

Techniques for Making Your GC Analysis More Repeatable, Reproducible and Robust

Mark Sinnott
Application Engineer
Agilent Technologies
June 10, 2009



Agilent Technologies

Agilent Restricted

Primary Areas of Concern

- **Sample**
- **Auto-Injector**
- **Inlet**
- **Column**
- **Detector**



Sample Preparation and Care

It is critical that the sample extract be handled in the most consistent manner possible with regard to the following variables:

- **Temperature**
- **Vial seal integrity**
- **pH**
- **Solvent purity**
- **Exposure to light**



Auto-Injector Setup

5uL syringe vs. 10uL syringe

Solvent washes before and after injection

Sample washes before injection

Sample pumps prior to injection

Plunger speed?

Viscosity delay?



Typical Auto-Injector Setup

5- μ L syringe with HP-Point

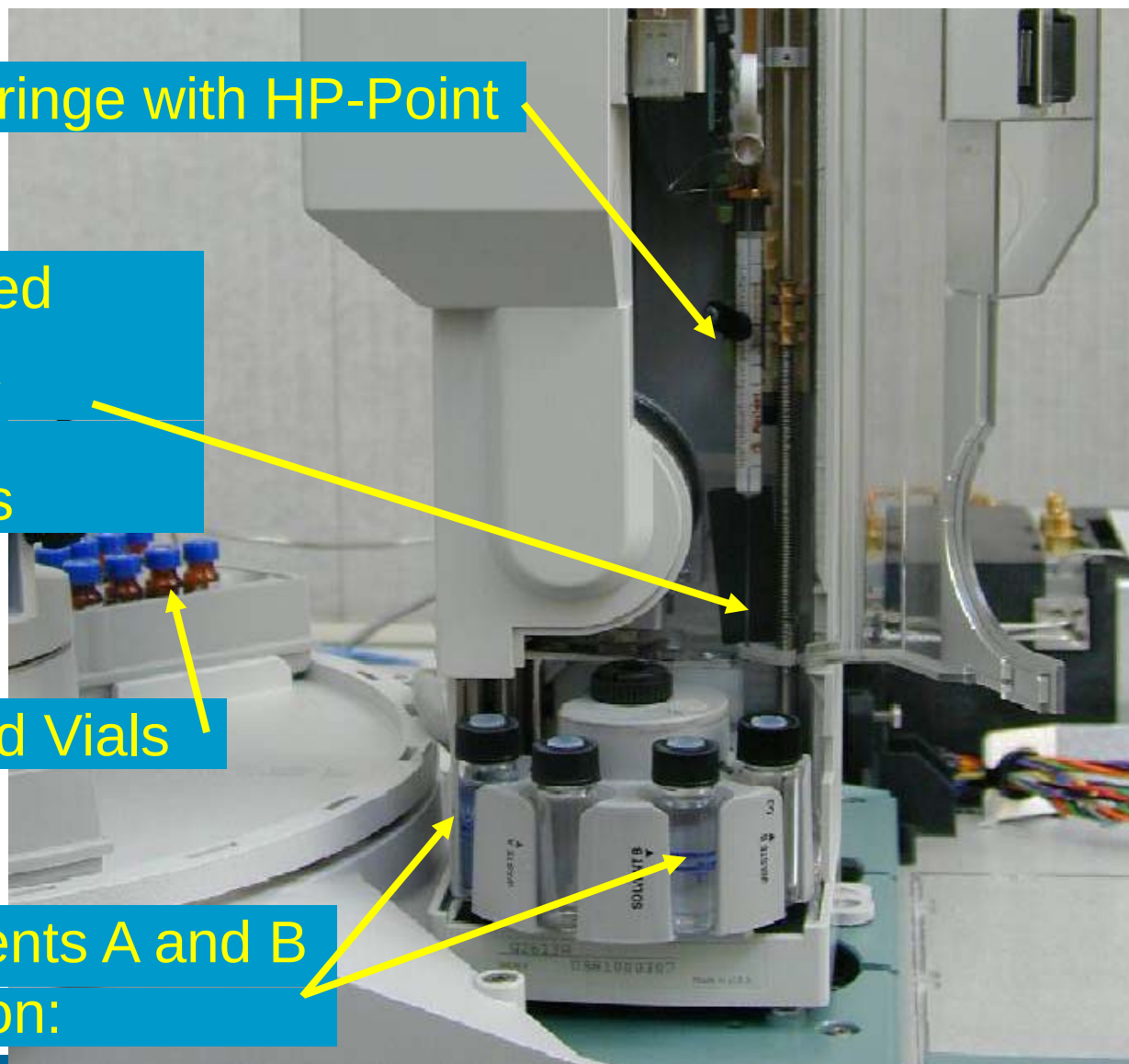
Fast injection speed

No viscosity delay

3-5 sample pumps

Use Agilent Certified Vials

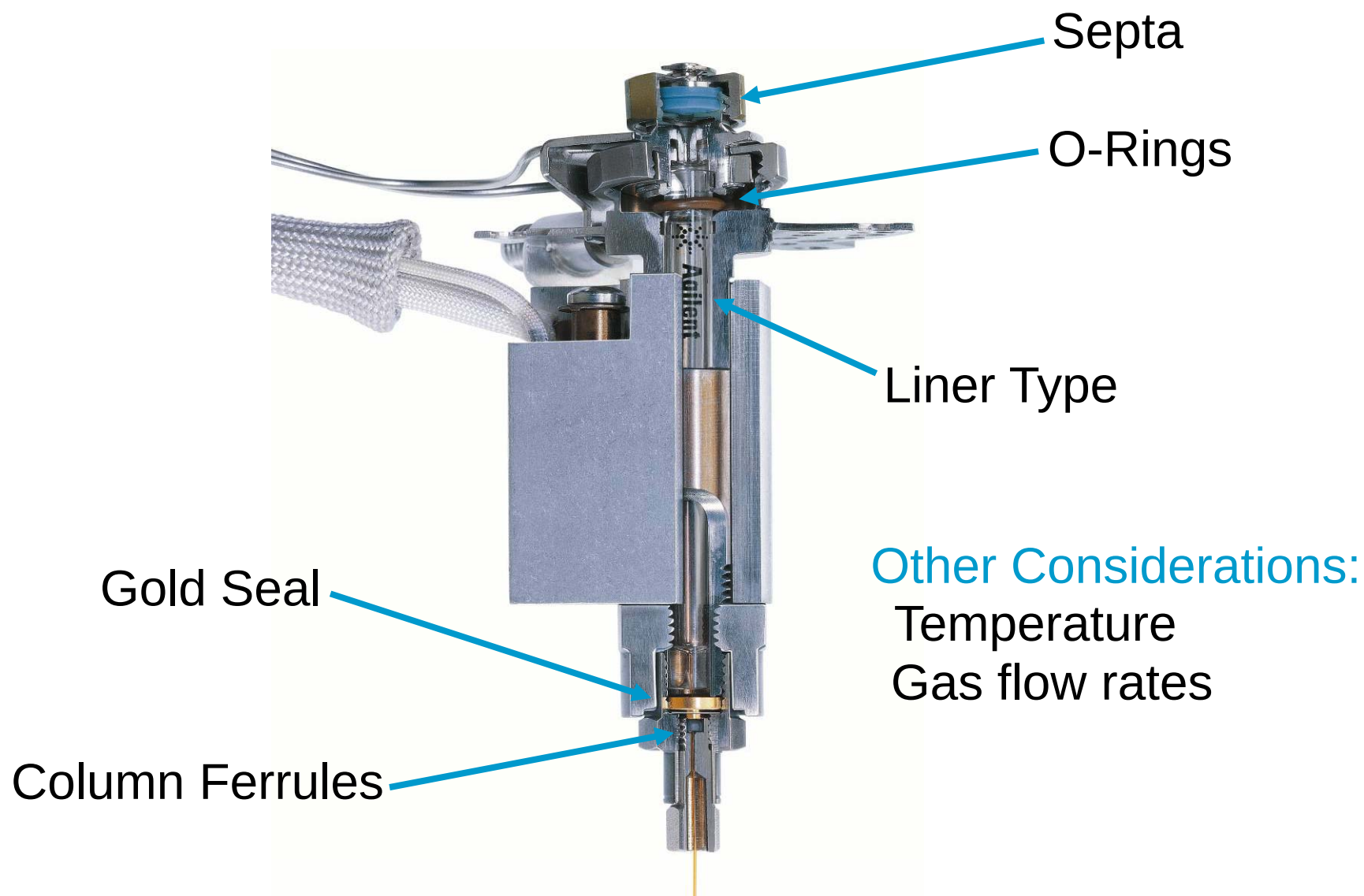
3 washes with solvents A and B
pre and post injection:



Agilent Technologies

Agilent Restricted

Inlet Considerations

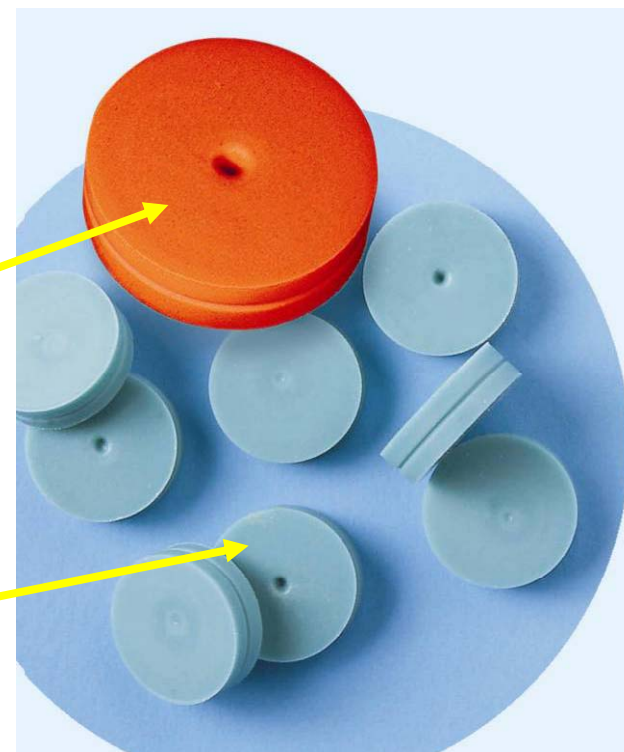


Preferred Inlet Septa

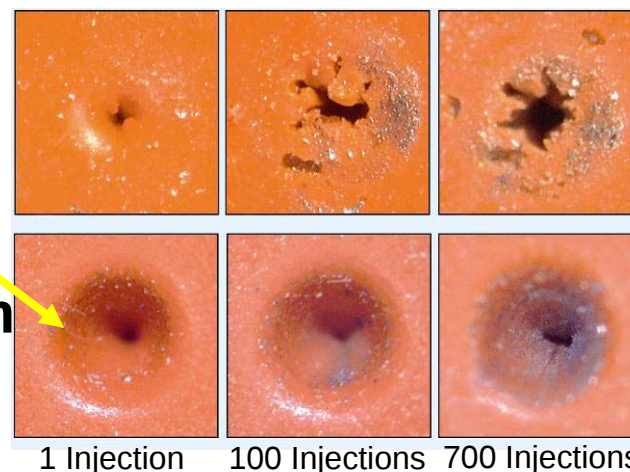
Use Bleed and Temperature Optimized (BTO) septa for inlet temperatures up to 400°C

Use Advanced Green septa for inlet temperature up to 350°C

The dimpled CenterGuide on Agilent septa greatly reduces coring related leak problems

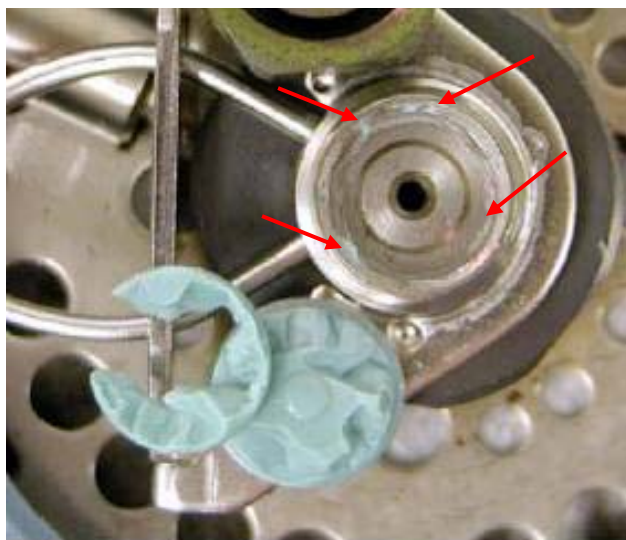


CenterGuide Septum



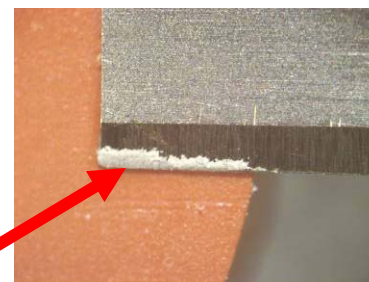
Tips to Maximize Septum Life, Minimize Septum Leaks

- Use Non-Stick septa, especially Agilent's Centerguide Septa with Proprietary Plasma Treatment

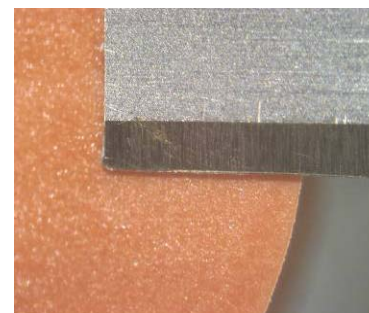


Their's

Talcum Powder!



