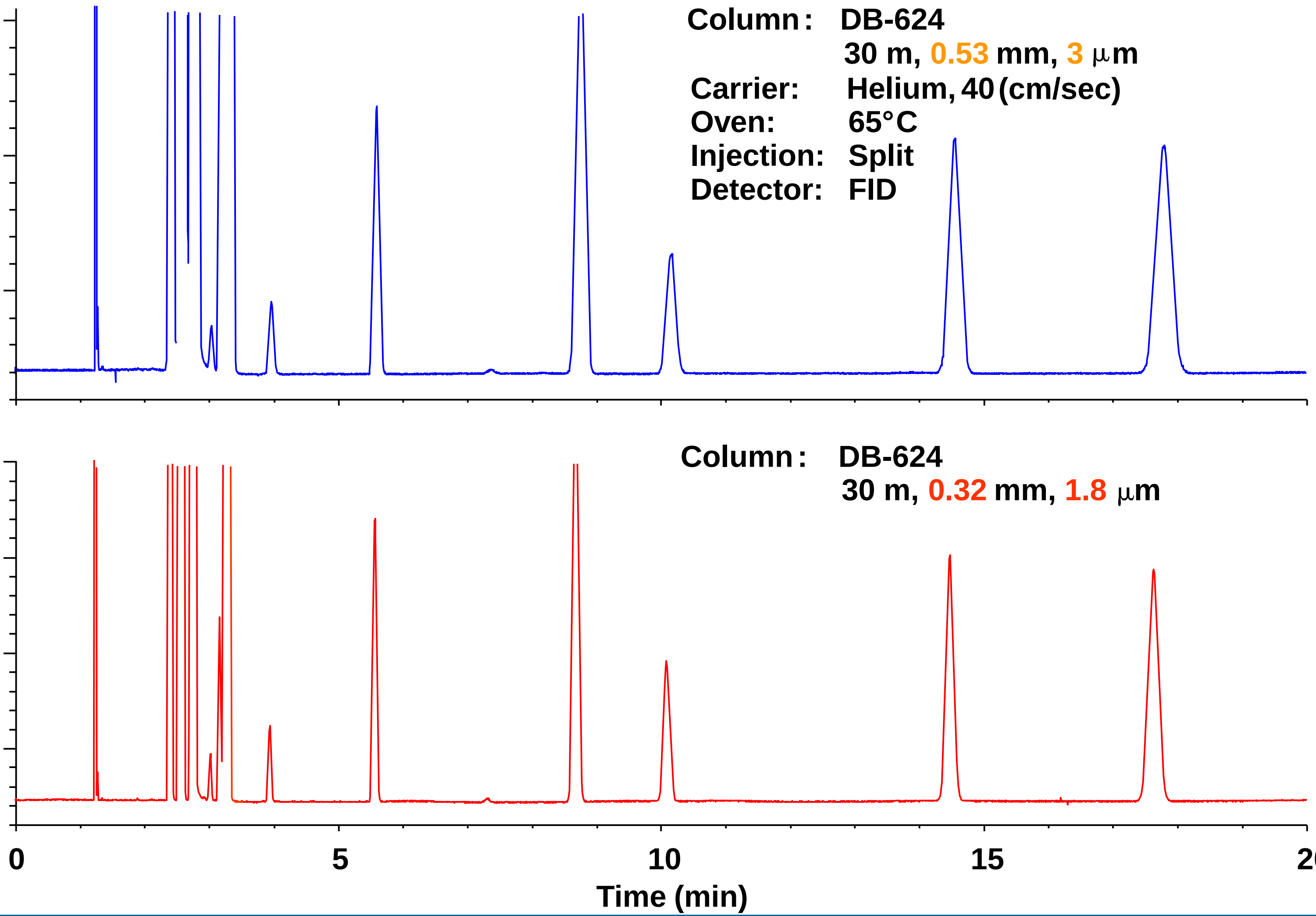
Column Diameter - Theoretical Efficiency

I.D. (mm)	n/m
0.05	23,160
0.10	11,980
0.18	6,660
0.20	5830
0.25	4630
0.32	3760
0.45	2840
0.53	2060

k = 5

Different Column I. D.

Equal Phase Ratios



Phase Ratio (β)

Film Thickness

Column Dimensions	Phase Ratio β
30 m x .53 mm x 3.0 μm	44
30 m x .32 mm x 1.8 μm	44

$$K_C = k\,\beta$$

$$\beta = \frac{r}{2d_f}$$

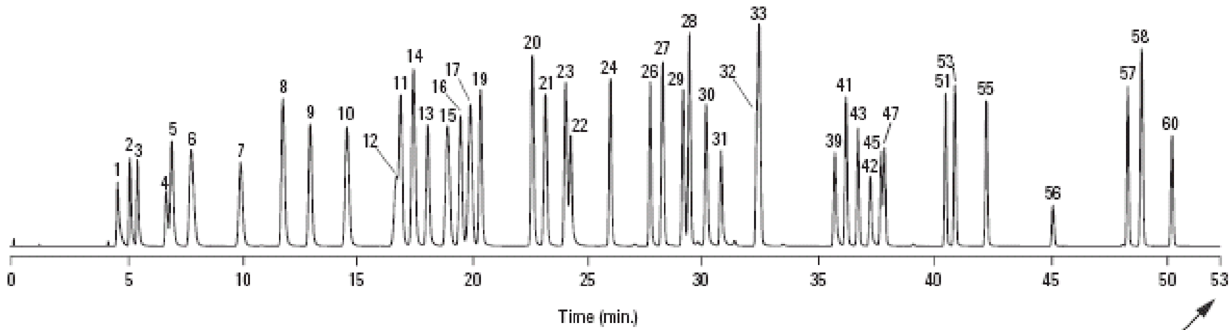
High Resolution Megabore

Same Resolution - Faster Analysis!

Conditions

Column: **DB-502.2, 105 m x 0.53-mm ID, 3.0 µm**
Part no.: 125-14A4
Carrier: Helium at 10 mL/min, measured at 35 °C
Oven: 35 °C for 10 min
35 °C - 200 °C at 4 °C/min
200 °C for 5 min

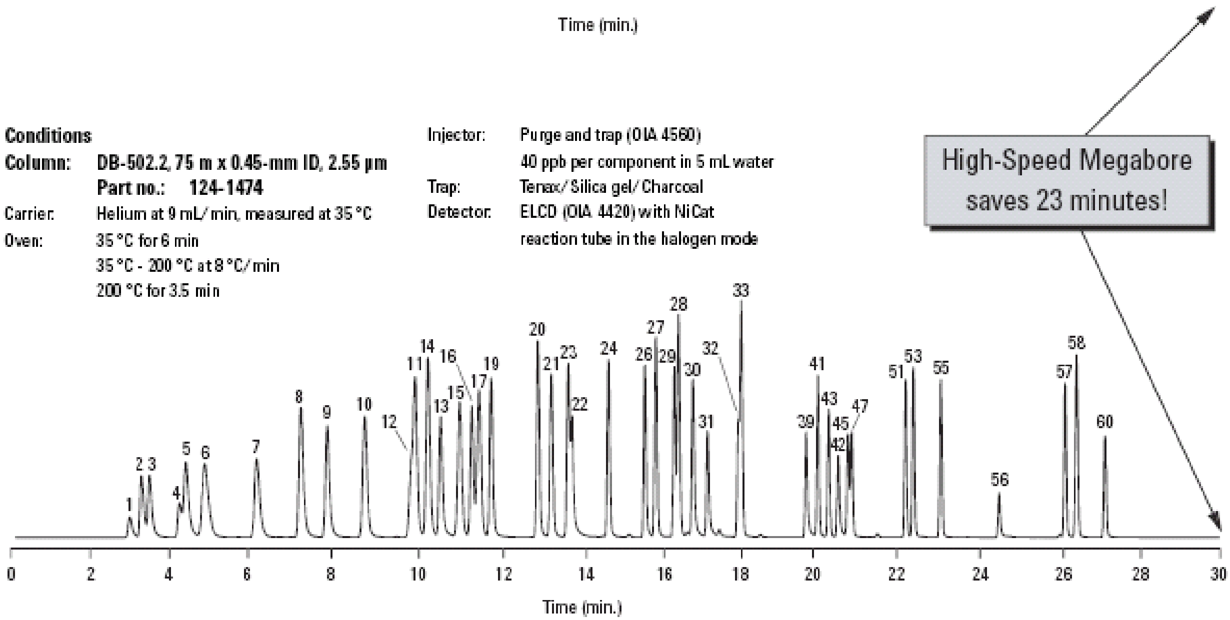
Injector: Purge and trap (OIA 4560)
40 ppb per component in 5 mL water
Trap: Tenax™/Silica gel/Charcoal
Detector: Electrolytic conductivity detector (ELCD) (OIA 4420) with NiCat™ reaction tube in the halogen mode



Conditions

Column: **DB-502.2, 75 m x 0.45-mm ID, 2.55 µm**
Part no.: 124-1474
Carrier: Helium at 9 mL/min, measured at 35 °C
Oven: 35 °C for 6 min
35 °C - 200 °C at 8 °C/min
200 °C for 3.5 min

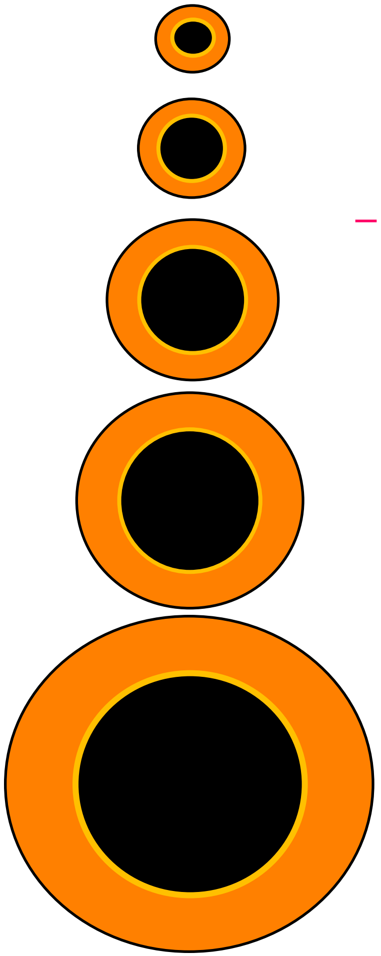
Injector: Purge and trap (OIA 4560)
40 ppb per component in 5 mL water
Trap: Tenax/Silica gel/Charcoal
Detector: ELCD (OIA 4420) with NiCat reaction tube in the halogen mode



"Increasing Sample Throughput With High-Speed Megabore"
Application note 5988-5271EN

Agilent Technologies

Column Diameter and Capacity



I.D. (mm)	Capacity (ng)
0.05	1-2
0.10	6-13
0.18	25-55
0.20	35-70
0.25	80-160
0.32	110-220
0.45	600-800
0.53	1000-2000

Like Polarity Phase/Solute
0.25 µm film thickness

Column Length and Efficiency (Theoretical Plates)

Length (m)	N
15	69,450
30	138,900
60	277,800

0.25 mm ID
n/m = 4630 (for k = 5)

More Meters = More Plates = More Resolution

Column Length and Resolution

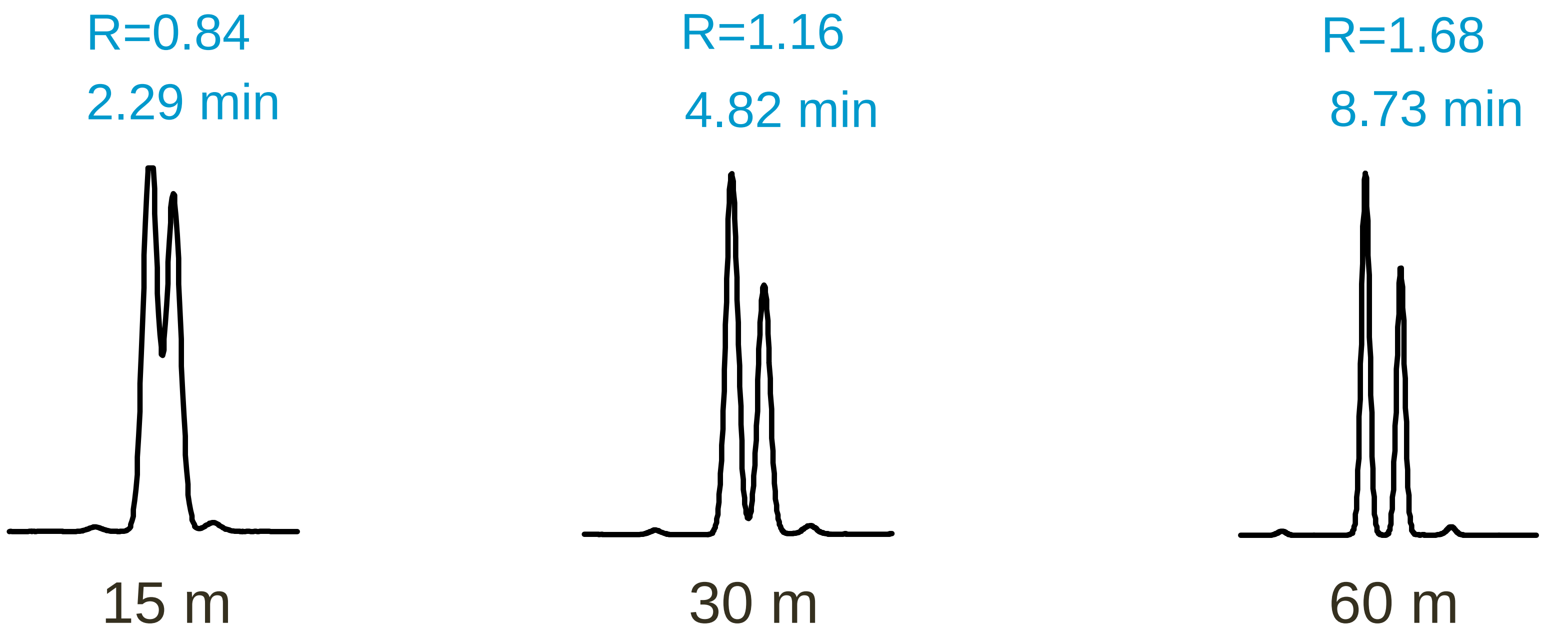
$$R \propto \sqrt{N} \propto \sqrt{L}$$

Length X 4 = Resolution X 2

$$t \propto L$$

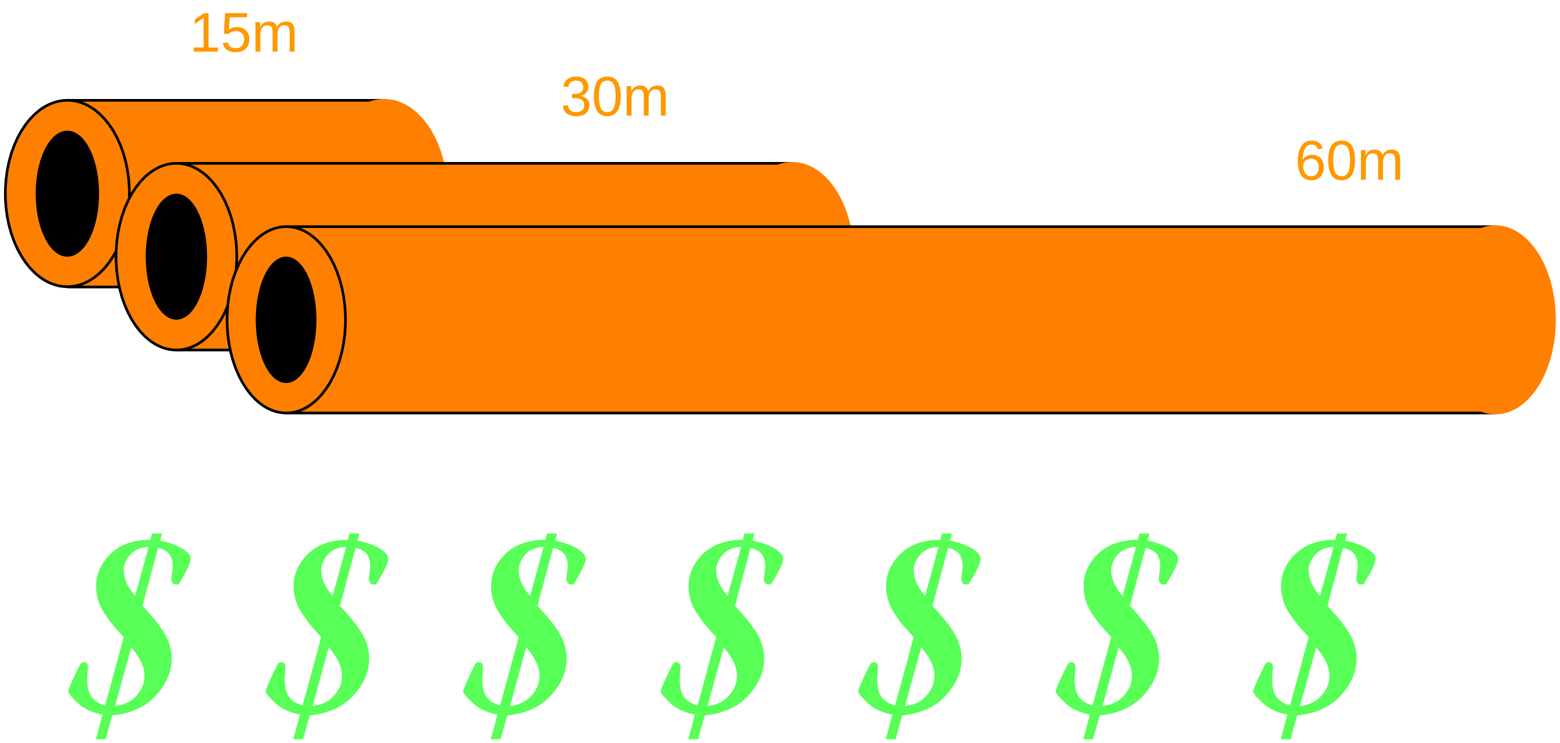
Upside = Cut a bunch off during routine inlet maintenance and not lose a lot of Resolution

Column Length vs. Resolution and Retention: Isothermal



Double the plates, double the time but not double the the resolution

Column Length and Cost



Resolution

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Efficiency $N = f\ (\text{gas}, L, r_c)$

Retention $k = f\ (T, d_f, r_c)$

Selectivity $\alpha = f\ (T, \text{phase})$

L = Length

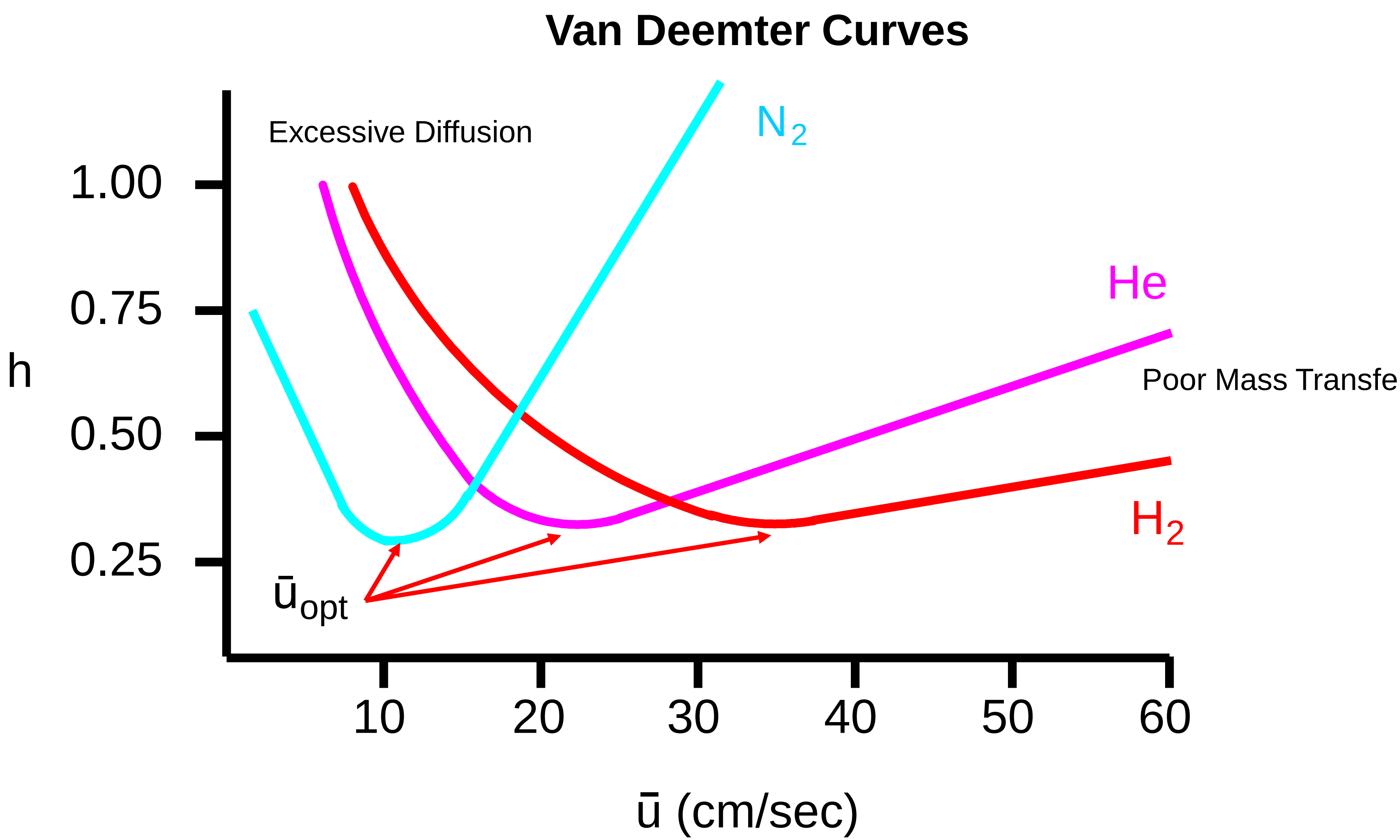
r_c = column radius

d_f = film thickness

T = temperature

Carrier Gas

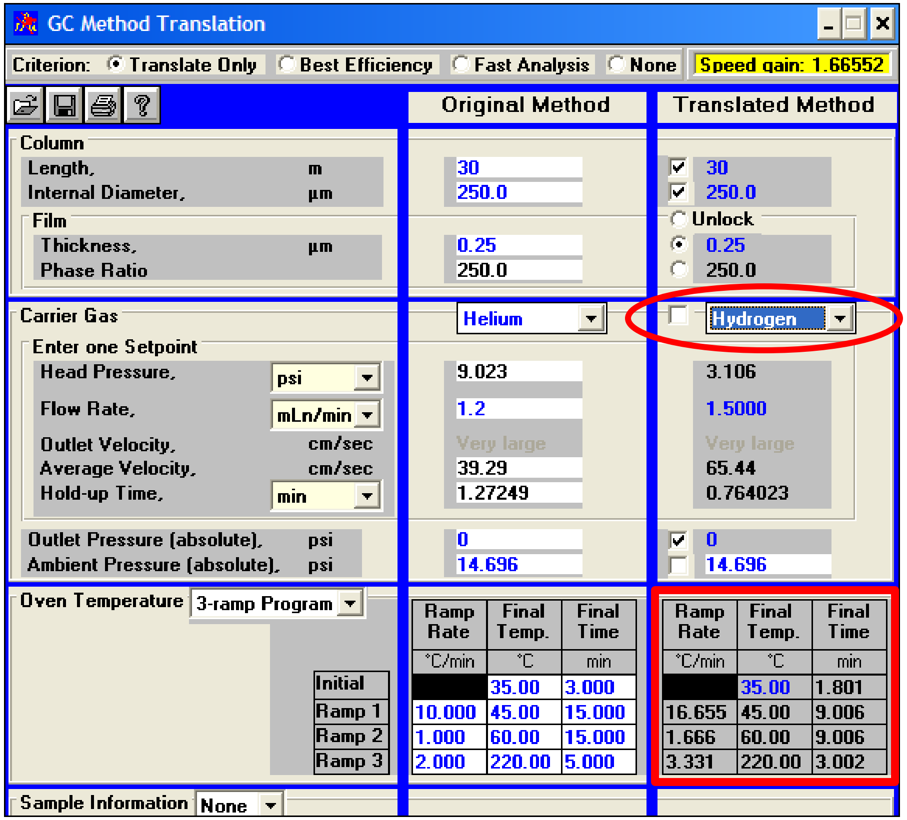
Maximum Resolution = Optimum Velocity = Slowest Velocity

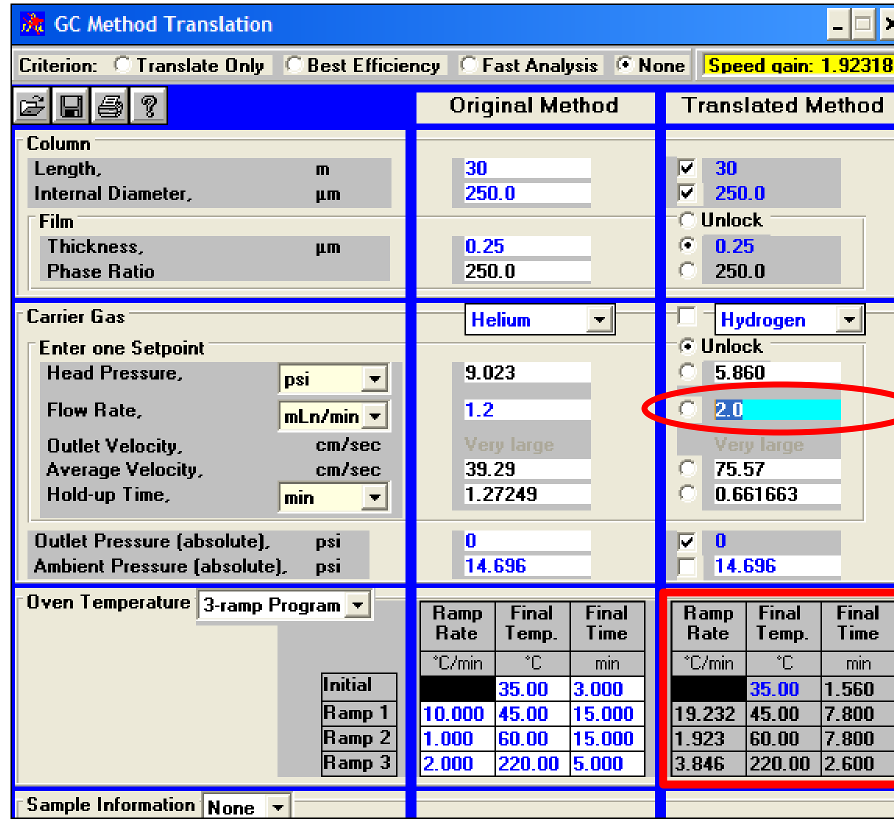


Carrier Gas

Type	Velocity Range (u_{opt} – OPGV)
Nitrogen	10-17 cm/sec
Helium	22-40 cm/sec
Hydrogen	35-55 cm/sec

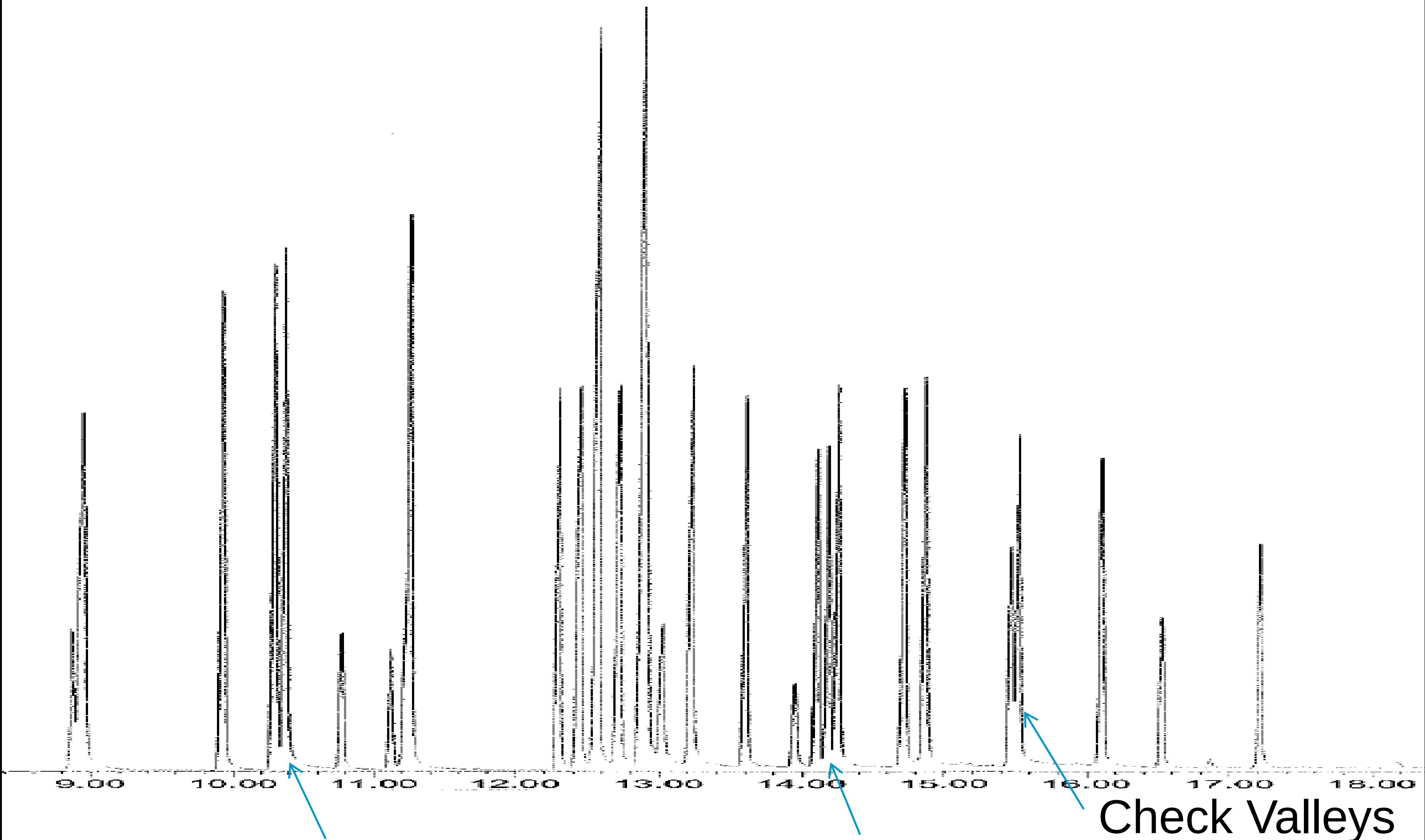
Changes in Column Dimensions, Gas Type or Velocity Require Changes in Temp Program Rates