Our's



- Stuck septa particles can cause sealing problems on next septum installation. Talc can cause activity/trap plugging problems



Agilent Technologies

Agilent Restricted

Septa vs GC Column Costs

Typical cost of 1 Premium Septum (list), \$1.25

Typical cost of 1 GC Column, 30 m x 0.25 mm ID, \$450.

Leaks affect flow rates causing inaccurate results.

“Don’t step over a dollar to pick up a dime!”

Proactively change inlet septa.



Liners - 3 Key Variables

- ✓ **Liner Volume**
- ✓ **Liner Treatments or Deactivation**
- ✓ **Special Characteristics (glass wool, cup, taper, etc.)**

When choosing a liner for your application, consider all three aspects to give you the best chromatography.

You must also determine what type of inlet is in your GC

Then consider the application itself, and the types of liners and injection techniques used for it:

 **Split**

 **On-Column**

 **Programmable
Temperature
Vaporization (PTV)**

 **Splitless**

 **Purge-Packed**



Agilent Technologies

Agilent Restricted

Inlet Liners – Volume Considerations

Glass Inlet Liners provide an “inert” space for liquid samples to be uniformly vaporized to a gas and moved to the column.

Liquid-gas phase change involves a significant change in volume.

Gaseous sample volume depends on

- Injection volume
- Solvent type
- Column head pressure
- Inlet temperature

These aspects should be optimized for your sample volume and application.

Solvent (1µL, ambient)	Volume (µL at 250°C and 20psig)
n-Hexane	140
Acetone	245
Acetonitrile	350
Methanol	450
Water	1010

See “A Practical Guide to the Care, Maintenance, and Troubleshooting of Capillary GC Systems”, Third Revised Edition, by Dean Rood, Wiley-VCH, New York, 2001.



Pressure/Flow Calculator Software

Hexane Looks Good

Solvent Vapor Volume Calculator [X]

Approximate vapor volume(ul): **195 ul**  **20 %**



Injection Volume (ul)
 **1.0** [Left] [Right]

Inlet Temp (C)
 **250** [Left] [Right]

Inlet Pressure
 **10** [Left] [Right]

Pressure Units
☐ KPa ☒ psi ☐ bar

Solvent Properties
Hexane [v]
Boiling Pt (C): 68.7
Density (g/cm3): 0.659
Mol Wt. (amu): 86.2
 Solvents

Injection Liner Volume (ul)
19251-60540 strai [v] 990
[Edit Liner list] Capacity limits (%)
75 100

Buttons: Print Help OK



Pressure/Flow Calculator Software

Water Can Be Trouble

Solvent Vapor Volume Calculator [X]

Approximate vapor volume(ul): **1414 ul** **Overload** **143%**

[Progress Bar]

Injection Volume (ul)
[Slider] **1.0** [Left] [Right]

Inlet Temp (C)
[Slider] **250** [Left] [Right]

Inlet Pressure
[Slider] **10** [Left] [Right]

Pressure Units
☐ KPa ☒ psi ☐ bar

Solvent Properties

Water [Dropdown]

Boiling Pt (C): 100
Density (g/cm3): 0.998
Mol Wt. (amu): 18.02

 **Solvents**

Injection Liner **Volume (ul)**
19251-60540 strai [Dropdown] **990**

Edit Liner list **Capacity limits (%)**
75 100

Print **Help** **OK**



Pressure/Flow Calculator Software

Water– Reduce Injection Volume

Solvent Vapor Volume Calculator [X]

Approximate vapor volume(ul): **707 ul**  **71 %**



Injection Volume (ul)
 **.5** [◀ ▶]

Inlet Temp (C)
 **250** [◀ ▶]

Inlet Pressure
 **10** [◀ ▶]

Pressure Units
☐ KPa ☒ psi ☐ bar

Solvent Properties
Water [▼]
Boiling Pt (C): 100
Density (g/cm3): 0.998
Mol Wt. (amu): 18.02
 Solvents

Injection Liner Volume (ul)
19251-60540 straight [▼] 990
[Edit Liner list] Capacity limits (%)
75 100

Print **Help** **OK**



Liner Treatments or Deactivation

Minimizes possibility of active sample components from adsorbing on active sites on the liner or glass wool surface.

Unwanted sample adsorption leads to tailing peaks and loss of response for active analytes.

Although not necessary for all applications, deactivated liners provide added insurance against possible sample adsorption.

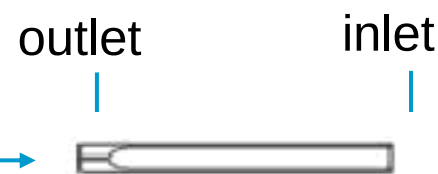
Deactivation of borosilicate glass liners is often done with a silylating reagent like Dimethyldichlorosilane (DMDCS) or by coating with a siloxane (as capillaries are made).



Special Characteristics

Some liners have special features that are necessary for different injection techniques. For example:

Taper (gooseneck), minimizes sample contact with gold seal. →



Dual taper, also minimizes sample contact with inlet weldment and reduces potential for backflash. →



Glass wool and shelf to hold it in place, prevents non-volatiles from reaching column and removes residual sample from needle. Glass wool should be deactivated. →



Jennings cup, normally used for efficient sample mixing in split inlets, reduces sample discrimination and prevents non-volatiles from reaching the column. Not for very dirty samples. →

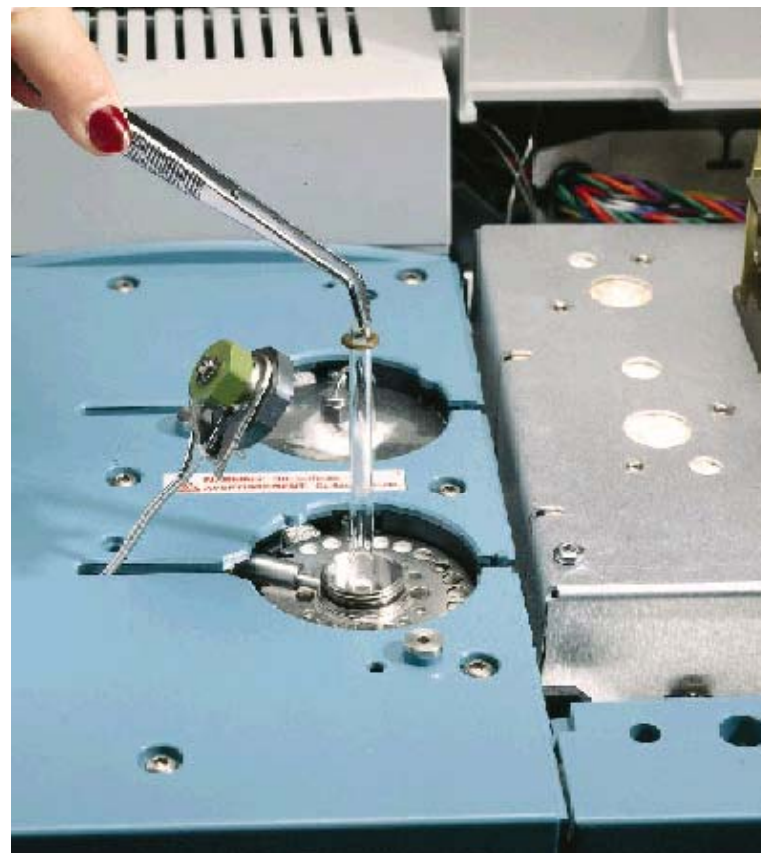


Press fit (direct) connection end to hold capillary column firmly (virtually all sample goes onto the column). Side hole needed for Electronic Pressure Control with direct connect liners. →



Split Injection Overview

- Most common injection technique
- Reduces the amount of sample that reaches the column (majority of sample exits the inlet via the split vent)
- Used primarily for highly concentrated samples (0.1 – 20mg/mL) and large sample volumes (up to 4 μL) .
- Highly efficient injection technique
- Must be inserted in inlet so bottom does not contact gold seal (need carrier flow access to split vent)



Split Injection Liners

Liner	Part No.	Comments
	19251-60540	Simplest split liner, glass wool, no-deactivation, large volume, 990 μ L volume. Use for general purpose applications for compounds with low glass adsorption activity. Also used for Splitless mode.
 Glass nub	5183-4647	Glass wool (held near needle entrance to remove residual sample on needle), deactivated, 870 μ L volume. Glass nub ensures that gap remains below liner for split injection. Efficient, for most applications, including active compounds. Fail-safe insertion into injection port. Needle length is important.
	18740-80190	Liner with Jennings cup, no glass wool, 800 μ L volume. Use for general purpose applications, high and low MW compounds. Reduces inlet discrimination.
	18740-60840	Liner with Jennings cup, glass wool, and column packing, 800 μ L volume. For dirty samples, traps non-volatiles and particulates well. For high and low MW compounds. Not recommended for use with EPC.



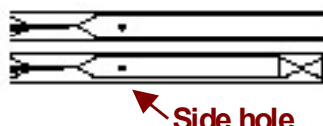
Splitless Injection Overview

For Trace Level Analysis

- Use split/splitless injection port in the splitless mode (split vent closed).
- The dilute sample is injected, the sample is volatilized, and majority of analytes condense on column.
- Later, the split vent is opened and residual solvent is vented.
- Timing, carrier and split vent flows, and oven temperature program are important.
- Sample has longer residence time in the heated inlet giving more opportunity to vaporize high boiling sample components compared to split injection.



Splitless Injection Liners

Liner	Part No.	Comments
	5181-3316	Single taper, deactivated, 900 μ L volume. Taper isolates sample from metal seal, reducing breakdown of compounds that are active with metals. For trace samples, general application.
	5062-3587	Single taper, deactivated, with glass wool, 900 μ L volume. Glass wool aides volatilization and protects column. For trace (dirty) samples.
	5181-3315	Double taper, deactivated, 800 μ L volume. Taper on inlet reduces chance for backflash into carrier gas lines. High efficiency liner for trace, active samples.
 Side hole	G1544-80730 G1544-80700	Direct connect liners, single and dual taper, deactivated. Capillary column press fits into liner end, eliminating sample exposure to inlet. Ultimate protection for trace, active samples. Side hole permits use with EPC.



Liner Maintenance

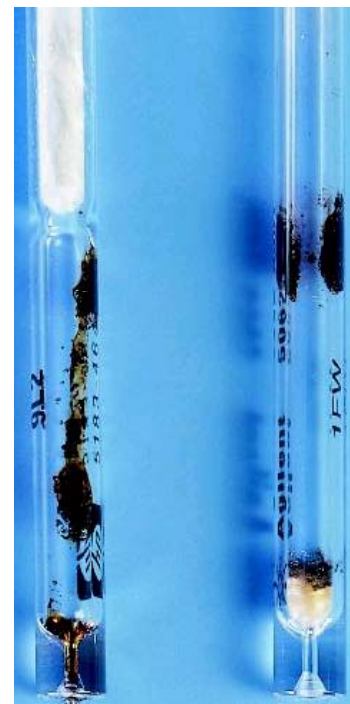
Liners become contaminated with use, collecting non-volatiles, salts, excess reagents, etc., or become damaged/cracked.

Should inspect and replace liners often.

Handle with gloves and forceps.

Insert into or remove liners only from cool injection ports.

Replacing with a new liner is recommended, to ensure reproducibility



Liner Maintenance (contd.)

Advantages of cleaning liners yourself:

- Reduced cost

Disadvantages:

- Time-consuming
- Liners with special features (glass wool, cup, etc.) are difficult to clean
- Reproducibility of liner is compromised
- Removing or inserting glass wool may create significant active sites in glass



Best advice -- keep a supply of new liners on-hand!



Liner Troubleshooting

Many chromatographic problems are blamed on the column. Often, a dirty liner is the culprit.

Symptoms include:

- ✉ Poor peak shape
- ✉ Irregular baselines
- ✉ Poor resolution
- ✉ Poor response



Do liner types really matter?

They do, especially for active compounds like:

- ✉ phenols
- ✉ organic acids
- ✉ pesticides
- ✉ amines
- ✉ drugs of abuse, etc.

Phenols, for example....in a separation of EPA method 8270 compounds



Liner Conclusions

Agilent inlet liners can be used with a broad range of samples and analytes and chromatographic response depends heavily on liner type.

To choose a liner, first consider:

- Type of inlet in your GC
- Concentration and type of sample
 - high conc. - use Split
 - trace analytes - use Splitless or PTV
 - broad range - use Split/Splitless or PTV – general purpose
 - heat-sensitive and high boiling point compounds - use On-Column or PTV



Liner Conclusions (contd.)