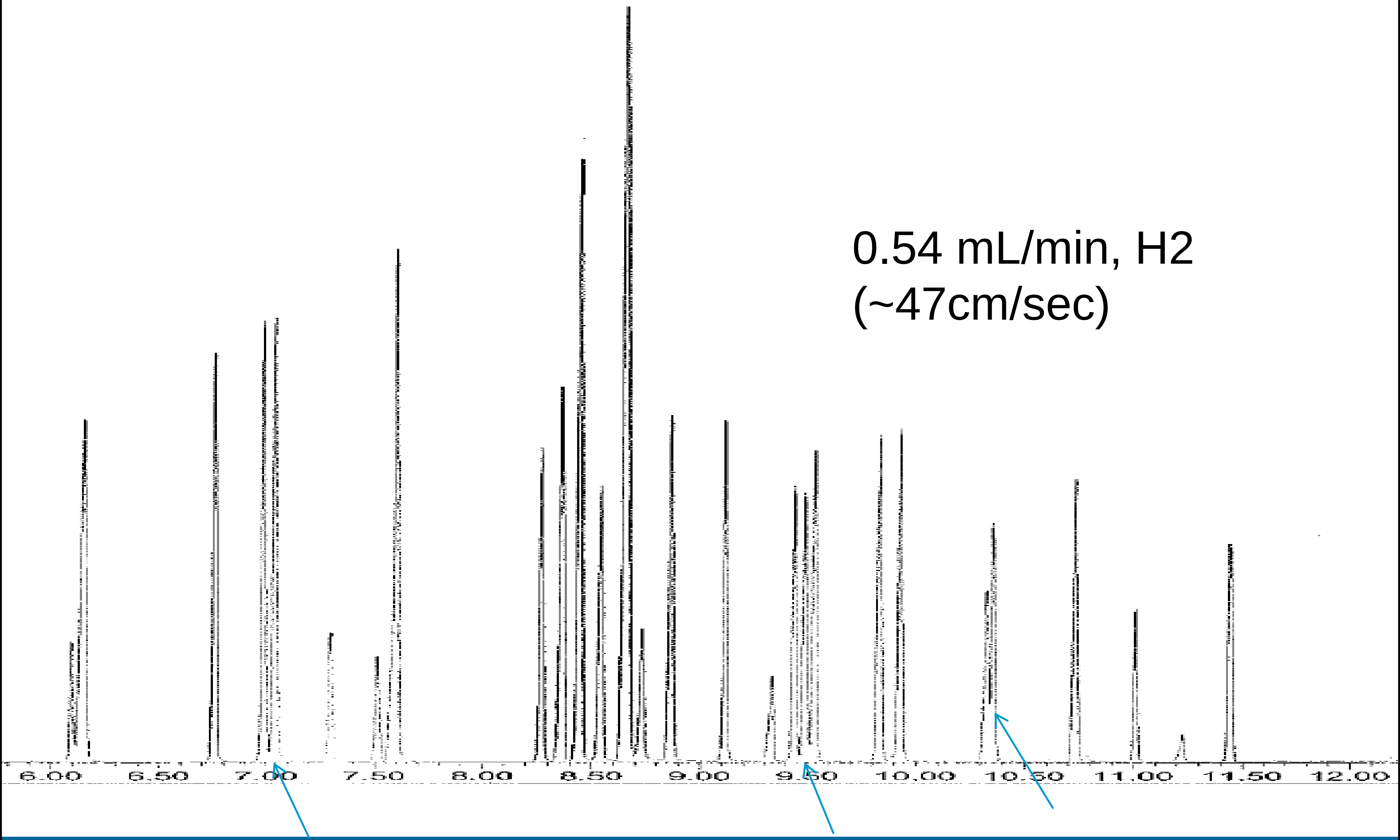


Method Translation Software to the Rescue!

Phenol's – 30m x 0.25mm ID, 0.25µm, DB-5ms



Phenol's – 20m x 0.18mm ID, 0.18µm, DB-5ms



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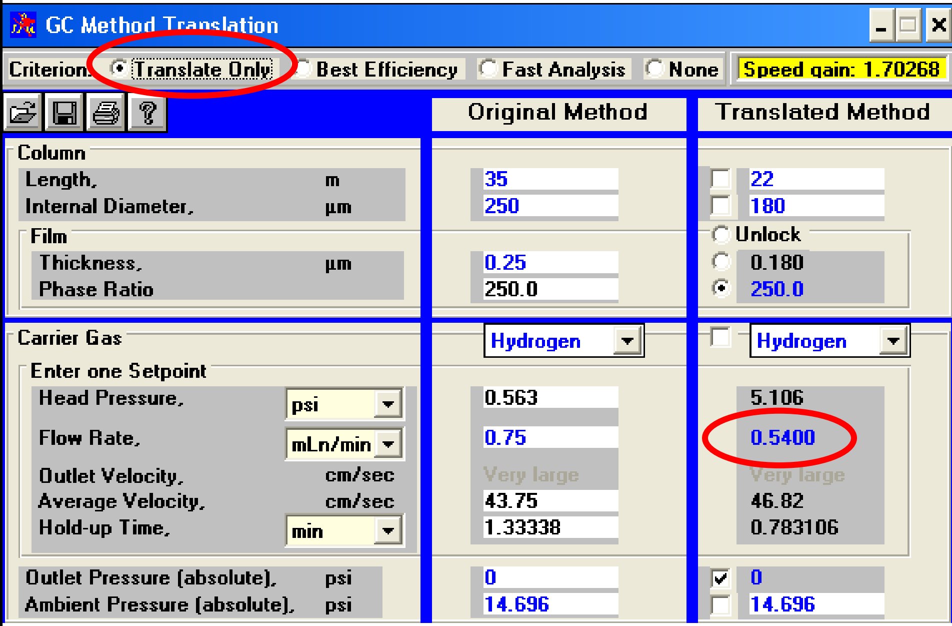
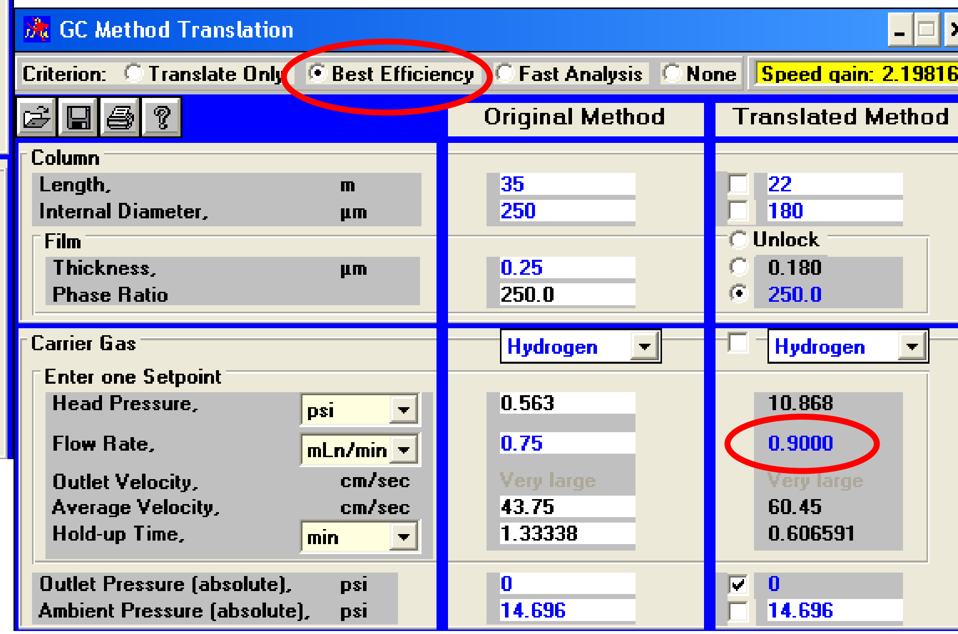
Slower than Best Efficiency? – must go faster!





(Sorry customer doesn't want temperature program revealed)

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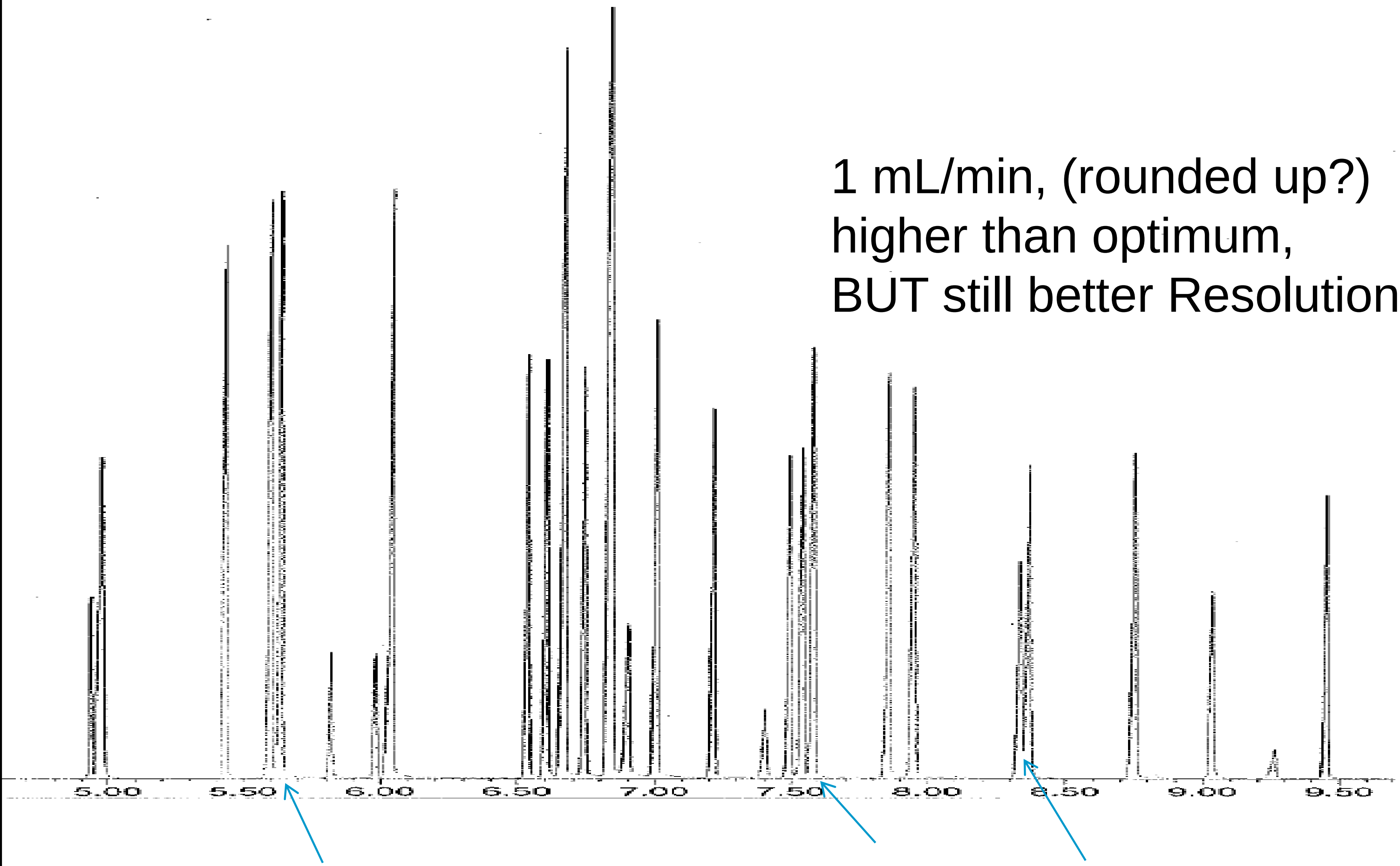
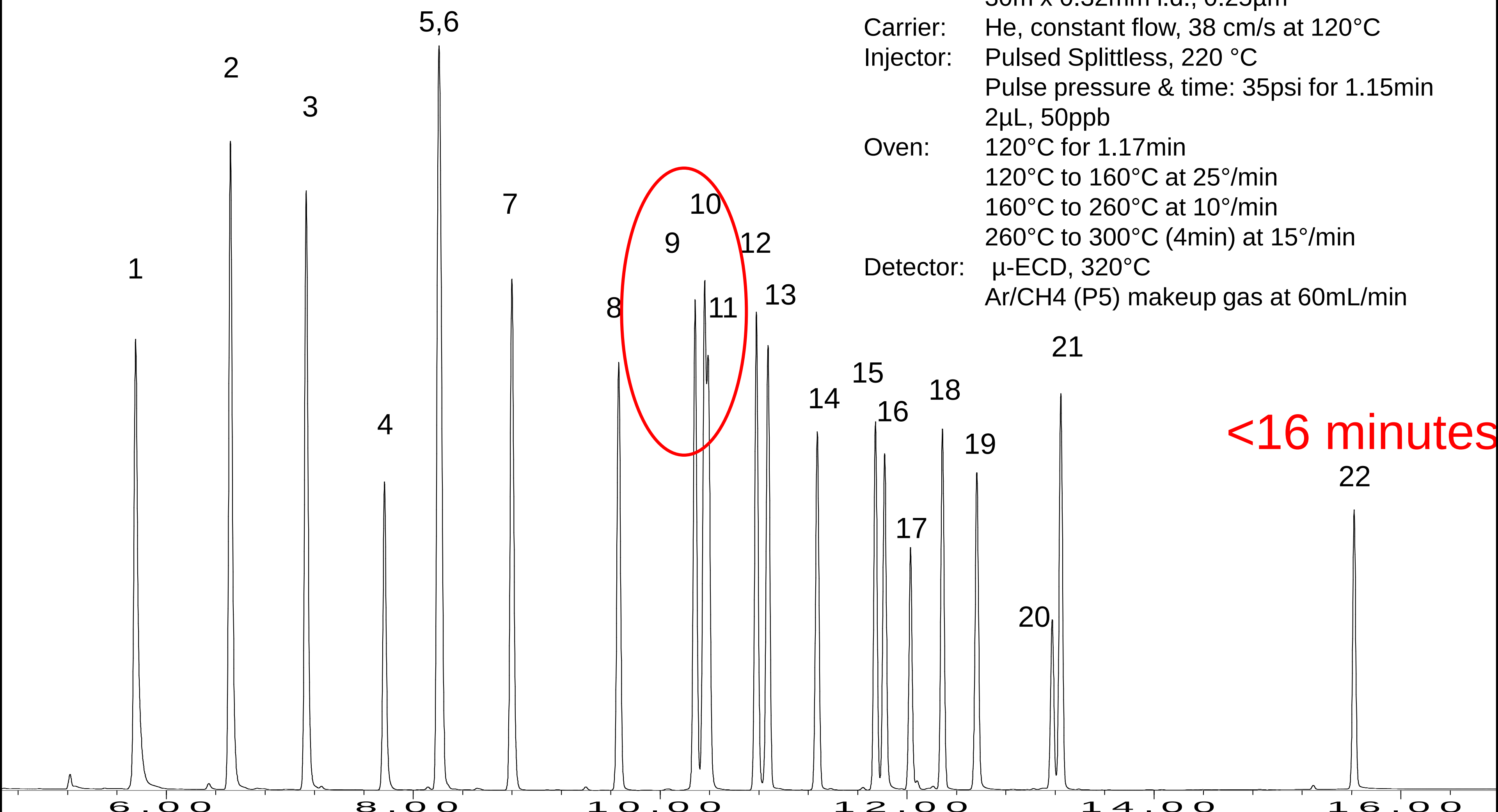
Phenol's – 20m x 0.18mm ID, 0.18µm, DB-5ms



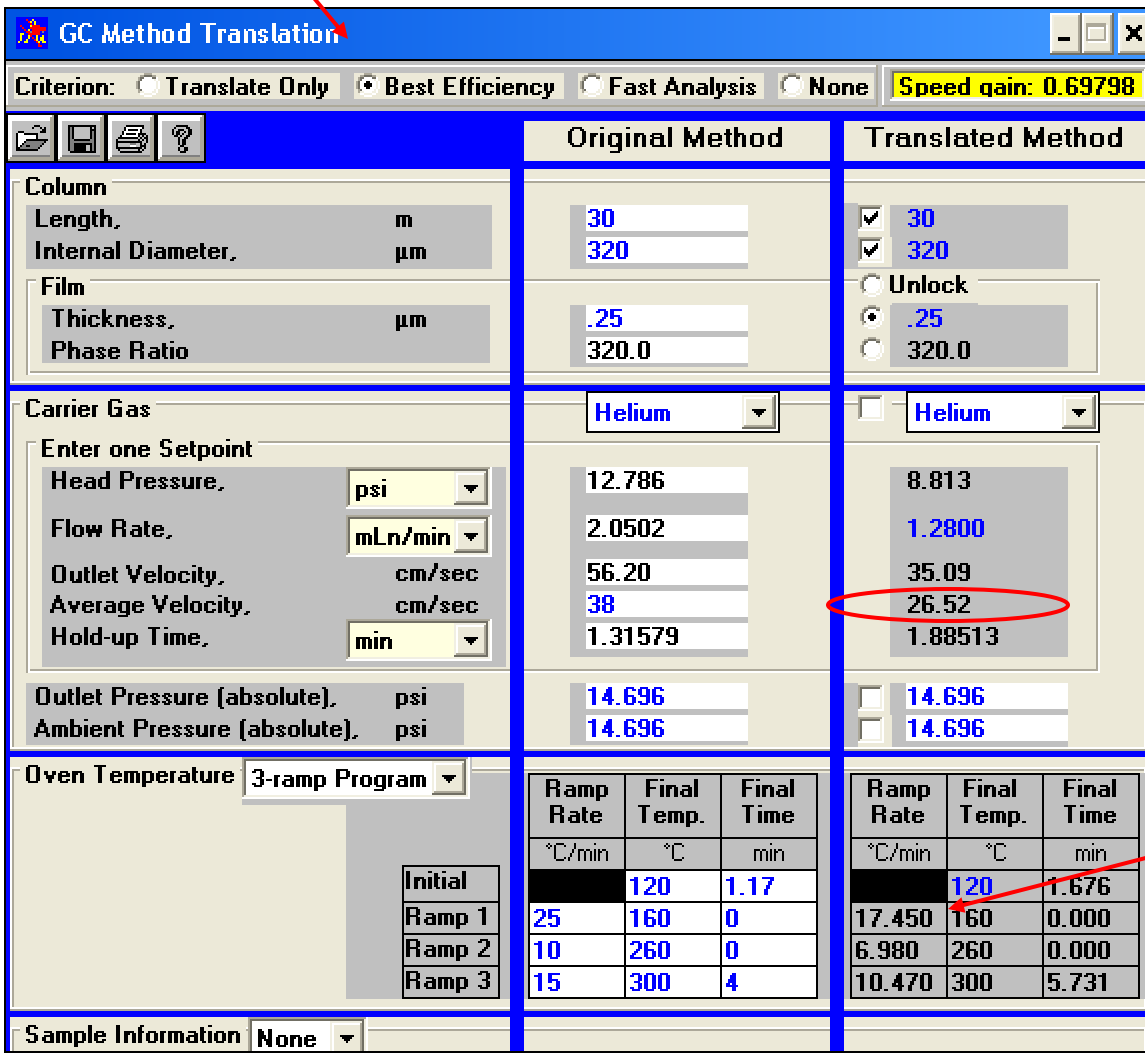
1 mL/min, (rounded up?) higher than optimum, BUT still better Resolution!

Original Method

Column:	DB-XLB
	30m x 0.32mm i.d., 0.25µm
Carrier:	He, constant flow, 38 cm/s at 120°C
Injector:	Pulsed Splittless, 220 °C
	Pulse pressure & time: 35psi for 1.15min
	2µL, 50ppb
Oven:	120°C for 1.17min
	120°C to 160°C at 25°/min
	160°C to 260°C at 10°/min
	260°C to 300°C (4min) at 15°/min
Detector:	µ-ECD, 320°C
	Ar/CH4 (P5) makeup gas at 60mL/min



Best Efficiency

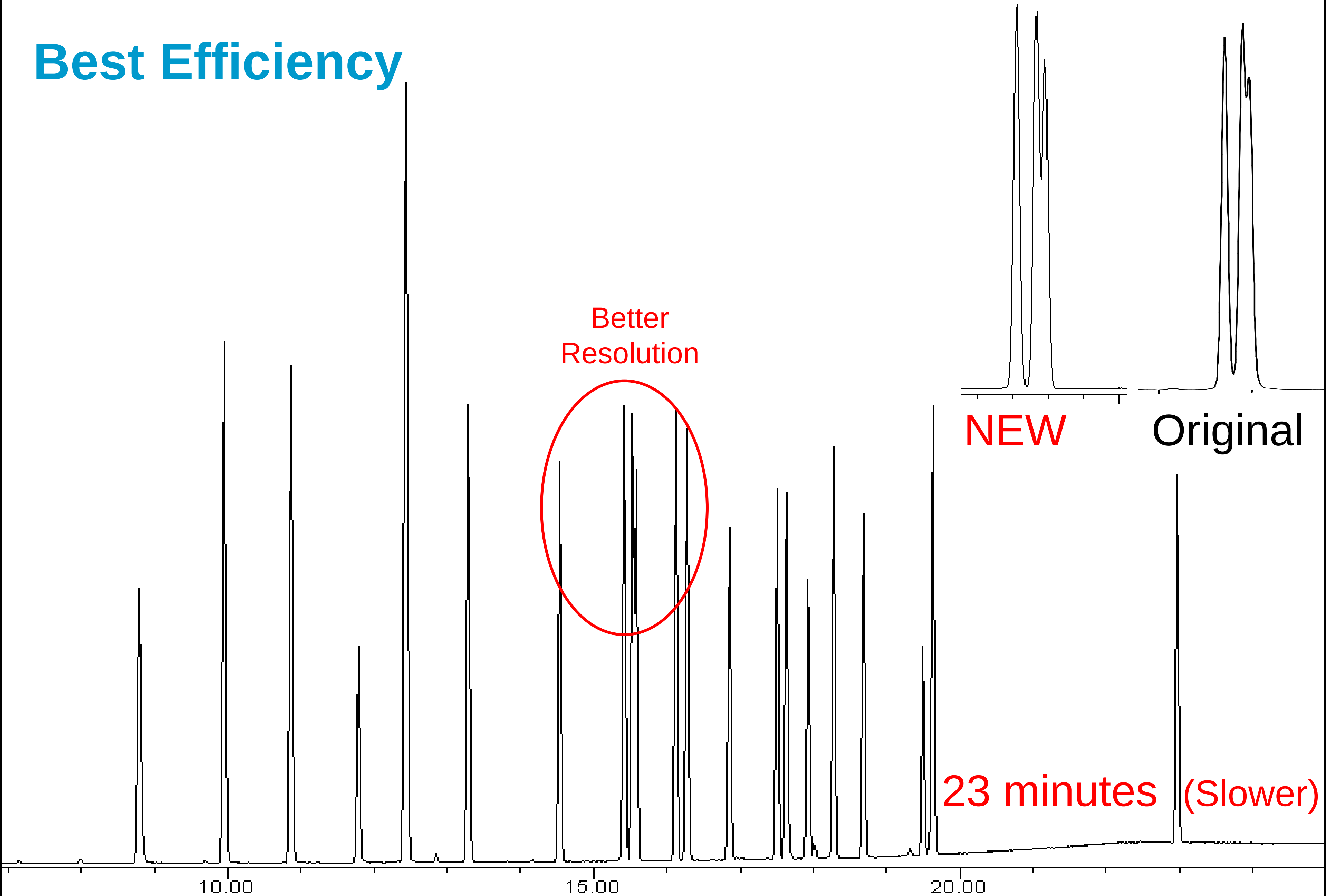


New Velocity (SLOWER)

New Temp. Program

(SLOWER)

Best Efficiency



Resolution

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Retention	$k = f\ (T, d_f, r_c)$	r_c = column radius
Selectivity	$\alpha = f\ (T, \text{phase})$	d_f = film thickness
		T = temperature

Temperature, the TRUMP card

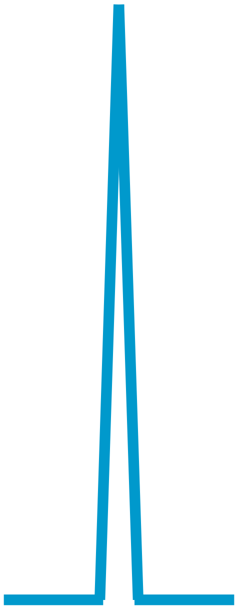
Column Temperature
Optimizing Temperature Programs

- Most powerful variable
- Changes Selectivity and Retention
- Natural log (ln) relationship between retention and temperature
- Most difficult to predict and develop
- Often involves trial and error (Sorry)

Peak Tailing

- High Resolution is worth nothing if the peaks start tailing and/or disappearing!

Peak Tailing – Why does it Happen

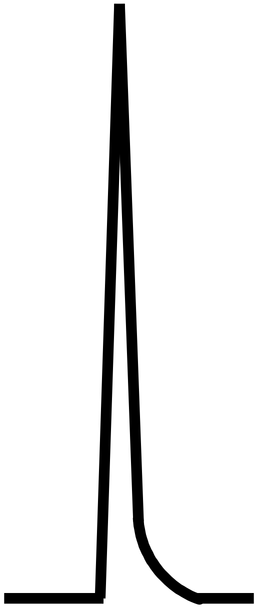


Turbulent Flow Problem

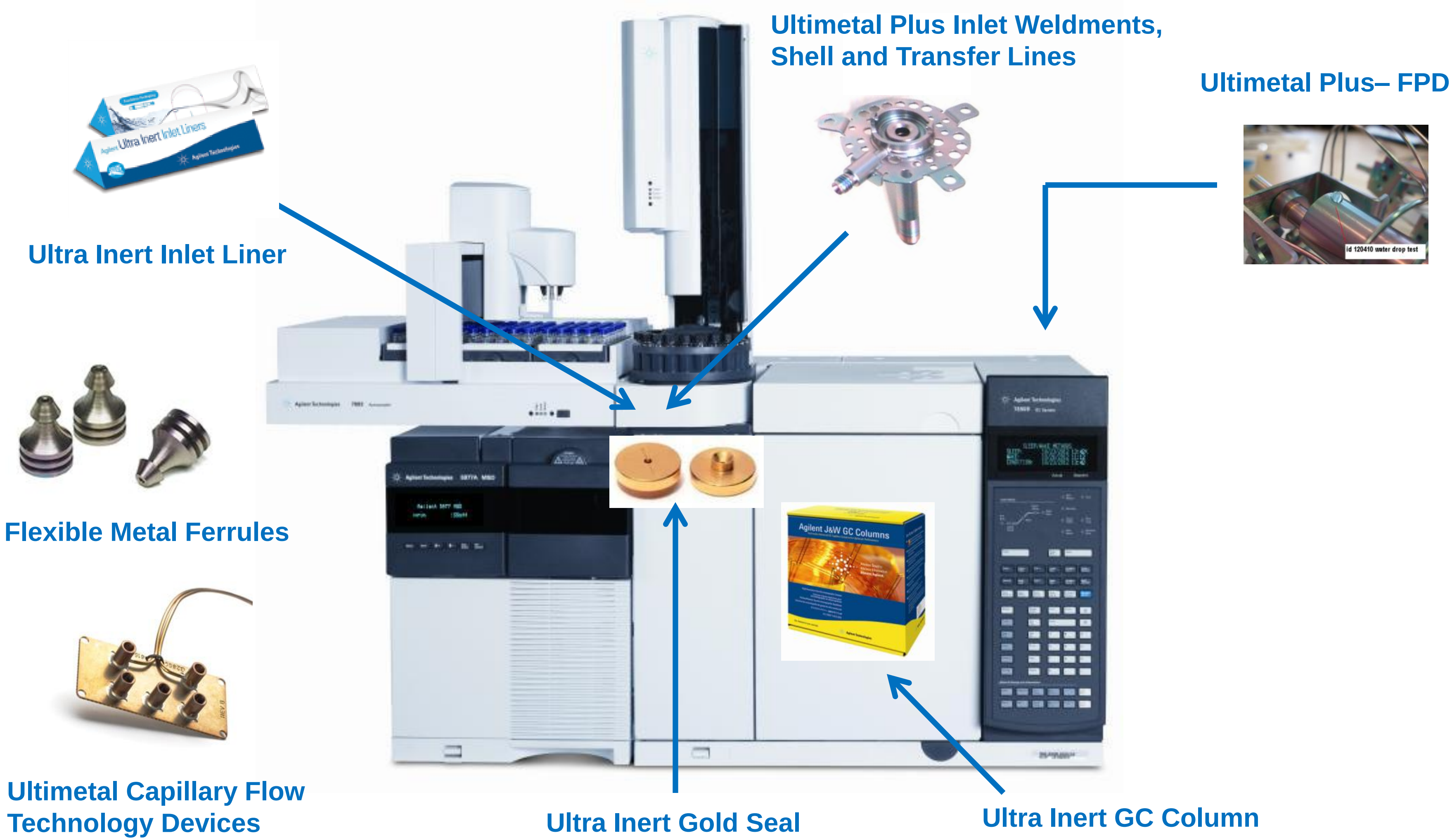
-Dead volume, obstruction, poor installation, or severe column contamination