Next, consider

- Sample size, solvent, cleanliness, and potential analyte activity - helps to choose special liner features (cup, wool, taper, etc.) and liner volume that are necessary for your application.

Finally, optimize chromatographic conditions for the best separation.

Remember to check liner condition often and replace when necessary to minimize downtime.

Good chromatography starts with the inlet. Choose the correct liner for your application.



Liner Conclusions (contd.)

Flip Top for Split/Splitless Injection Ports

- 30 sec liner change out
- No more hunting for that “funny looking” wrench!
- Saves fingers from getting burned
- Increases instrument up time



GC Column Advances

Last several years have seen modest advances in GC column technology

- Column bleed
- Custom columns
- Customized stationary phases
- Application specific columns
- High temperature phases
- Dependability and reproducibility
- New line of Ultra Inert (UI) columns

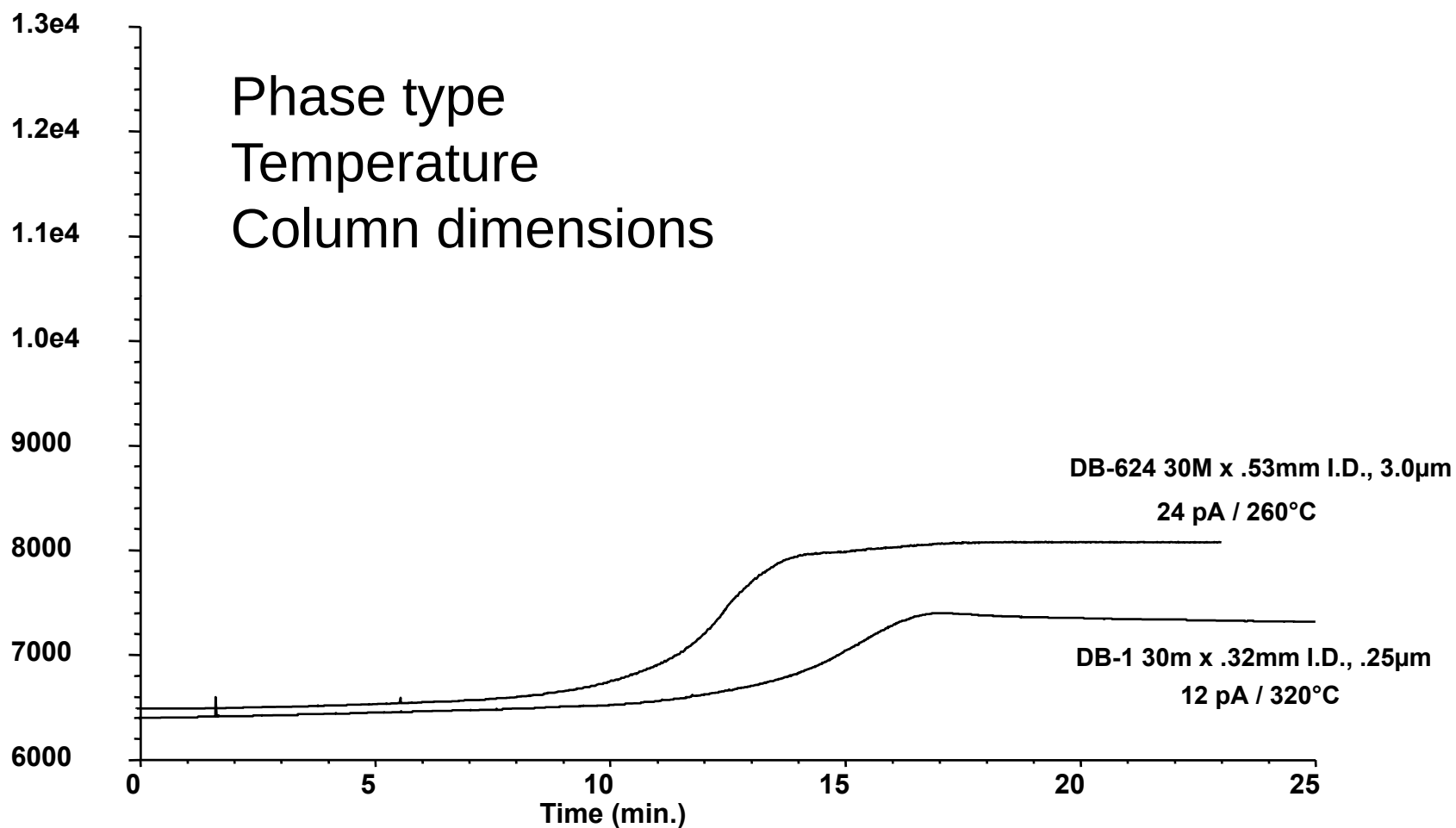


What Is Normal Column Bleed?

Normal background signal generated by the elution of normal degradation products of the column stationary phase



Column Bleed Is Influenced By:



What Is A Bleed Problem?

An abnormal elevated baseline at high temperature

IT IS NOT

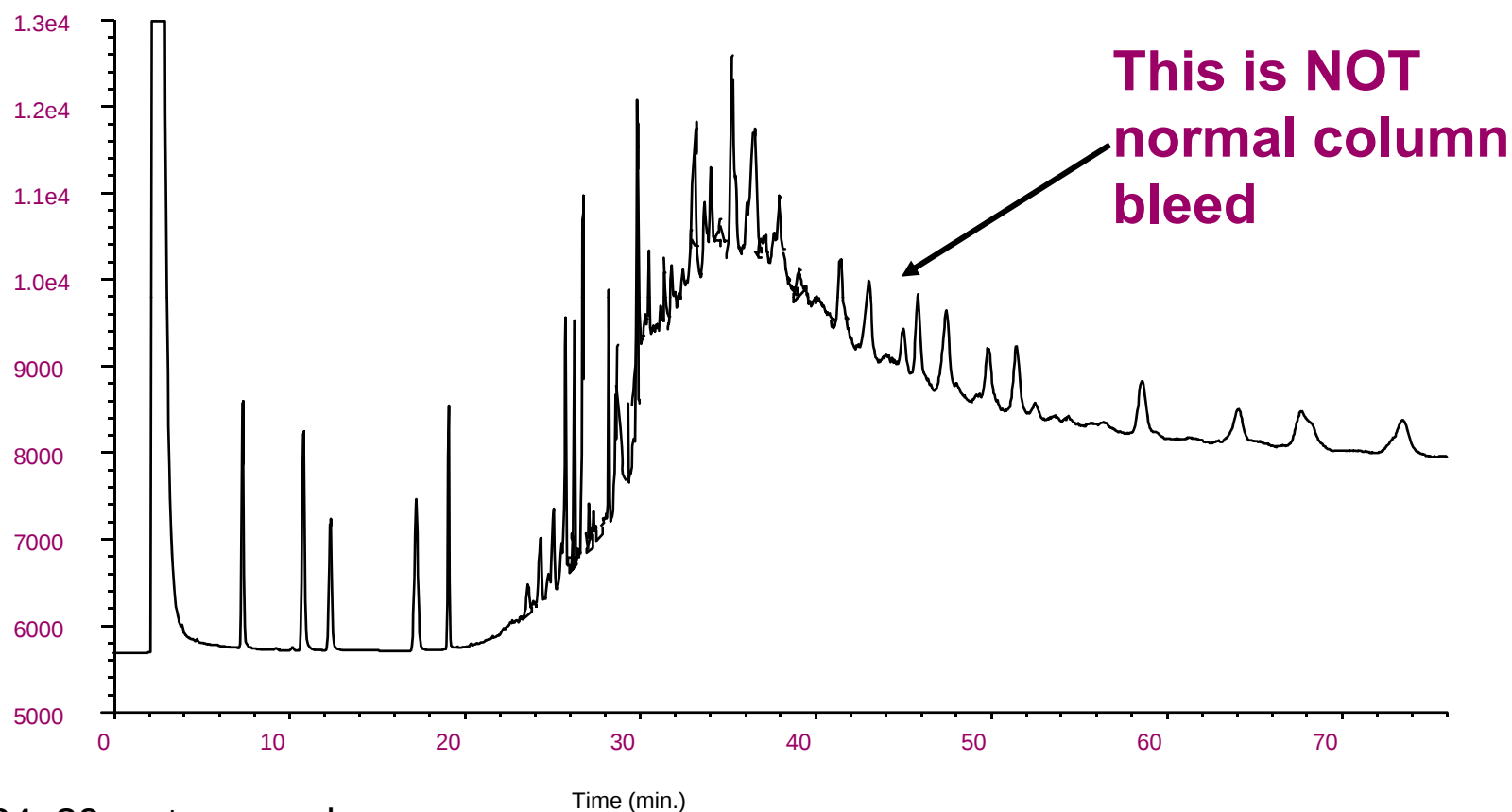
A high baseline at low temperature

Wandering or drifting baseline at any temperature

Discrete peaks



Example Of Column Contamination



DB-624, 30 meter megabore

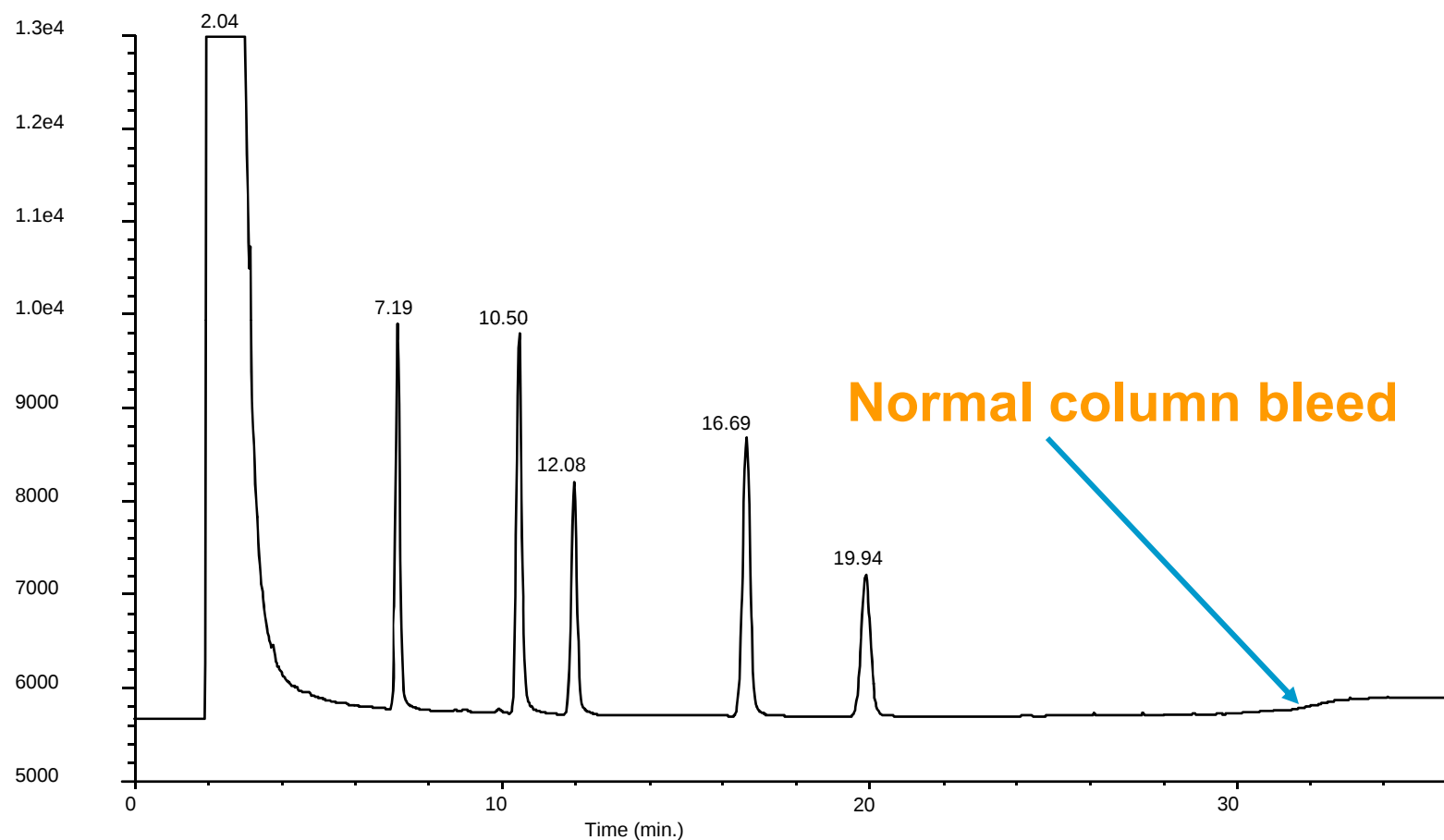
Temperature program // 35°C, hold 1.50 min // 30°/min to 65°C,
hold 15 min // 20°/min to 260°, hold 50 min



Agilent Technologies

Agilent Restricted

Same Column After Inlet And Column Maintenance



*Temperature program // 35°C, hold for 1.50 min //
30°/min to 65°C, hold 15 min // 20°/min to 260°C for 5 min



Agilent Technologies

Agilent Restricted

What Should You Look For In a Quality GC Column?

How demanding are the test probes?