Active Sites in Injector, Column, and/or Flow Path

- Reversible adsorption of active compounds (-OH, -NH, -SH)



Agilent Inert Flow Path Solution



Top 5 Inertness Tips

1. Use Good Gas Purifiers
2. Regular Inlet Maintenance
3. Use Inert Liners, Seals, Ferrules, etc. in Inlet

3. Select an Ultra Inert Column

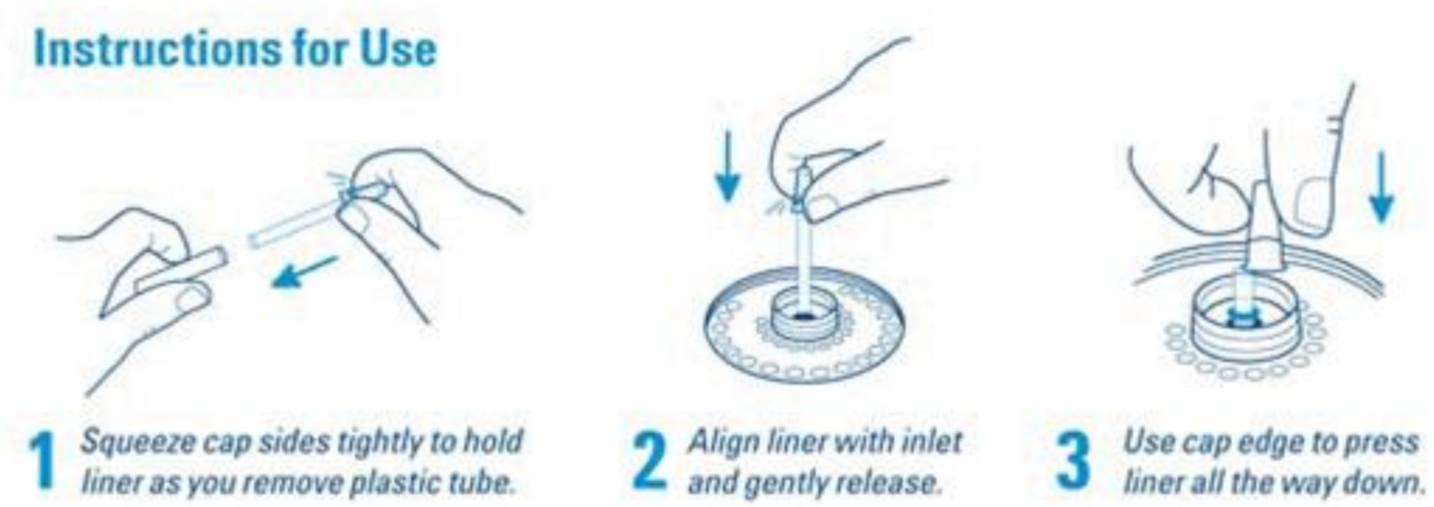
4. Maintain an Inert Detector

Agilent Ultra Inert Inlet Liners

Touchless Packaging

Easy installation of new, clean liner without risk of contamination from touching.

Includes non-stick plasma treated o-ring



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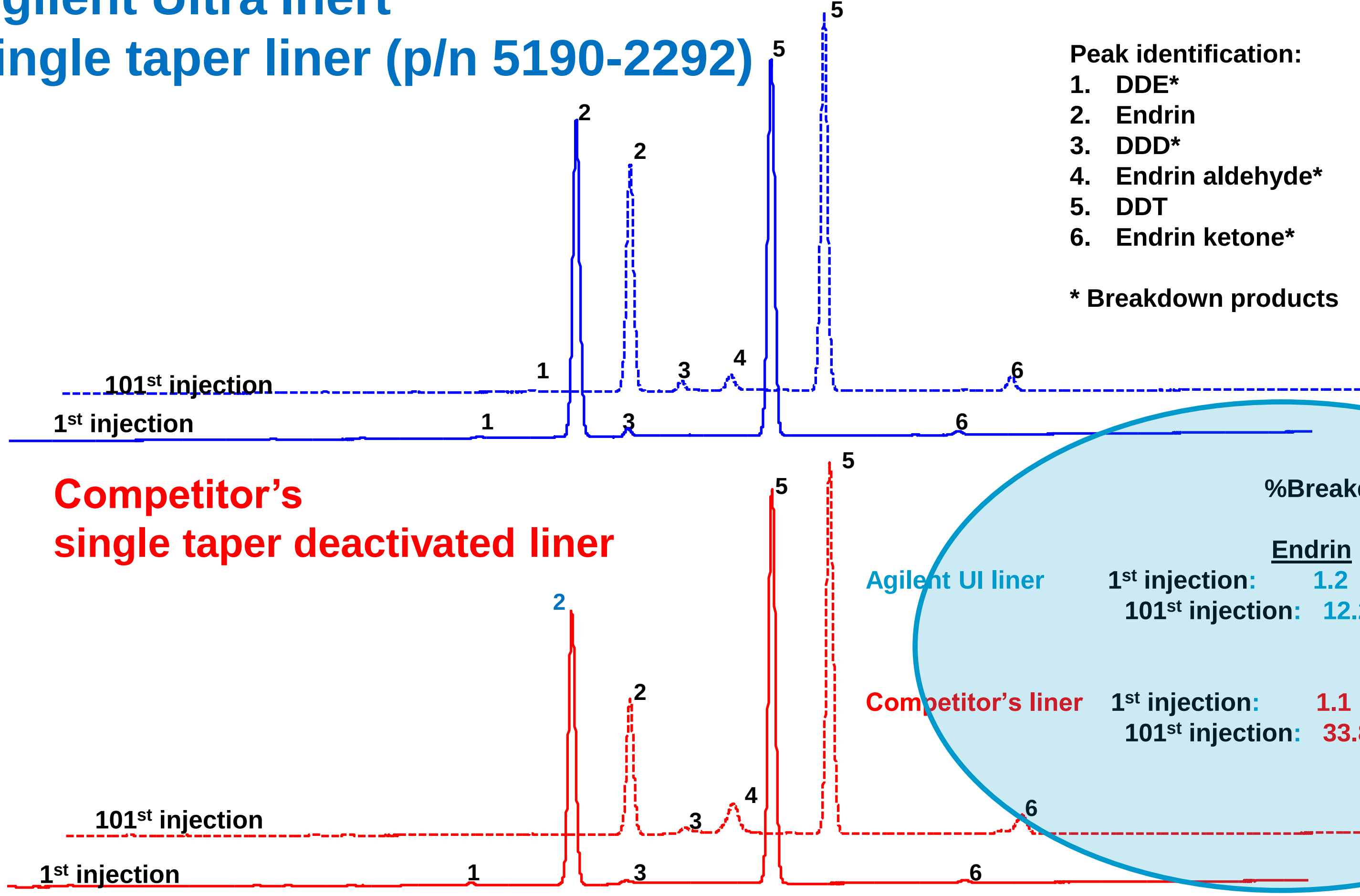
Endrin Decomposition Test : Robustness

Agilent Ultra Inert single taper liner (p/n 5190-2292)

Peak identification:
1. DDE*
2. Endrin
3. DDD*
4. Endrin aldehyde*
5. DDT
6. Endrin ketone*

* Breakdown products

Competitor's single taper deactivated liner



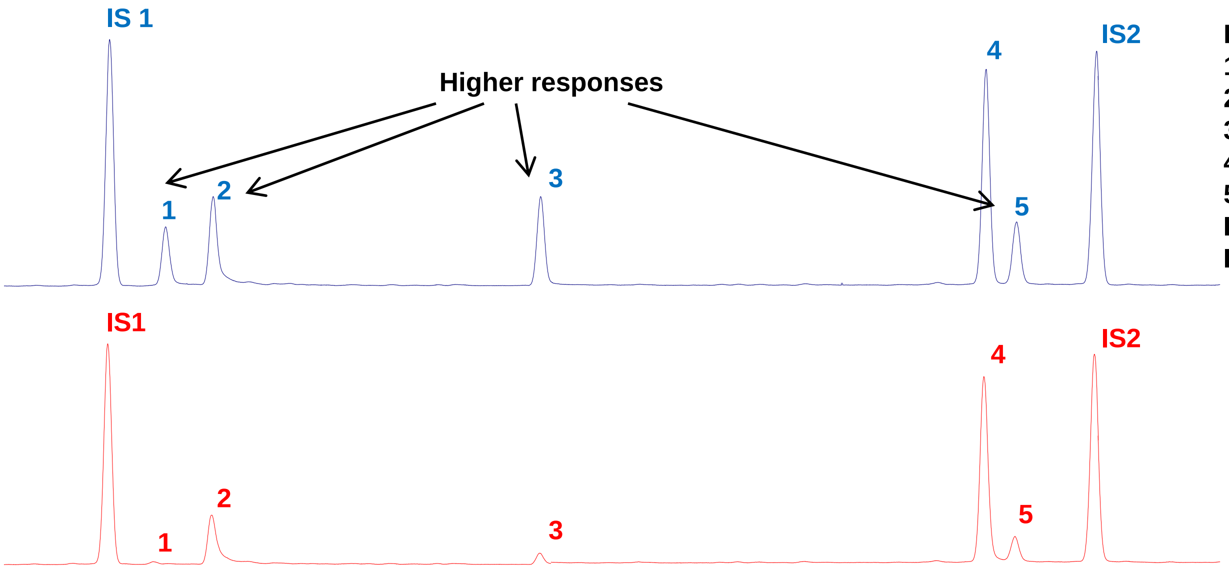
%Breakdown

		Endrin	DDT
Agilent UI liner	1st injection:	1.2	2.5
	101st injection:	12.2	3.0
Competitor's liner	1st injection:	1.1	1.7
	101st injection:	33.8	2.2

Ultra Inert deactivation passes Endrin/DDT decomposition test after 100 injections

Semi-Volatiles Suitability

Agilent Ultra Inert single taper liner **with wool** (p/n 5190-2293)



Peaks:
1. 2,4-Dinitrophenol
2. 4-Nitrophenol
3. 4,6-Dinitro-2-methylphenol
4. 4-Aminobiphenyl
5. Pentachlorophenol

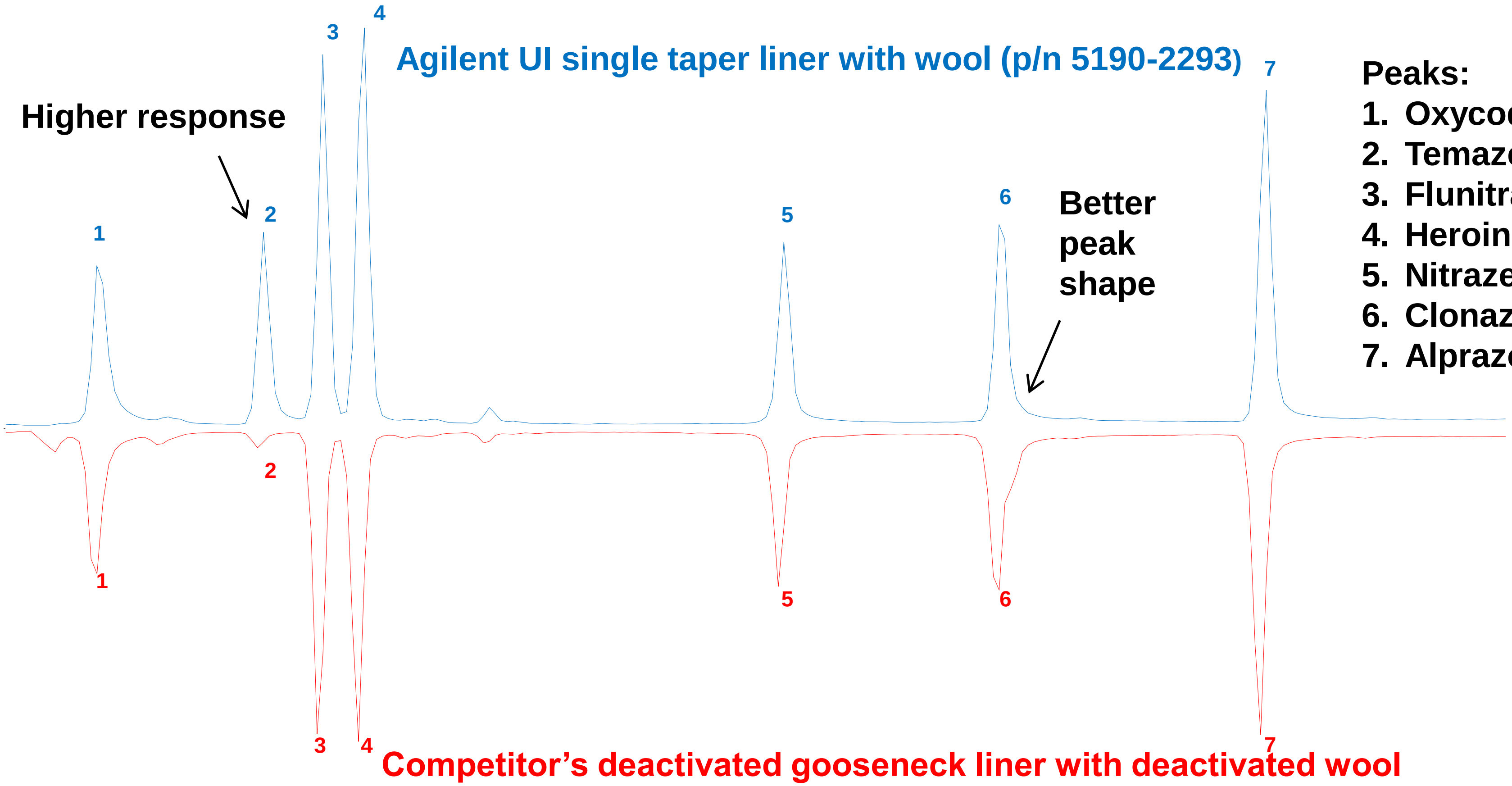
IS1. Acenaphthene-d10
IS 2. Phenanthrene-d10

Competitor's deactivated gooseneck liner with deactivated wool

Even with glass wool

Ultra Inert liners provide high responses for sensitive semivolatile acidic compounds.

Basic Drug Suitability



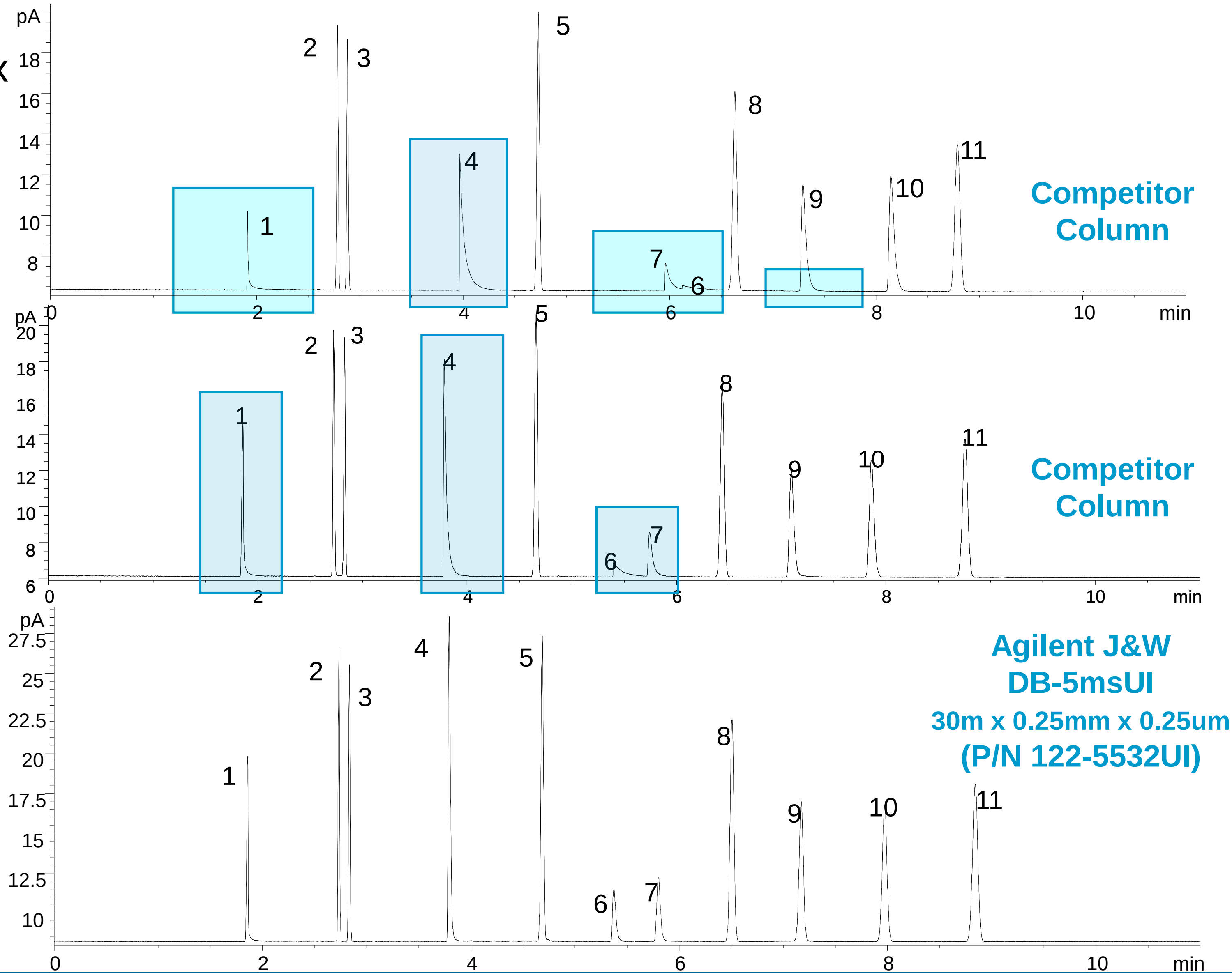
Peaks:
1. **Oxycodone**
2. **Temazepam**
3. **Flunitrazepam**
4. **Heroin**
5. **Nitrazepam**
6. **Clonazepam**
7. **Alprazolam**

Drug of abuse are shown on GC/MS SIM chromatograms 5 ng of checkout standards on column

DB-5ms Ultra Inert vs. Competitors

Ultra Inert Test Mix

1. 1-Propionic acid
2. 1-Octene
3. n-Octane
4. 4-Picoline
5. n-Nonane
6. Trimethyl phosphate
7. 1,2-Pentanediol
8. n-Propylbenzene
9. 1-Heptanol
10. 3-Octanone
11. n-Decane



Semivolatiles Prone to Peak Tailing (App. Note 5990-3416EN)

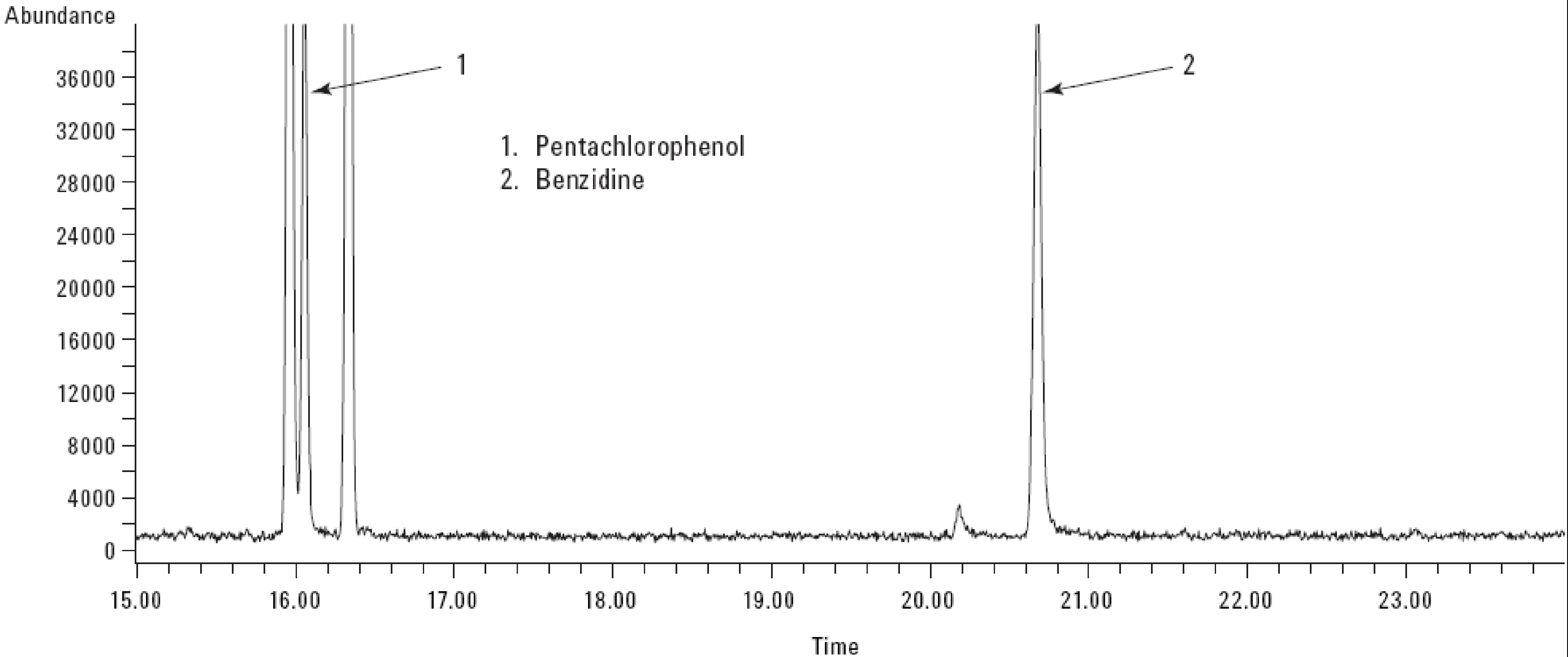


Figure 2. *Enlarged section of the total ion chromatogram for a 1-µL injection of 1.0 µg/mL EPA 8270 short mix standard. The peaks of interest noted in the figure are two semivolatiles that are prone to peak tailing. Chromatographic conditions are listed in Table 1.*

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Semivolatiles Prone to Peak Tailing (App. Note 5990-3416EN)

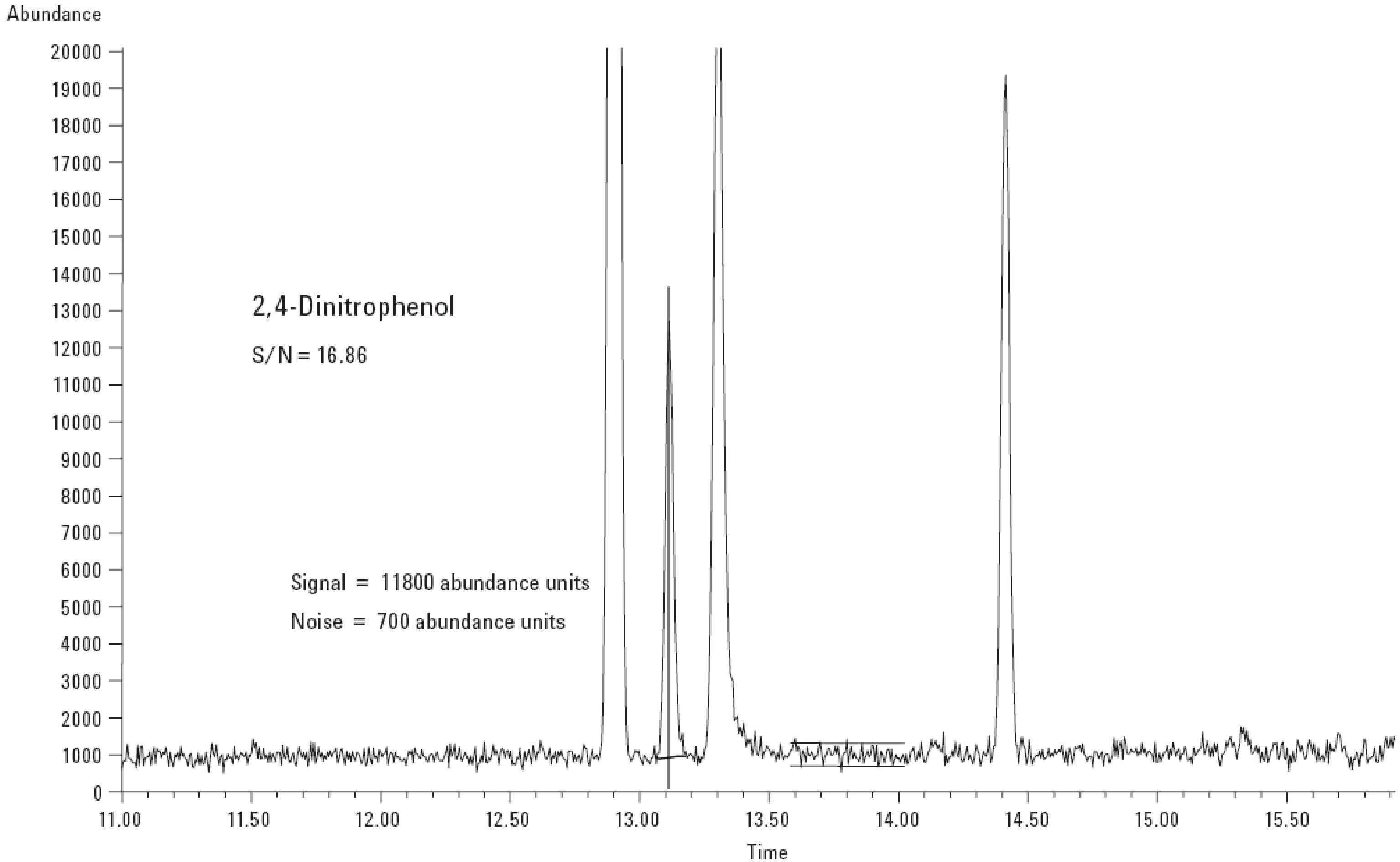
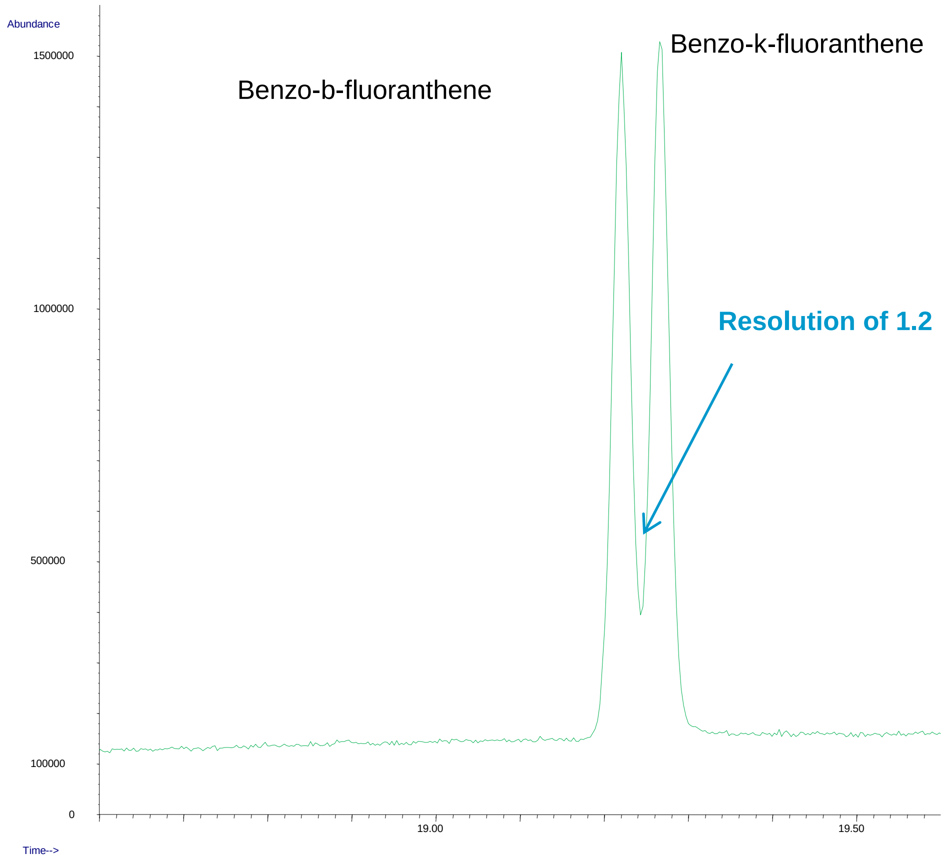


Figure 4. *Enlarged section of the total ion chromatogram (scan mode) for a 1-µL injection of 1 µg/mL EPA Method 8270 short mix standard on an Agilent J&W HP-5ms Ultra Inert 30 m × 0.25 mm × 0.25 µm capillary GC column (p/n 19091S-433UI). The peak in the figure is 2,4-dinitrophenol, one of the more demanding semivolatiles. This injection represents an on-column loading of 1 ng per component. Chromatographic conditions are listed in Table 1.*

Resolution of benzo-b & k fluoranthene isomers



As good as it gets…it only gets worse!