Do the probes used in the QC test emulate your analyses?

When looking at a “replacement” column for existing methods on a different column brand, does the manufacture’s test adequately test the stationary phase functionality (selectivity, film thickness)

What temperature is the test performed? Isothermal or programmed?



What Should You Look For In a Quality GC Column?

If bleed is measured/stated, how and at what temperature was it measured?

If comparing two columns, remember “don’t mix apples and oranges” when drawing conclusions.

Everything looks the same “from the cheap seats”, so take a close up look at small pictures in brochures and advertisements



QC Test Mixture Components

Compounds

Hydrocarbons

Alcohols

FAME's, PAH's

Acids

Bases

Purpose

Efficiency

Retention

Activity

Retention

Acidic Character

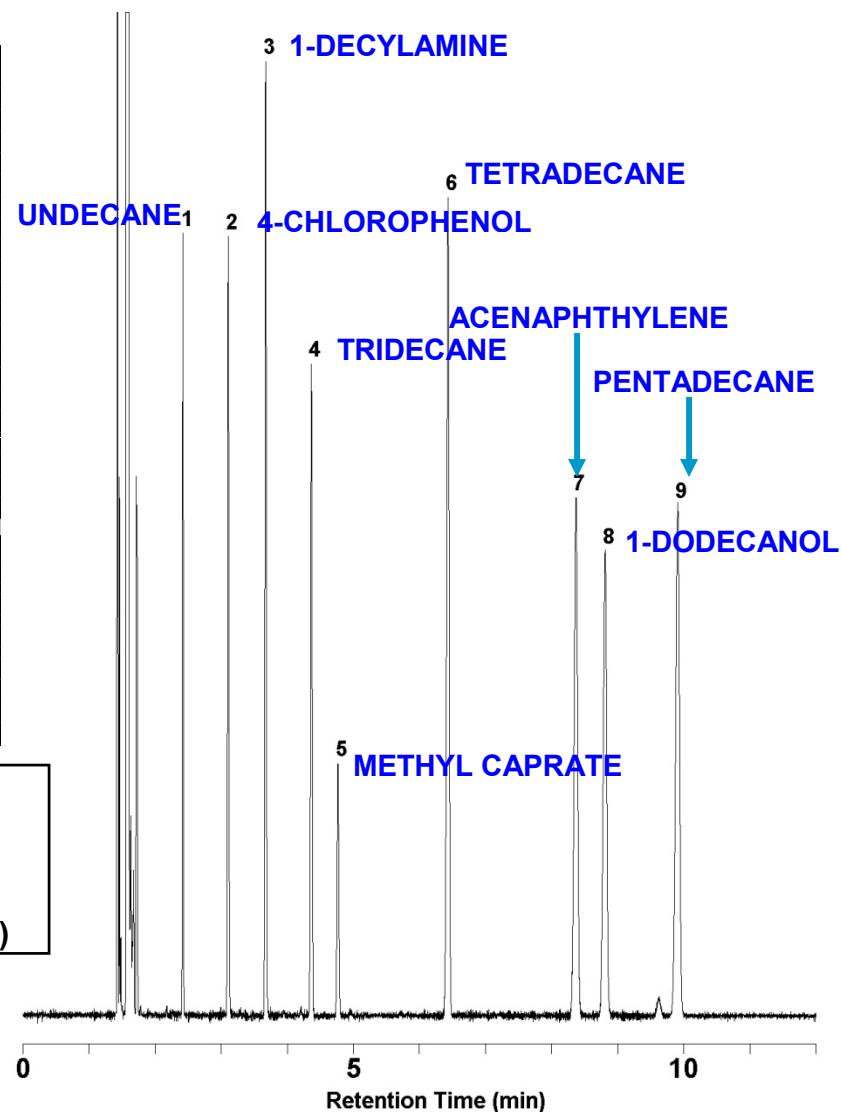
Basic Character



Comprehensive Testing--Demanding Criteria

Performance Results		Compound Identification			
Theoretical Plates/Meter:		Retent. Time	Part. Ratio	1/2-Width	
PENTADECANE	4326	1. UNDECANE	2.419	.69	.014
		2. 4-CHLOROPHENOL	3.105	1.17	.021
		3. 1-DECYLAMINE	3.672	1.57	.023
		4. TRIDECAENE	4.364	2.06	.027
		5. METHYL CAPRATE	4.766	2.34	.030
		6. TETRADECANE	6.433	3.50	.041
Retention Index:		7. ACENAPHTHYLENE	8.370	4.86	.056
METHYL CAPRATE	1324.1	8. 1-DODECANOL	8.811	5.17	.058
ACENAPHTHYLENE	1462.0	9. PENTADECANE	9.910	5.94	.065
1-DODECANOL	1473.7				
Peak Height Ratio:					
1-DODECANOL, TETRADECANE	0.57				
1-DECYLAMINE, TRIDECAENE	1.47				
4-CHLOROPHENOL, TRIDECAENE	1.19				

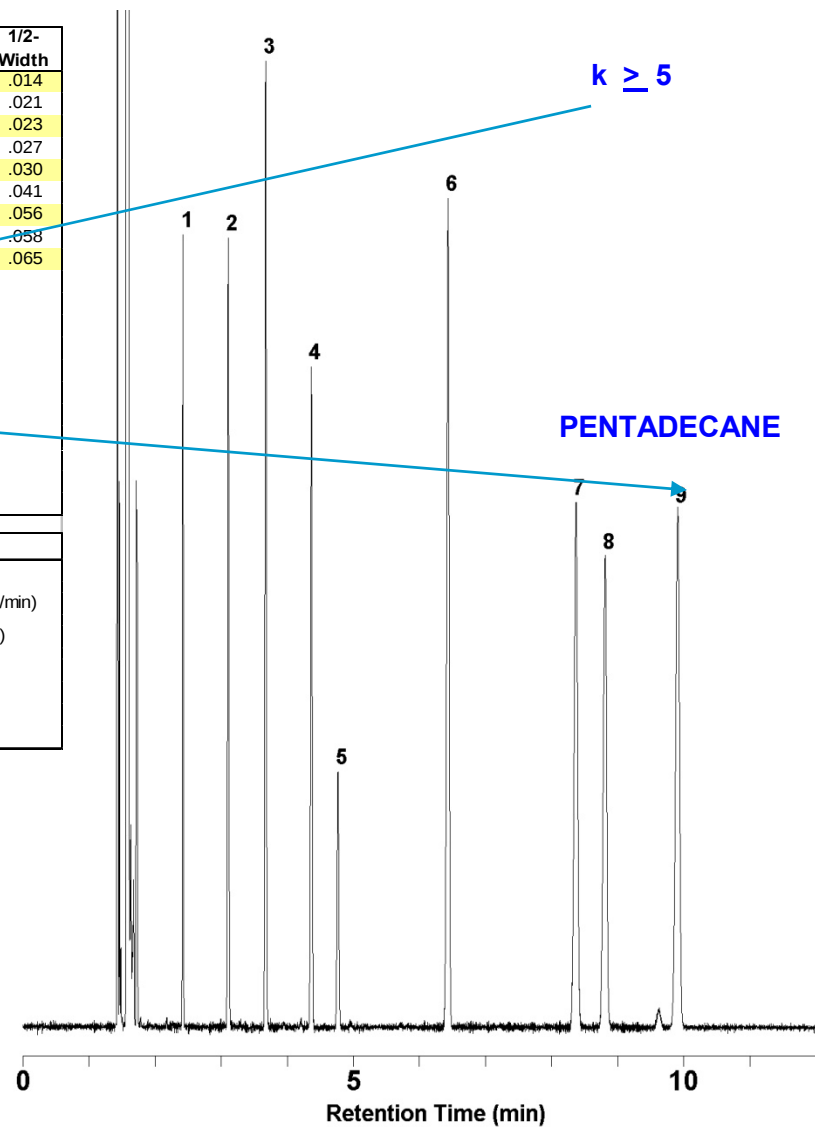
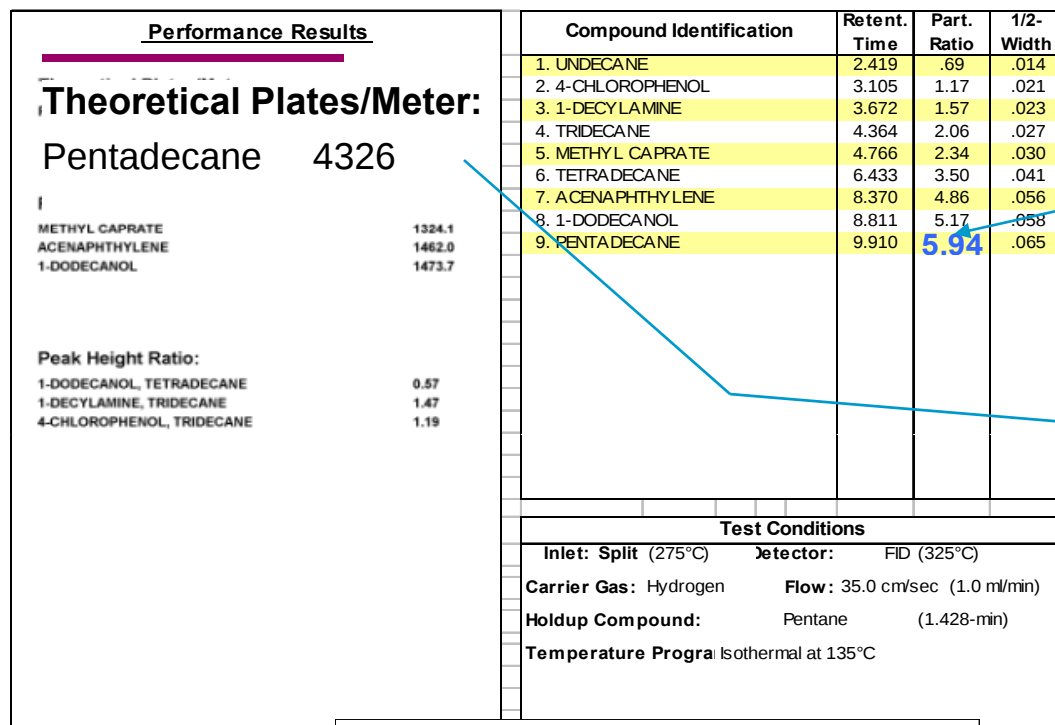
Catalog: 19091S-433	Stationary Phase: HP-5MS
Serial: US2469142H	Description: 30 m x 0.25 mm x 0.25 µm
Temperature Limits: -60°C to 325°C (350°C Pgm)	



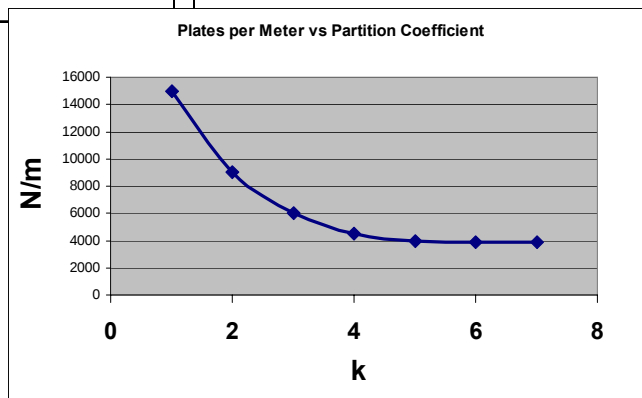
Agilent Technologies

Agilent Restricted

Exacting Pass Fail Criteria--Efficiency (N/m)



Measured
Isothermally
Probe $k \geq 5$

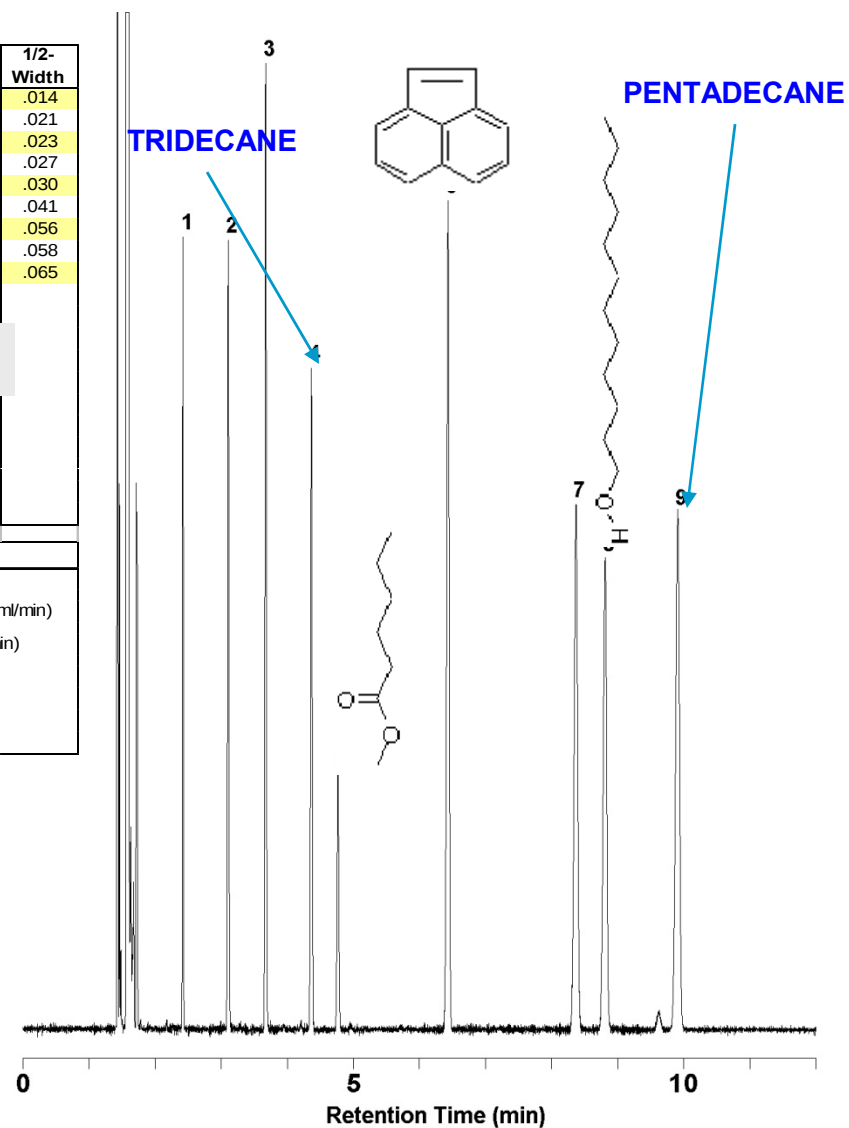


Agilent Technologies

Agilent Restricted

Demanding Criteria--Selectivity (RI)