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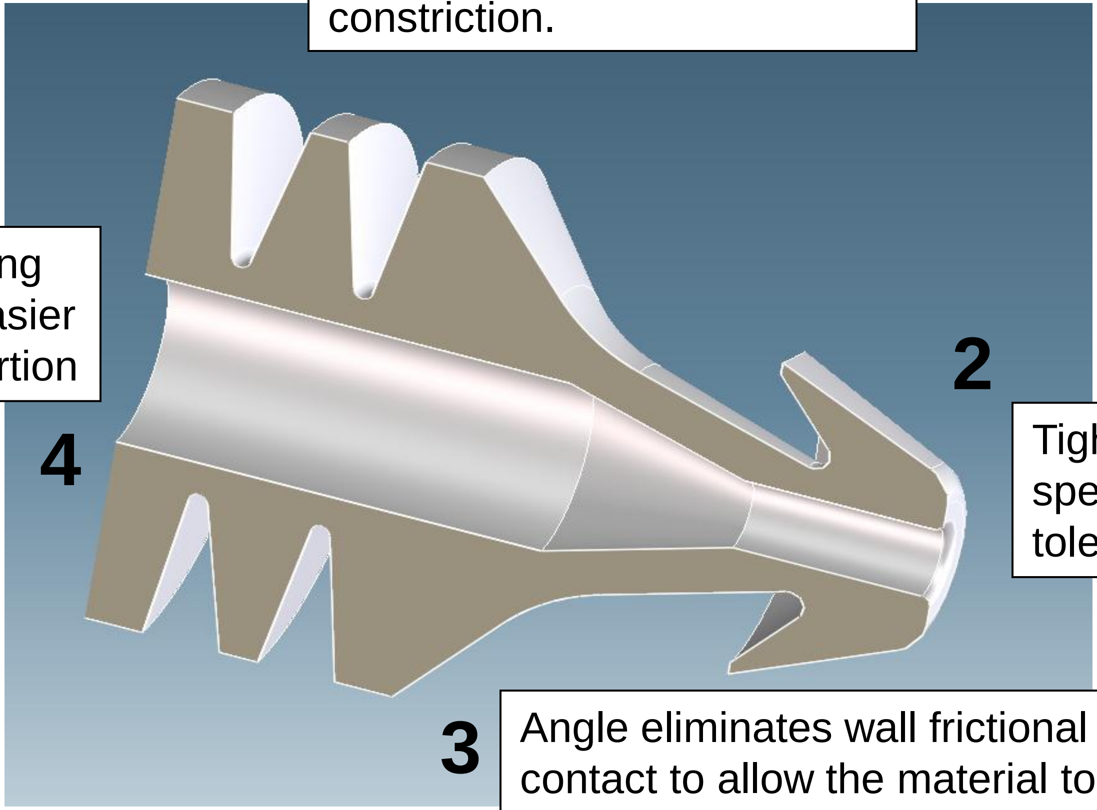
New Flexible Metal Ferrule - Design Features

1 Grooves
reduce bulk stiffness during compression and improves ID constriction.



4 Wider opening allows for easier column insertion

2 Tighter ferrule ID specification and tolerances (30%)

3 Angle eliminates wall frictional contact to allow the material to flow into the ID area for larger range of constriction.

Current vs. New Agilent Flexi-Metal Ferrule

Current Ferrule

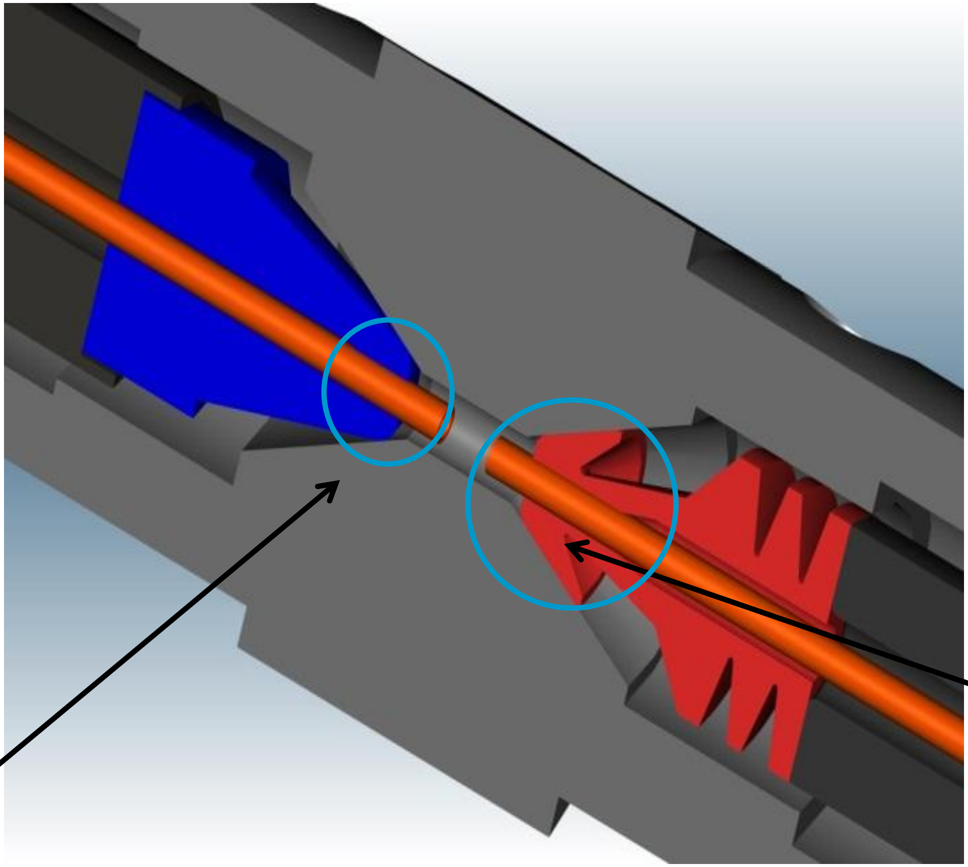
Stiff- prone to cause damage

Poor installation
Can lead to:

- column crushing
- leakage
- Tread damage

Small ID compression
(40 to 60um)
seals at tip

Tight pinch point



Agilent Flexi-Metal Ferrule

More forgiving

Easier to use

Large ID compression
(100 to 120um)

Broad lamp shade sealing surface

Forgiving pinch point

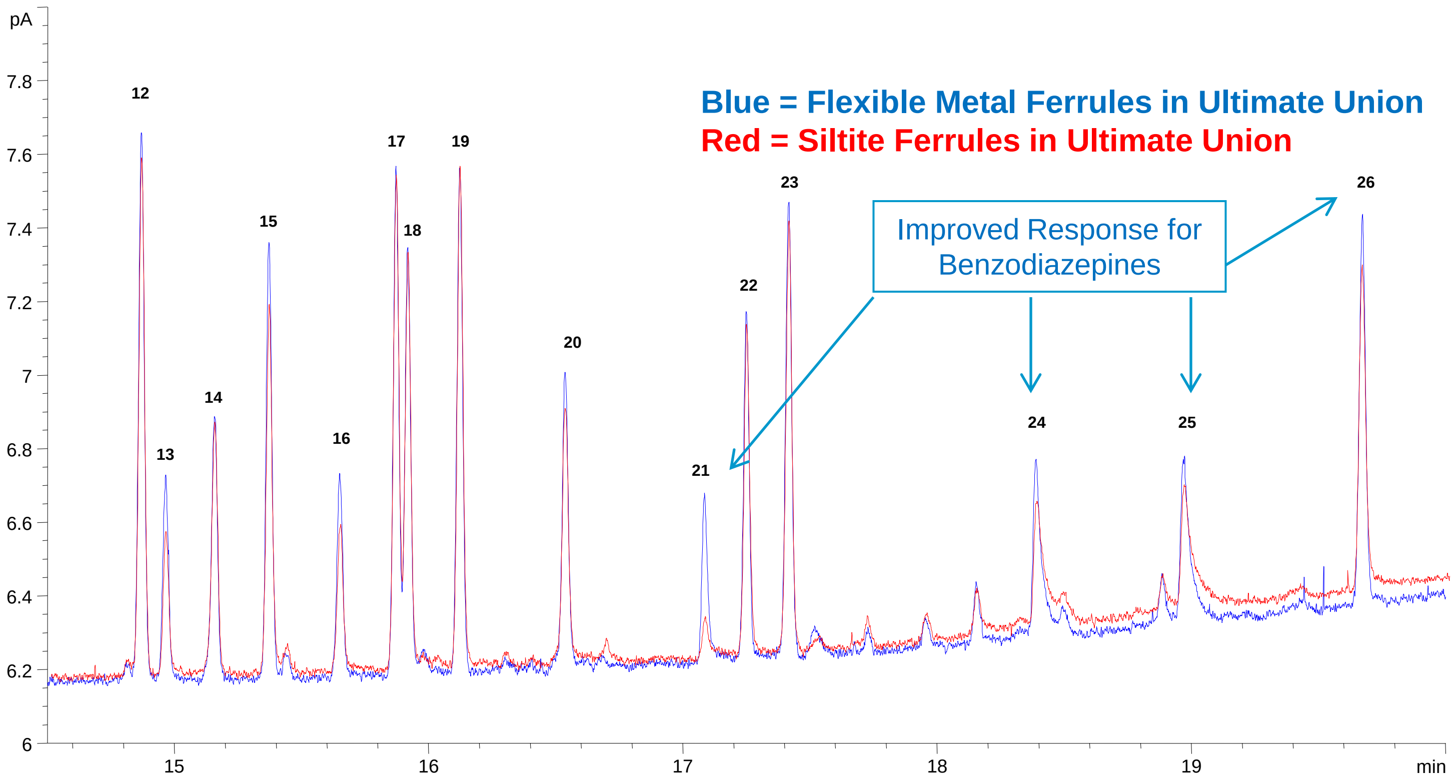
New Touchless Dial Packaging

Easy column insertion

No lost ferrules

No contamination risks

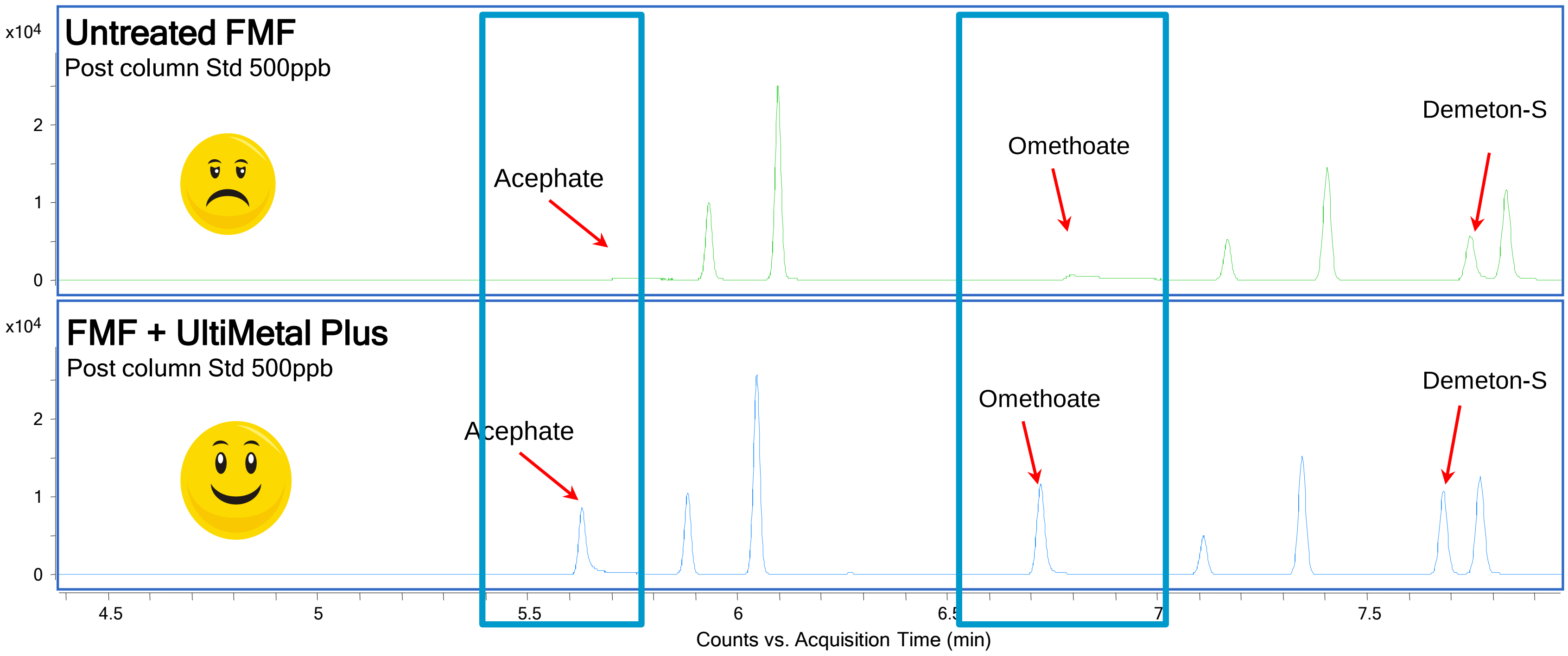
1 ng on Column Forensic/Toxicology Check Out Mix Flexible metal Ferrules vs. Siltite installed post column



12 SKF-525a, proadifen
13 Oxazepam
14 triphenyl phosphate
15 Codeine
16 Lorazepam
17 Diazepam
18 Hydrocodone
19 Dronabinol
20 Oxycodone
21 Temazepam
22 Flunitrazepam
23 Diacetylmorphine
24 Nitrazepam
25 Clonazepam
26 Alprazolam

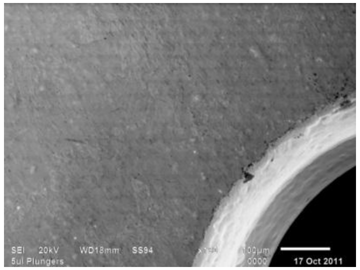
UltiMetal Plus on Flexible Metal Ferrule
Is deactivation needed for such small surface?