Performance Results		Compound Identification			
Theoretical Plates/Meter:		Retent. Time	Part. Ratio	1/2-Width	
PENTADECANE	4326	1. UNDECANE	2.419	.69	.014
		2. 4-CHLOROPHENOL	3.105	1.17	.021
		3. 1-DECYLAMINE	3.672	1.57	.023
		4. TRIDECANE	4.364	2.06	.027
		5. METHYL CAPRATE	4.766	2.34	.030
		6. TETRADECANE	6.433	3.50	.041
Retention Index:		7. ACENAPHTHYLENE	8.370	4.86	.056
METHYL CAPRATE	1324.1	8. 1-DODECANOL	8.811	5.17	.058
ACENAPHTHYLENE	1462.0	9. PENTADECANE	9.910	5.94	.065
1-DODECANOL	1473.7				
3 probes, 3 functional groups					
Pe					
1-DODECANOL, TETRADECANE	0.57				
1-DECYLAMINE, TRIDECANE	1.47				
4-CHLOROPHENOL, TRIDECANE	1.19				
Retention Index:					
Methyl Caprate	1324.1				
Acenaphthylene	1462.0				
1-Dodecanol	1473.7				
Test Conditions					
Inlet: Split (275°C)	Detector: FID (325°C)				
Carrier Gas: Hydrogen	Flow: 35.0 cm/sec (1.0 ml/min)				
Holdup Compound: Pentane	(1.428-min)				
Temperature Program: Isothermal at 135°C					

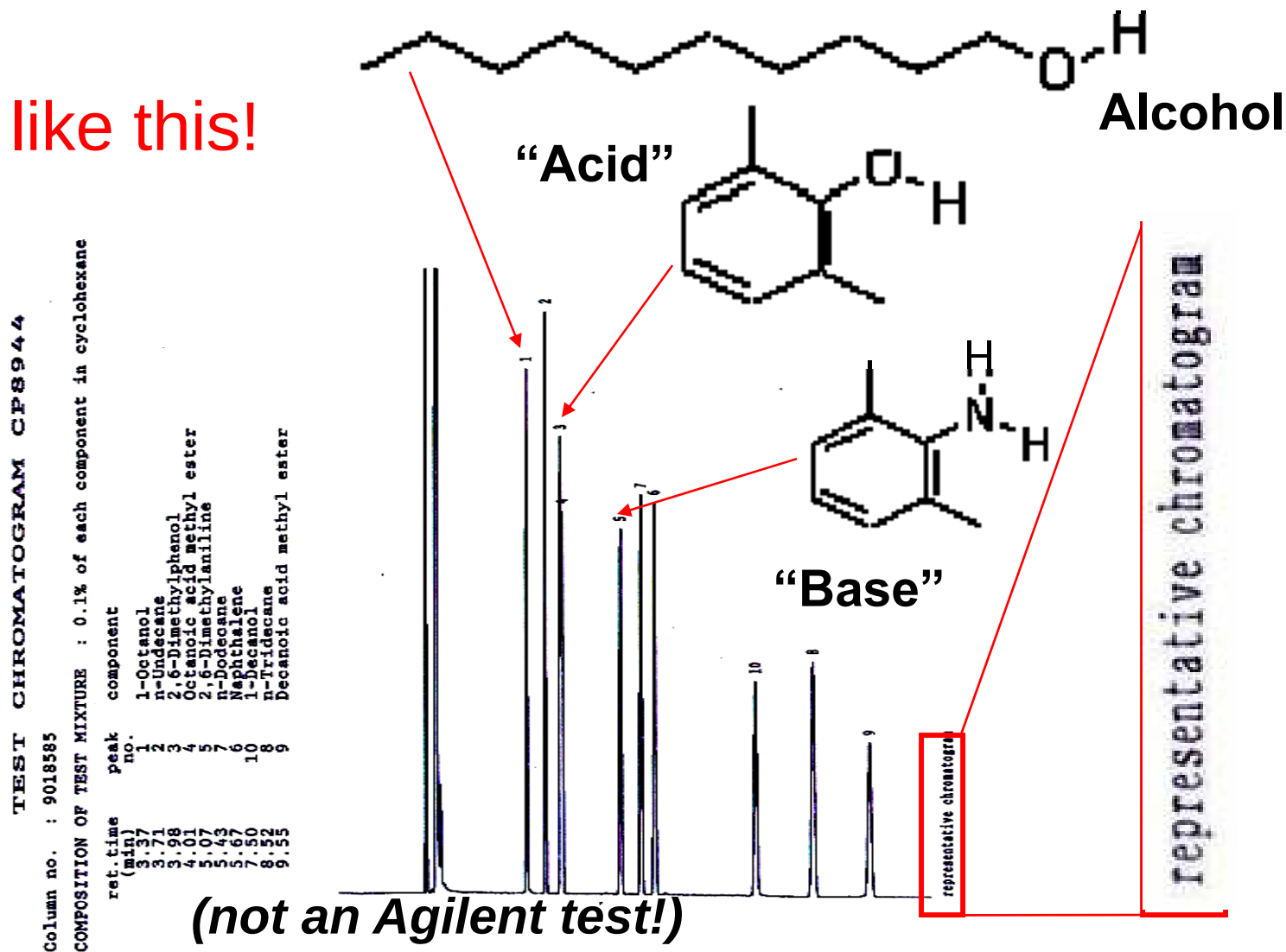


Agilent Technologies

Agilent Restricted

How is High GC column Inertness Assured?

Not like this!



Agilent Technologies

Agilent Restricted

How Agilent Assures High Inertness on the HP-5ms columns, with Every Test

Performance Results	
Theoretical Plates/Meter:	
PENTADECANE	3945
Retention Index:	
METHYL CAPRATE	1324.1
ACENAPHTHYLENE	1462.2
1-DODECANOL	1473.8
Peak Height Ratio:	
1-DODECANOL, TETRADECANE	0.54
1-DECYLAMINE, TRIDECAENE	1.43
4-CHLOROPHENOL, TRIDECAENE	1.12

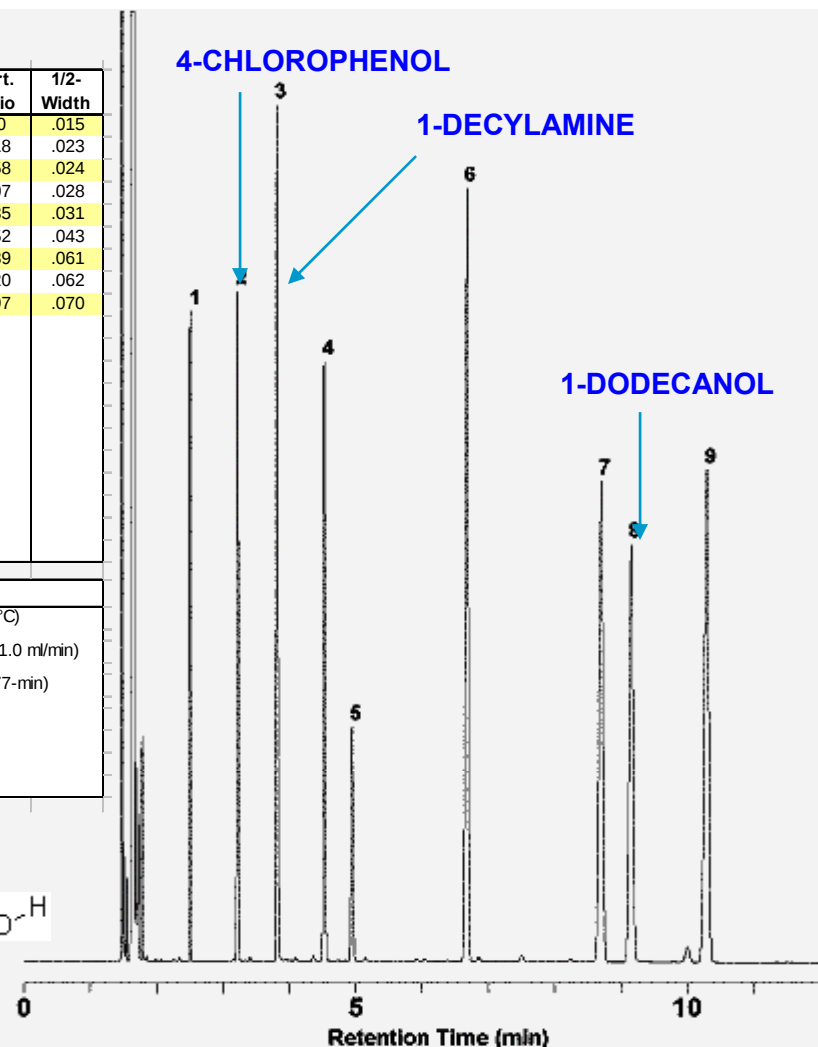
Compound Identification	Retent. Time	Part. Ratio	1/2-Width
1. UNDECANE	2.506	.70	.015
2. 4-CHLOROPHENOL	3.222	1.18	.023
3. 1-DECYLAMINE	3.813	1.58	.024
4. TRIDECAENE	4.528	2.07	.028
5. METHYL CAPRATE	4.946	2.35	.031
6. TETRADECANE	6.677	3.52	.043
7. ACENAPHTHYLENE	8.700	4.89	.061
8. 1-DODECANOL	9.155	5.20	.062
9. PENTADECANE	10.293	5.97	.070

Test Conditions	
Inlet: Split (275°C)	Detector: FID (325°C)
Carrier Gas: Hydrogen	Flow: 33.9 cm/sec (1.0 ml/min)
Holdup Compound: Pentane	(1.477-min)
Temperature Program: Isothermal at 135°C	

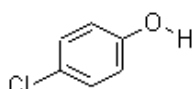
4-CHLOROPHENOL

1-DECYLAMINE

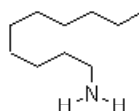
1-DODECANOL



Acid



Base



Alcohol

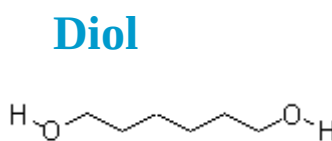
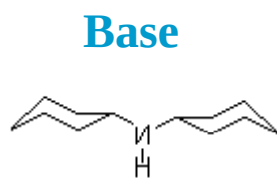
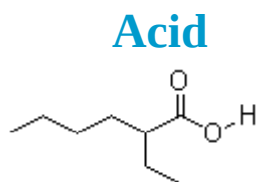
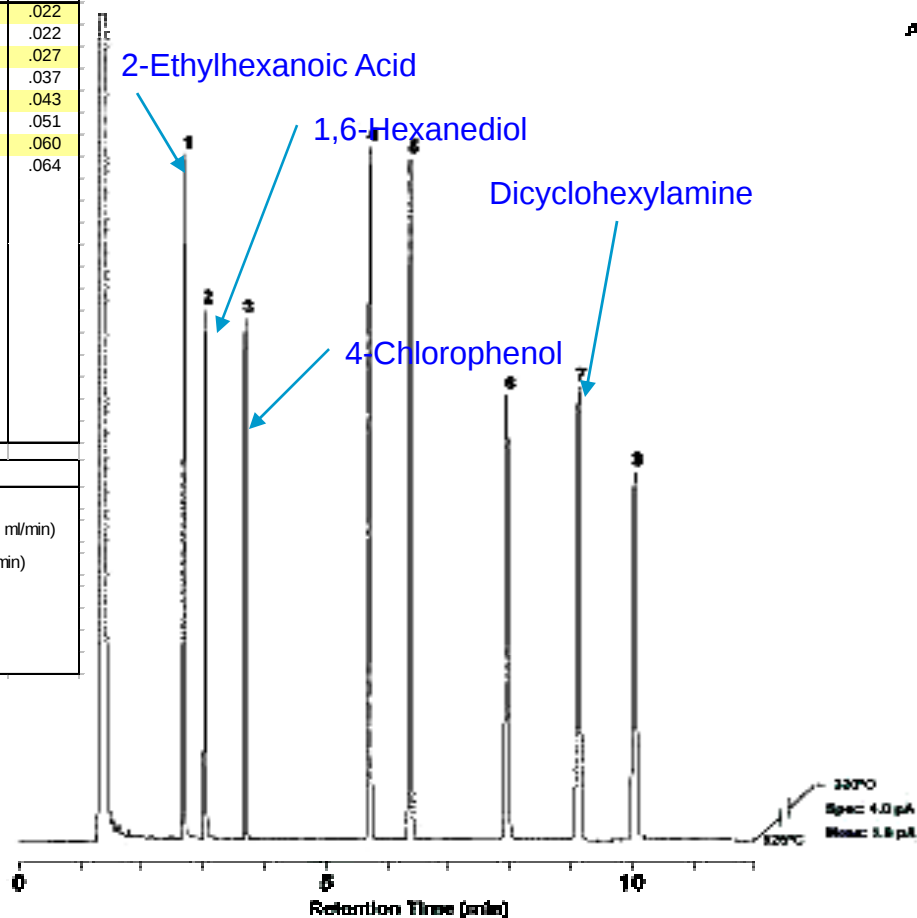


Agilent Technologies

Agilent Restricted

How Agilent Assures High Inertness on the DB-5ms columns, with Every Test

Performance Results		Compound Identification			
Theoretical Plates/Meter:			Retent. Time	Part. Ratio	1/2-Width
TETRADECANE	4165	1. 2-ETHYLHEXANOIC ACID	2.655	1.05	.022
		2. 1,6-HEXANEDIOL	2.990	1.31	.022
		3. 4-CHLOROPHENOL	3.635	1.80	.027
		4. TRIDECA NE	5.627	3.34	.037
UTE%:	89.9%	5. 1-METHYLNAPHTHALENE	6.283	3.84	.043
		6. 1-UNDECANOL	7.820	5.03	.051
		7. TETRA DECA NE	8.963	5.91	.060
		8. DICYCLOHEXYLAMINE	9.877	6.62	.064
Retention Index:					
1-METHYLNAPHTHALENE	1323.7				
1-UNDECANOL	1370.7				



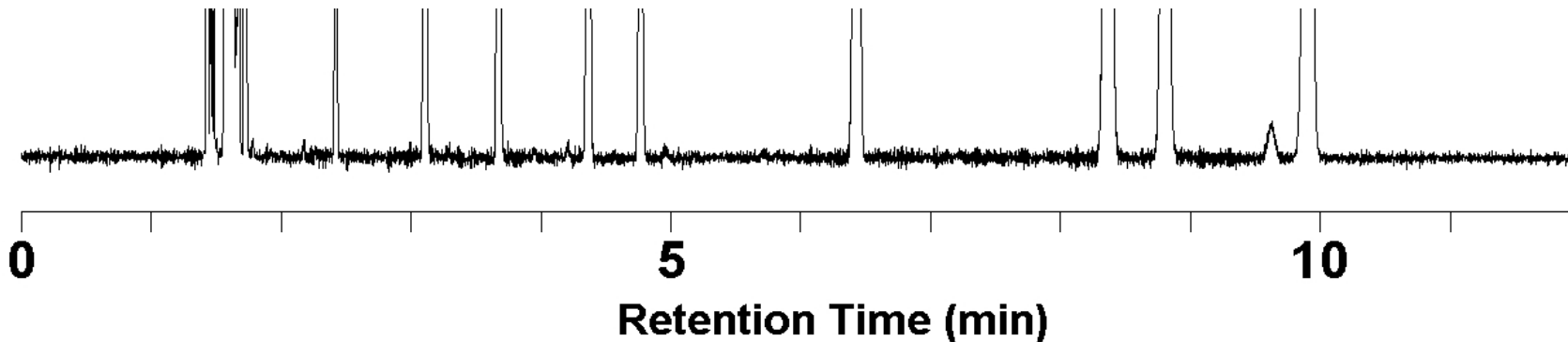
Agilent Technologies

Agilent Restricted

Demanding Criteria--Inerttness: A Closer Look

[illegible]

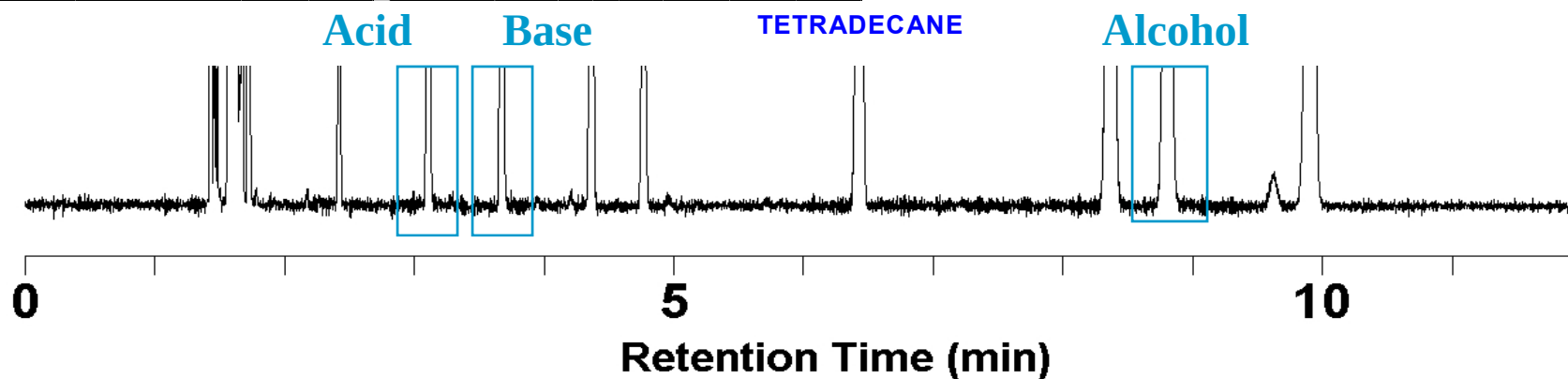
Can you tell which the active probes are?



Demanding Criteria--Inertness: A Closer Look

Performance Results		Compound Identification			
Theoretical Plates/Meter:		Retent. Time	Part. Ratio	1/2-Width	
PENTADECANE	4326	1. UNDECANE	2.419	.69	.014
		2. 4-CHLOROPHENOL	3.105	1.17	.021
		3. 1-DECYLAMINE	3.672	1.57	.023
		4. TRIDECANE	4.364	2.06	.027
		5. METHYL CAPRATE	4.766	2.34	.030
		6. TETRADECANE	6.433	3.50	.041
Retention Index:		7. ACENAPHTHYLENE	8.370	4.86	.056
METHYL CAPRATE	1324.1	8. 1-DODECANOL	8.811	5.17	.058
ACENAPHTHYLENE	1462.0	9. PENTADECANE	9.910	5.94	.065
1-DODECANOL	1473.7				
Peak Height Ratio:					
1-DODECANOL, TETRADECANE	0.57				
1-DECYLAMINE, TRIDECANE	1.47				
4-CHLOROPHENOL, TRIDECANE	1.19				
Peak Height Ratio		Test Conditions			
1-Dodecanol Tetradecan	0.57	Inlet: Split (275°C)	Detector: FID (325°C)		
1-Decylamine Tridecane	1.47	Carrier Gas: Hydrogen	Flow: 35.0 cm/sec (1.0 ml/min)		
4-Chlorophenol Tridecane	1.19	Holdup Compound: Pentane (1.428-min)	Temperature Program: Isothermal at 135°C		

Can't ID by peak tailing!



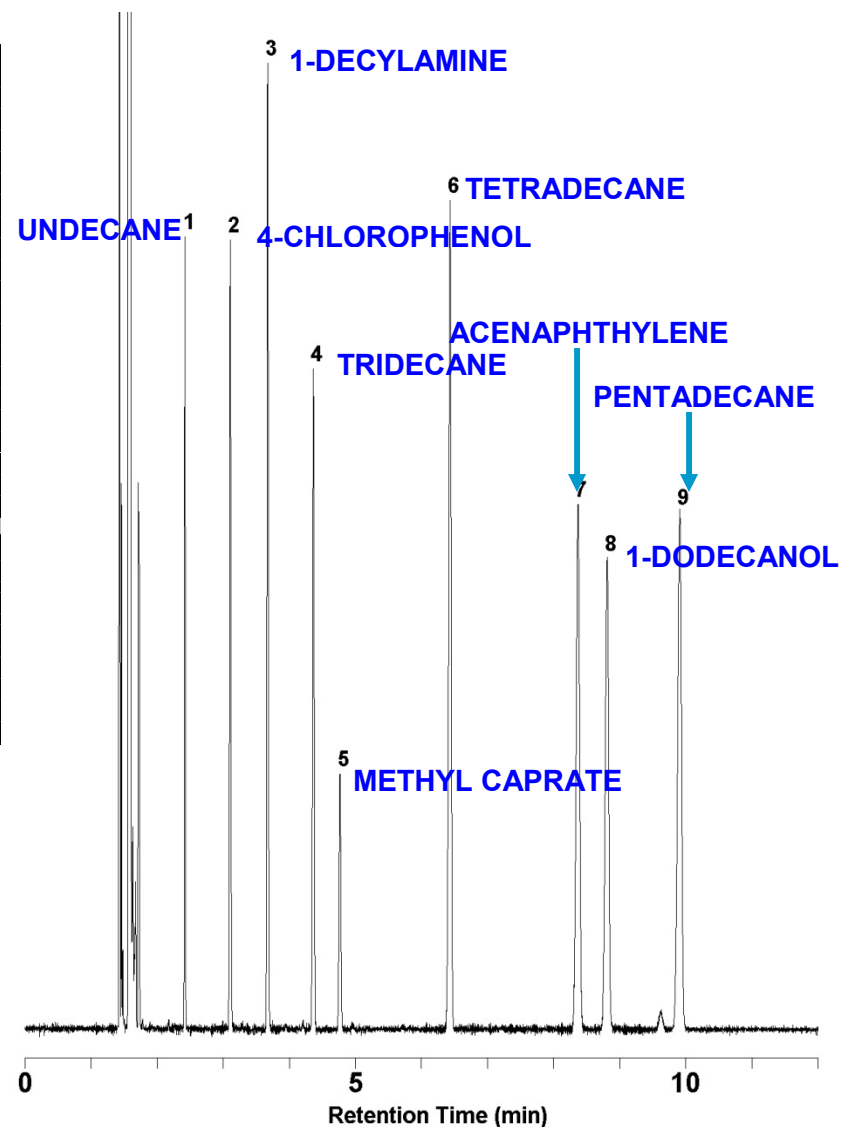
Agilent Technologies

Agilent Restricted

Demanding Criteria--Looks Flawless!

Performance Results		Compound Identification	Retent. Time	Part. Ratio	1/2-Width
Theoretical Plates/Meter: PENTADECANE	4326	1. UNDECANE	2.419	.69	.014
		2. 4-CHLOROPHENOL	3.105	1.17	.021
		3. 1-DECYLAMINE	3.672	1.57	.023
		4. TRIDECANE	4.364	2.06	.027
Retention Index: METHYL CAPRATE ACENAPHTHYLENE 1-DODECANOL	1324.1 1462.0 1473.7	5. METHYL CAPRATE	4.766	2.34	.030
		6. TETRADECANE	6.433	3.50	.041
		7. ACENAPHTHYLENE	8.370	4.86	.056
		8. 1-DODECANOL	8.811	5.17	.058
Peak Height Ratio: 1-DODECANOL, TETRADECANE 1-DECYLAMINE, TRIDECANE 4-CHLOROPHENOL, TRIDECANE	0.57 1.47 1.19	9. PENTADECANE	9.910	5.94	.065

Test Conditions	
Inlet: Split (275°C)	Detector: FID (325°C)
Carrier Gas: Hydrogen	Flow: 35.0 cm/sec (1.0 ml/min)
Holdup Compound: Pentane	(1.428-min)
Temperature Program: Isothermal at 135°C	