Enlarged chromatograms for sensitive pesticides

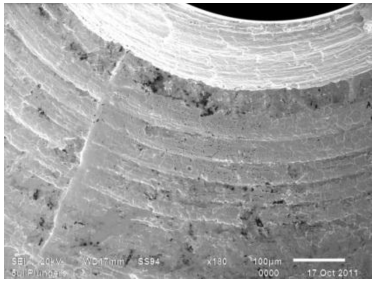


Agilent UI Gold Seal: Deactivated Gold Surface

- Soft gold plating is essential for proper sealing
- Ultra Inert chemistry blocks active sites
  (gold is NOT inert)
- Smooth surface doesn't leak



Agilent MIM seal

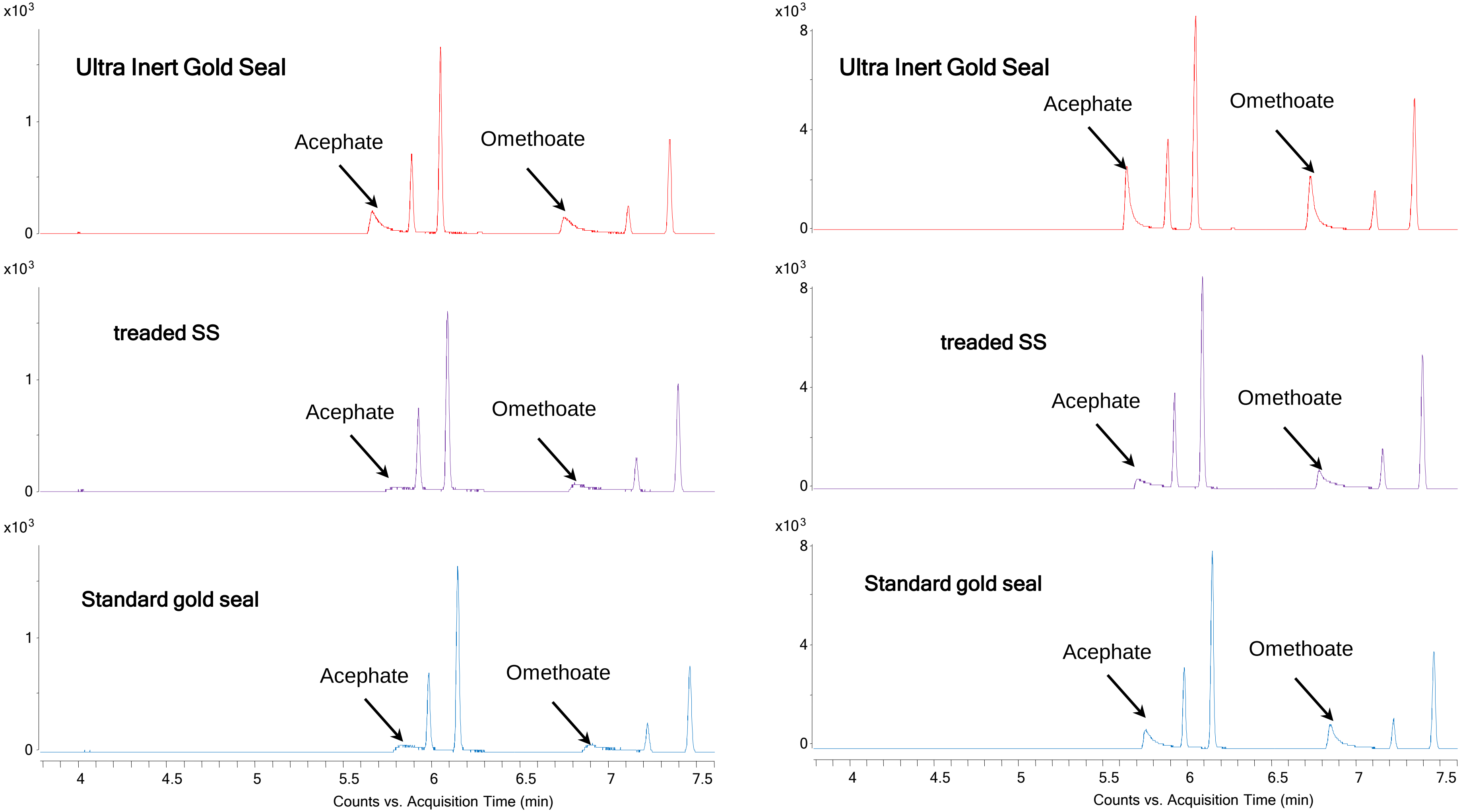


Alterative vendor machined seal

Reliable ppb and ppt measurements require attention to the little things!

Response Comparison for Sensitive Pesticides

100 ppb STD | **500 ppb STD**



New Product: Agilent Inert Inlet

UltiMetal Plus treatment for
inert surface 7890 inlet shell & top weldments

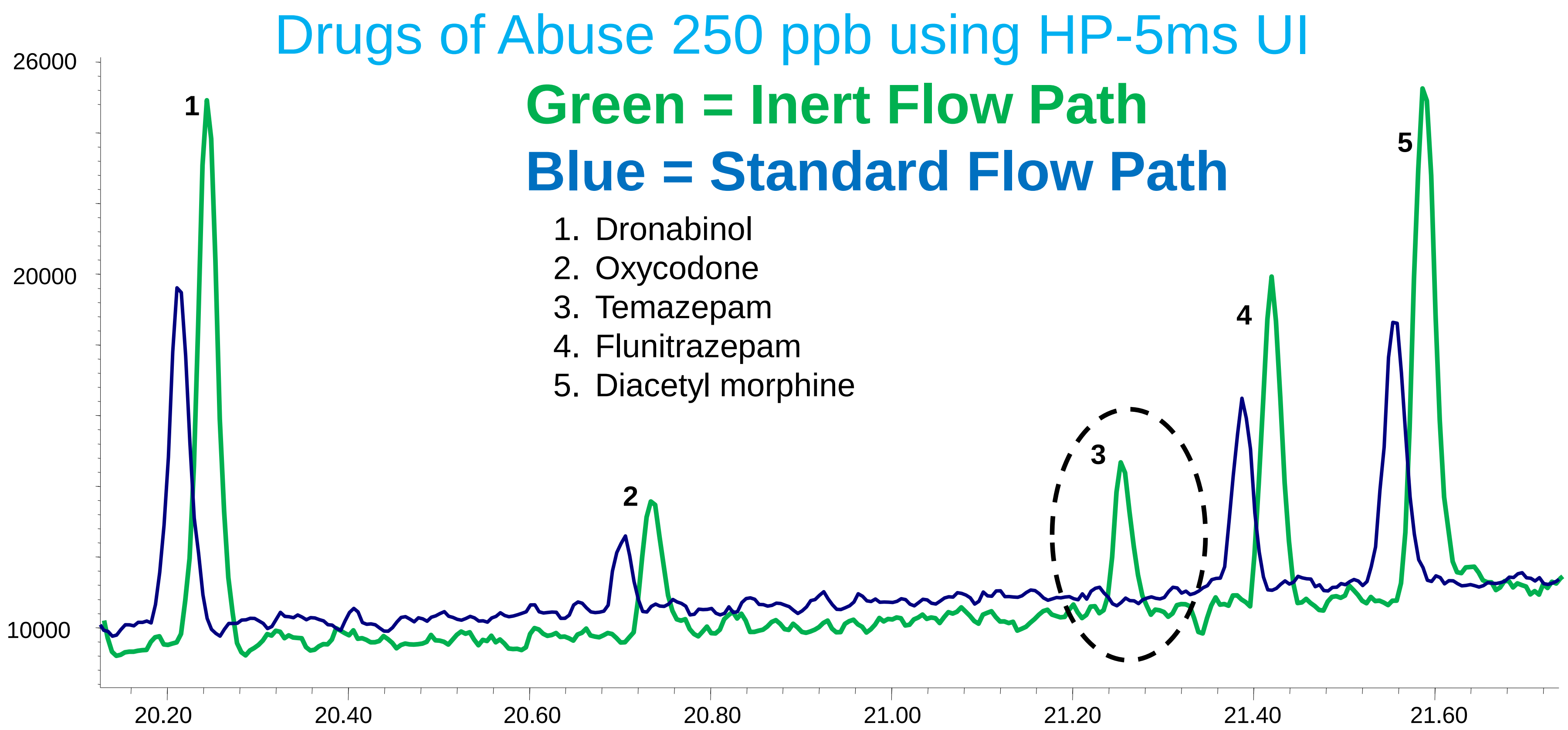
•Limits adsorption/degradation of active analytes

•GC/MS and GC-ECD pesticide analysis

or Any Active Analyte Analyses

Agilent's proven proprietary Ultimetal Plus surface treatment

Putting It All Together –
Inert Flow Path vs. Standard Flow Path



Column:	Agilent J&W HP-5ms UI 30 m x 0.25 mm x 0.25 um
Oven:	100°C 4 min hold, 10°/min to 280°C, 6 °/min to 300°C (4.67 min hold),
Carrier :	Helium 52.7 cm/s (2 mL/min) set at 100°C, EPC-Constant Flow
Inlet:	Pulsed Splitless 35 PSI pulse until 0.73 min, 0.75 min purge 50 ml/min, gas saver 20, ml/min at 2 min
Inlet liner:	Ultra Inert with wool / Standard single taper liner with wool (p/n 5190-3165)
Gold Seal:	UI Gold Seal / Standard gold seal
Detector:	MSD Scan mode 40 t0 450 m/z, 230 °C source temp, 150 °C Quad temp, 310 °C transfer line

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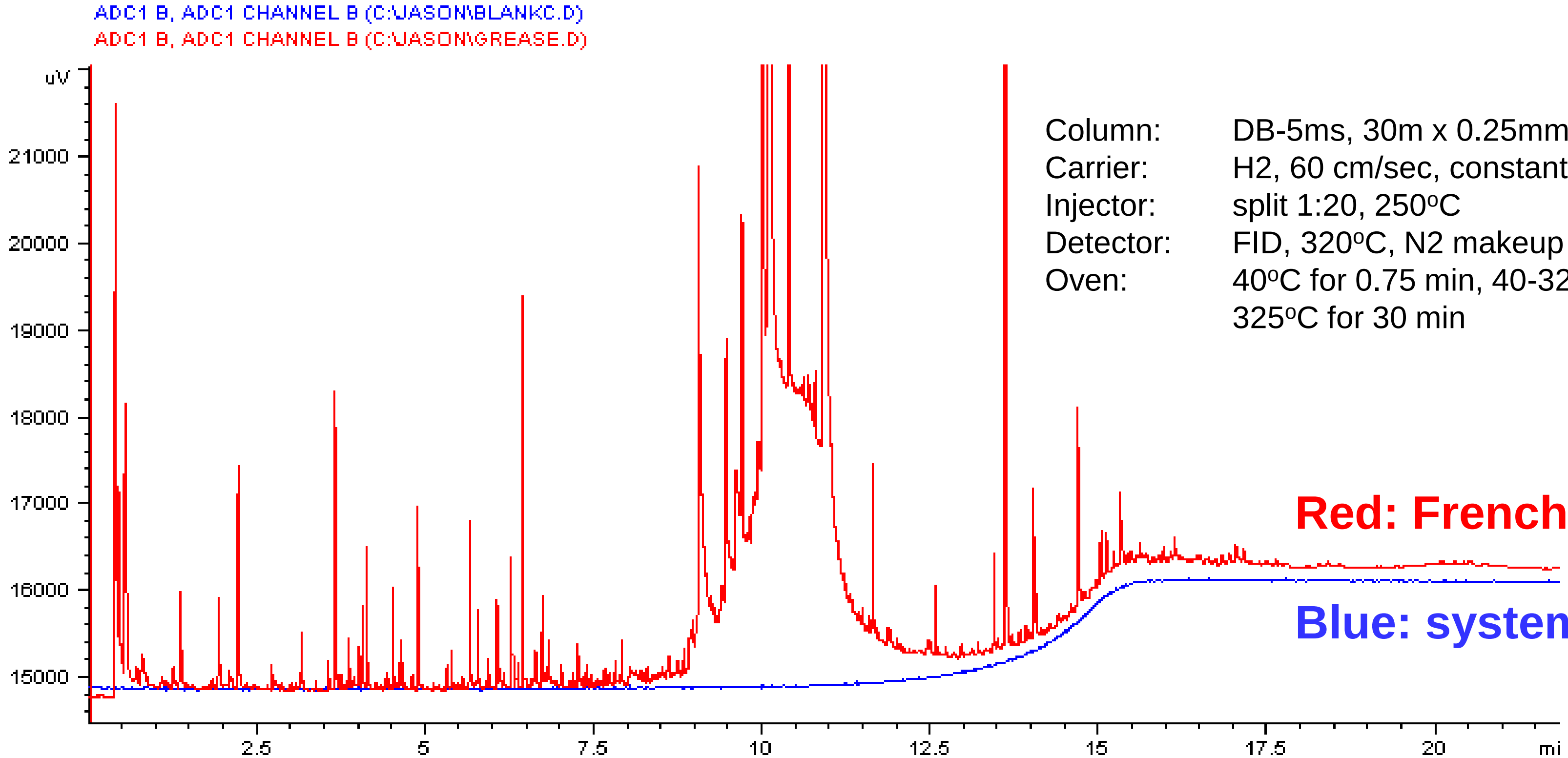
So Remember…

1. Use Good Gas Purifiers
2. Do Regular Inlet Maintenance
3. Use Inert Liners, Seals, Ferrules, etc. in Inlet

3. Select an Ultra Inert Column

4. Maintain an Inert Detector

AND Don't Touch Anything!!!



Column: DB-5ms, 30m x 0.25mm, 0.25um
Carrier: H_2, 60 cm/sec, constant flow
Injector: split 1:20, 250°C
Detector: FID, 320°C, N_2 makeup gas
Oven: 40°C for 0.75 min, 40-325°C at 20°C/min, 325°C for 30 min

Red: French Fry

Blue: system blank

Procedure:
(1) Held French fry for 5 seconds.
(2) Fingertip was wiped with paper towel to remove as much of the offending material as possible.
(3) Lightly touched the part of the column sticking up above the ferrule.
(4) Installed column into injector.
(5) Set oven temperature to 40°C.
(6) Started oven temperature program as soon as oven reached 40°C.

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Conclusions for Improving GC Resolution

- Application Specific Stationary Phase - tuned for max R
- Smaller Diameter - loss in capacity, so small changes
- Longer Columns, More Plates - big changes needed since it has a small effect and it increases analysis times
- Optimum Carrier Gas Velocity - slowest carrier gas velocity
- Inert Flow Path for Active Peaks - tailing loses resolution
- Inlet Maintenance - dirtier the sample, higher the frequency

ANY QUESTIONS?

Technical Support
1-800-227-9770, 3

E-mail: gc_column_support@Agilent.com