Agilent Technologies

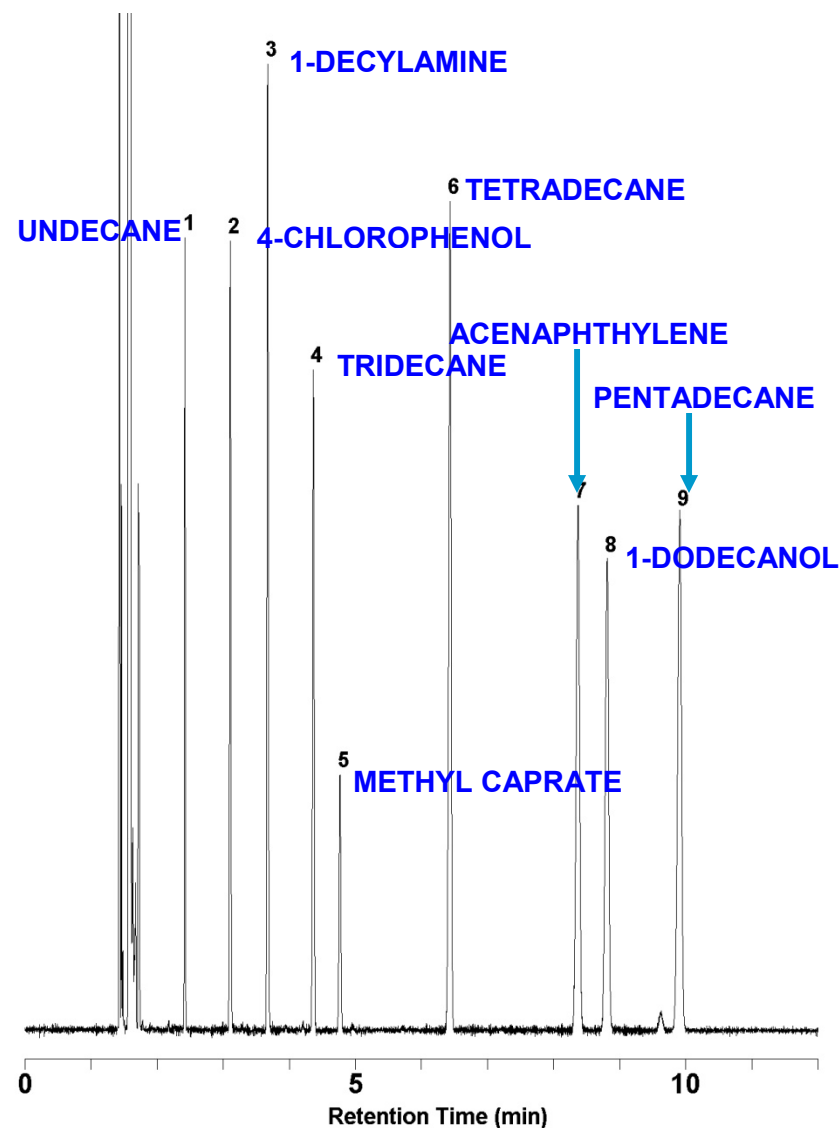
Agilent Restricted

Demanding Criteria--And It **WILL NOT** Ship

Failed

High Bleed High: 14.4 Low: 8.9

Bleed Spec is 4 pA, this one was 5.5 pA

[illegible]

Catalog: 19091S-433
Serial: US2469141H

Stationary Phase: HP-5MS
Description: 30m x 0.250mm x 0.25µm
Temperature Limits: -60°C to 325°C (350°C Pgm)

Performance Results

Theoretical Plates/Meter:
PENTADECANE 4243

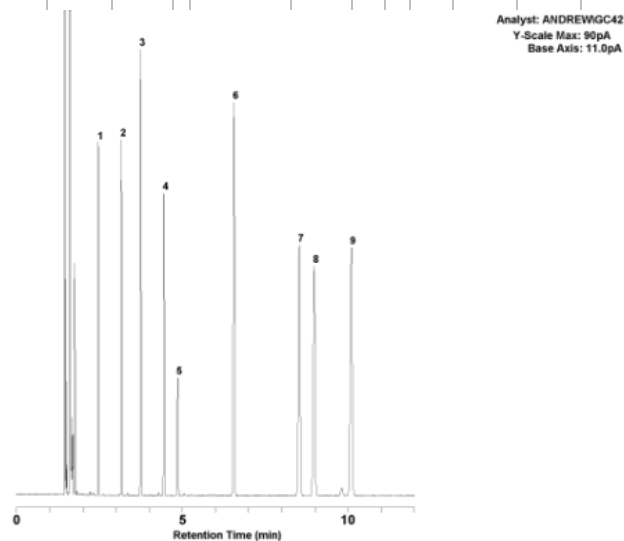
Retention Index:
METHYL CAPRATE 1324.1
ACENAPHTHYLENE 1462.0
1-DODECANOL 1473.7

Peak Height Ratio:
1-DODECANOL, TETRADECANE 0.58
1-DECYLAMINE, TRIDECANE 1.47
4-CHLOROPHENOL, TRIDECANE 1.17

Compound Identification	Retent. Time	Part. Ratio	1/2-Width
1. UNDECANE	2.467	.69	.015
2. 4-CHLOROPHENOL	3.166	1.17	.023
3. 1-DECYLAMINE	3.744	1.57	.024
4. TRIDECANE	4.451	2.06	.028
5. METHYL CAPRATE	4.861	2.34	.031
6. TETRADECANE	6.558	3.50	.042
7. ACENAPHTHYLENE	8.530	4.86	.060
8. 1-DODECANOL	8.980	5.17	.058
9. PENTADECANE	10.099	5.94	.067

Test Conditions

Inlet: Split (275°C) Detector: FID (325°C)
Carrier Gas: Hydrogen Flow: 34.3 cm/sec (1.0 ml/min)
Holdup Compound: Pentane (1.456-min)
Temperature Program: Isothermal at 135°C



Catalog: 19091S-433
Serial: US2469142H

Stationary Phase: HP-5MS
Description: 30m x 0.250mm x 0.25µm
Temperature Limits: -60°C to 325°C (350°C Pgm)

Performance Results

Theoretical Plates/Meter:
PENTADECANE 4326

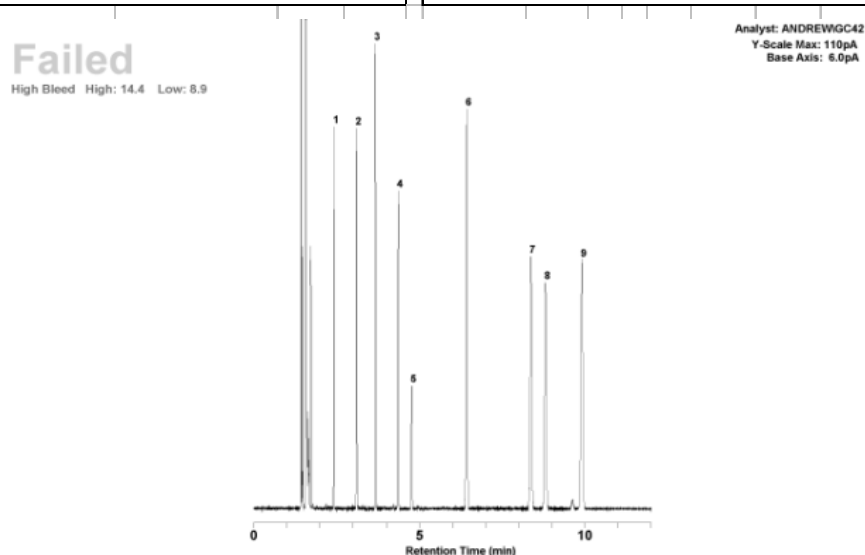
Retention Index:
METHYL CAPRATE 1324.1
ACENAPHTHYLENE 1462.0
1-DODECANOL 1473.7

Peak Height Ratio:
1-DODECANOL, TETRADECANE 0.57
1-DECYLAMINE, TRIDECANE 1.47
4-CHLOROPHENOL, TRIDECANE 1.19

Compound Identification	Retent. Time	Part. Ratio	1/2-Width
1. UNDECANE	2.419	.69	.014
2. 4-CHLOROPHENOL	3.105	1.17	.021
3. 1-DECYLAMINE	3.672	1.57	.023
4. TRIDECANE	4.364	2.06	.027
5. METHYL CAPRATE	4.766	2.34	.030
6. TETRADECANE	6.433	3.50	.041
7. ACENAPHTHYLENE	8.370	4.86	.056
8. 1-DODECANOL	8.811	5.17	.058
9. PENTADECANE	9.910	5.94	.065

Test Conditions

Inlet: Split (275°C) Detector: FID (325°C)
Carrier Gas: Hydrogen Flow: 35.0 cm/sec (1.0 ml/min)
Holdup Compound: Pentane (1.428-min)
Temperature Program: Isothermal at 135°C



Agilent Technologies

Agilent Restricted

Column Manufacturing-Failed Columns



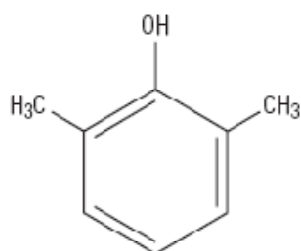
Agilent Technologies

Agilent Restricted

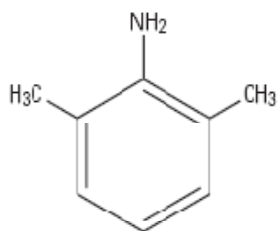
Even more demanding probes

Agilent's standard probes are already demanding, but what about even more demanding probes?

Chemical Structures

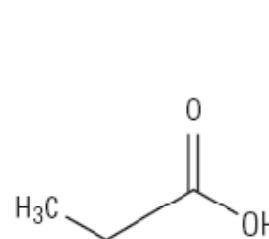


2, 6-Dimethylphenol

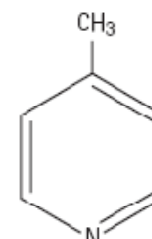


2, 6-Dimethylaniline

Weak probe molecules: The acidic and basic portions of these molecules are shielded by the two methyl groups on their phenyl rings, making them less probative.



1-Propionic acid



4-Picoline

Strong probe molecules: The probes in Agilent's Über One Test Probe Mixture are highly probative of the stationary phase and surface. Note, too, that the active end of each compound is available to interact with any active sites on the column.



Agilent Technologies

Agilent Restricted



New Agilent J&W Ultra Inert Capillary GC Columns

Raising the Bar for
CONSISTENT Column
Inertness Performance



Agilent Technologies

Agilent Restricted

How important is Column inertness to overall Flowpath Inertness?

GC Flowpath Surface Areas

	l (cm)	d (cm)	pi	Surface Area (cm ²)
Liner	7	0.2	3.142	4.4
Seal	0.4	0.8	3.142	1.0
Column	3000	0.025	3.142	235.6



High Inertness Advantages

Low Bleed is only **Half the Story**.

Inertness..."What goes in, comes out," or the **lack of activity**.

High Inertness enables greater GC and GC/MS sensitivity at trace levels, especially for active analytes.

Increase in S/N from:

- Improvement in peak shape for active compounds
- Increase in amount of analytes eluted from column

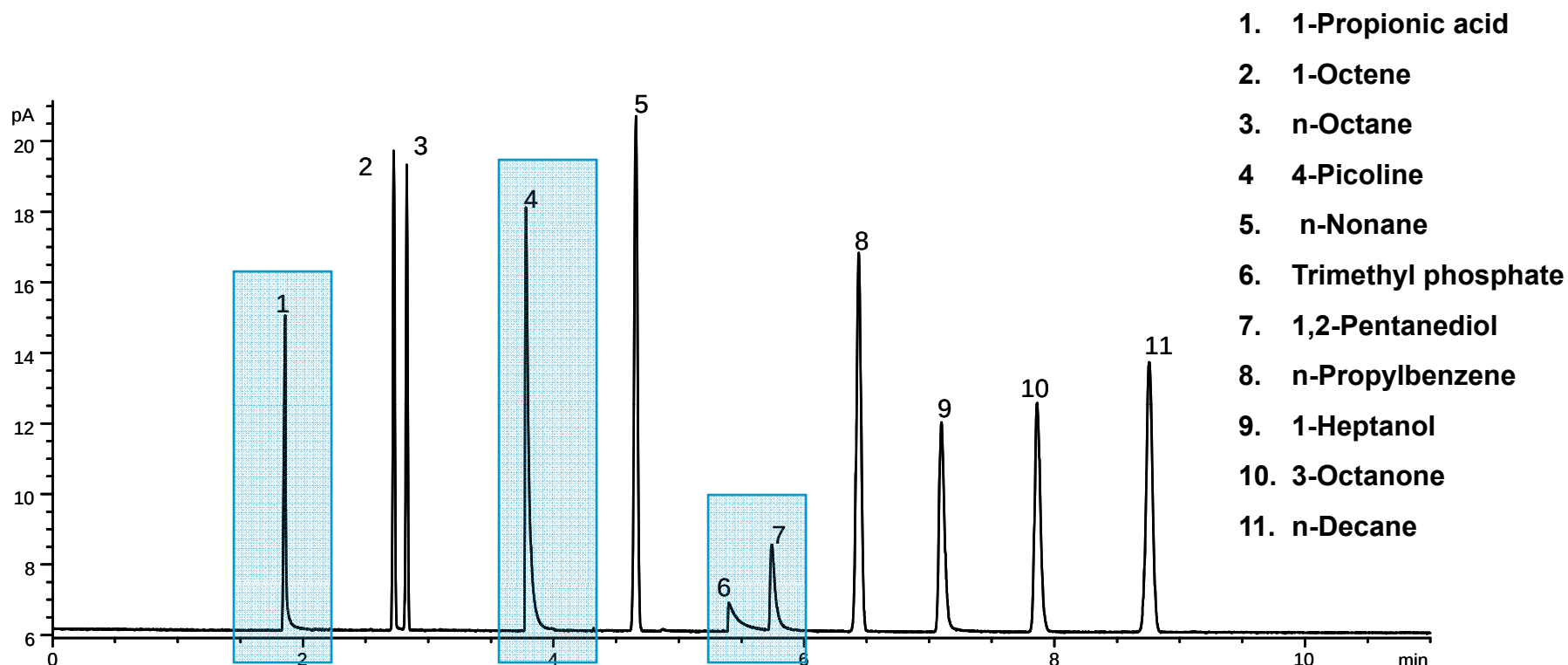


Ultra Inert Columns – Innovation from Agilent

- **Ultra inert columns are built on top of the existing Agilent J&W GC/MS columns**
 - Better column inertness and
 - Same low bleed profile you have come to expect
- **Ultra inert columns are the result of continuous process improvements through Six- σ and Lean Manufacturing programs at our Folsom manufacturing site.**
- **Originally introduced at ISCCE 2008:**
- **HP/DB-5ms column family in various formats**
- **HP/DB-1ms column family in various formats**
 - More phase families to follow



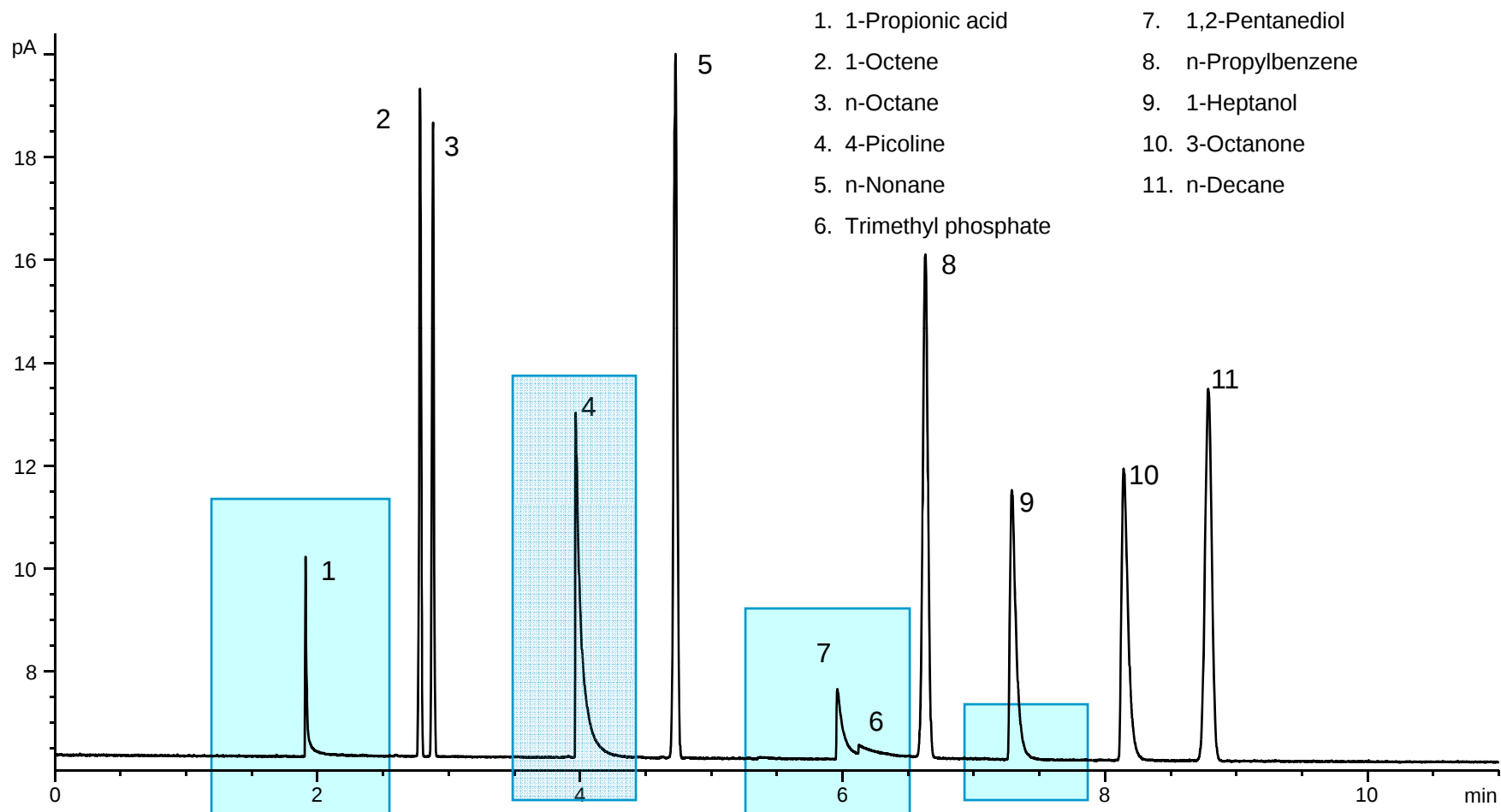
Über test results on a competitor's “good column”



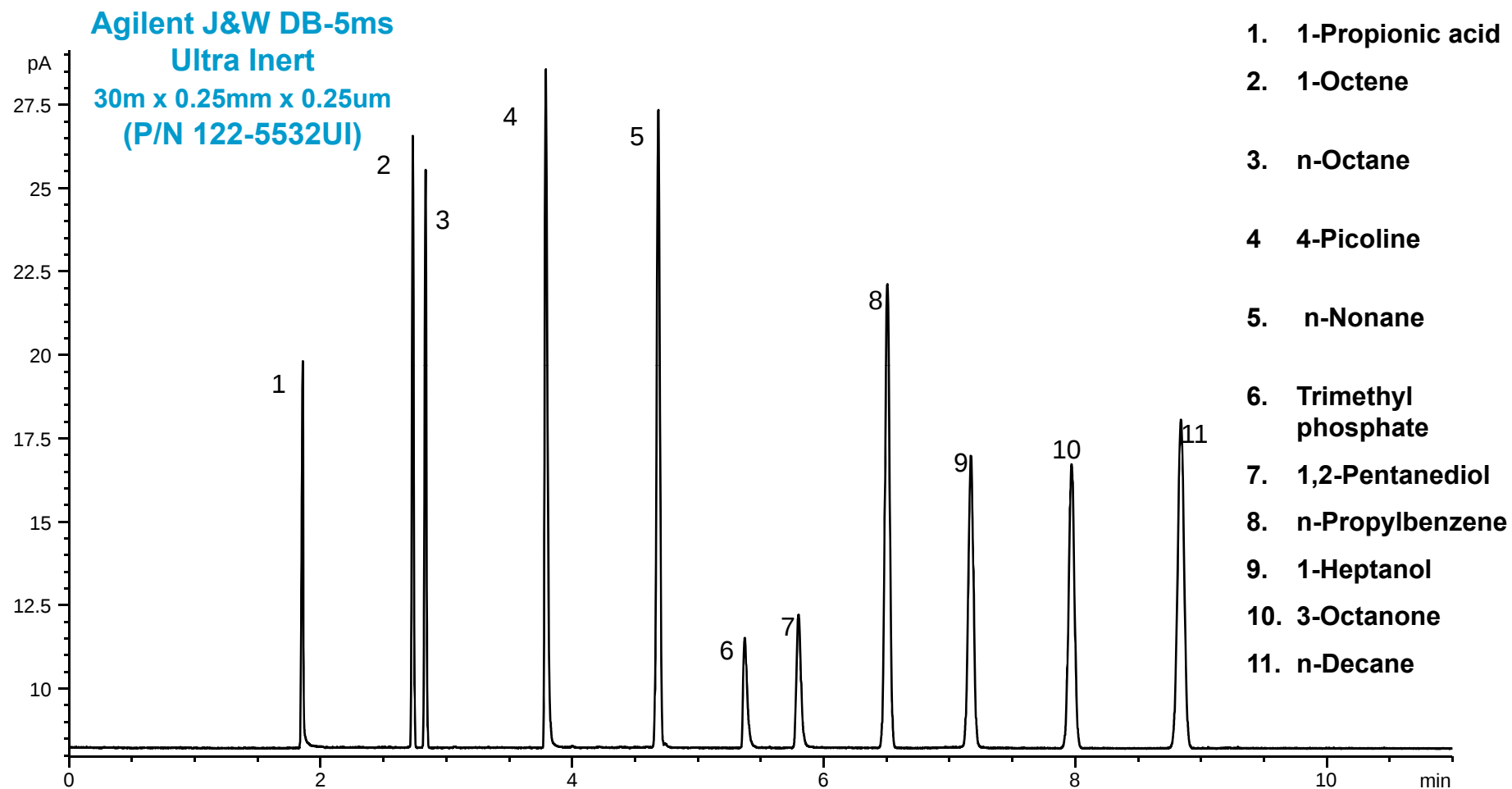
- Competitor column showed very poor performance when tested against the Über One test mix.
- Less demanding test probes masked the column activity for this column
 - The same column performed well with Grob-type test mix and DB-5ms test mix



Über test results on a competitors “good column”



Ultra Inert Test Mix on Agilent J&W DB-5ms Ultra Inert

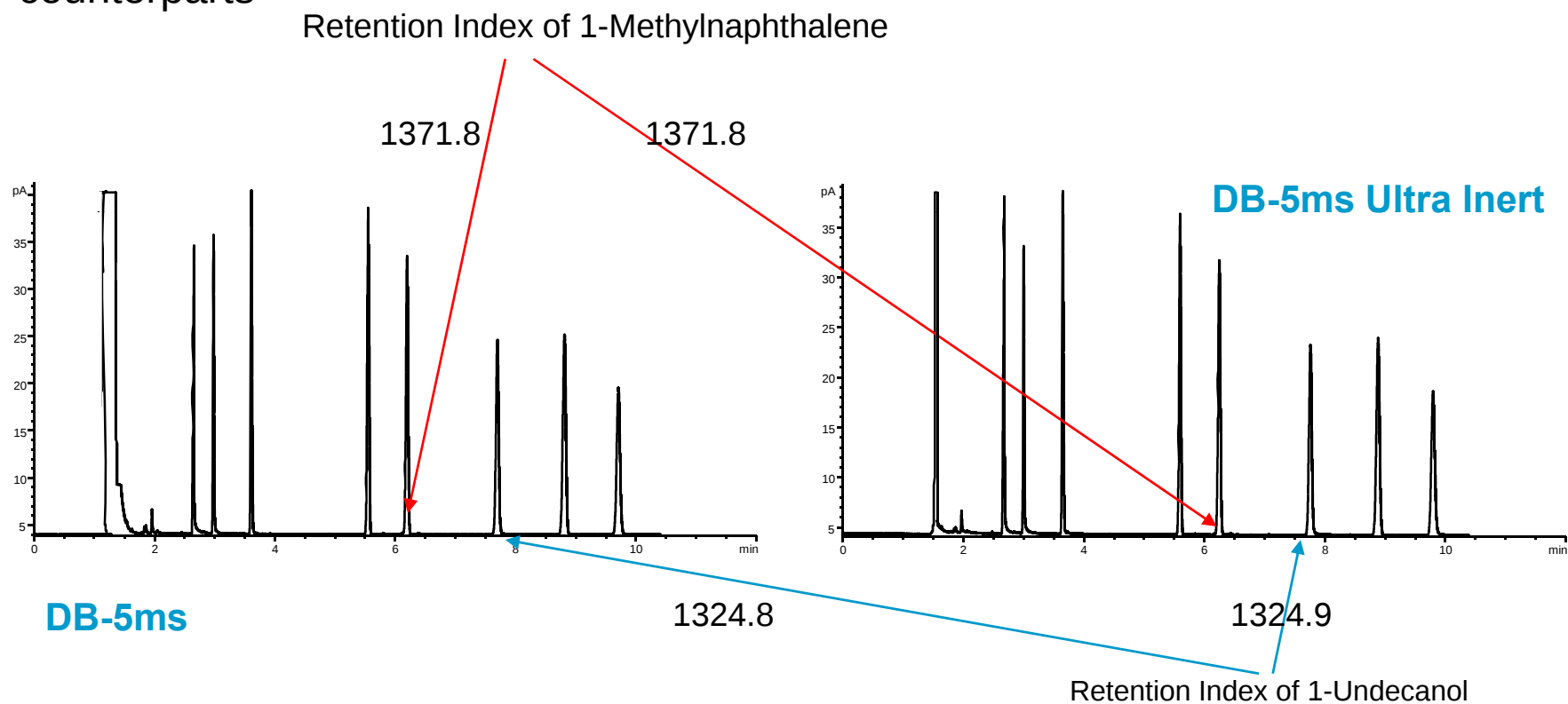


- Nice Peak Shapes for challenging active compounds
- Increased peak heights for accurate integration and detection of trace samples
- Routine analysis of demanding analytes now feasible



Same Selectivity – No Method Re-Development

- DB-5ms Ultra Inert columns have the same selectivity as their DB-5ms counterparts
- HP-5ms Ultra Inert columns have the same selectivity as their HP-5ms counterparts



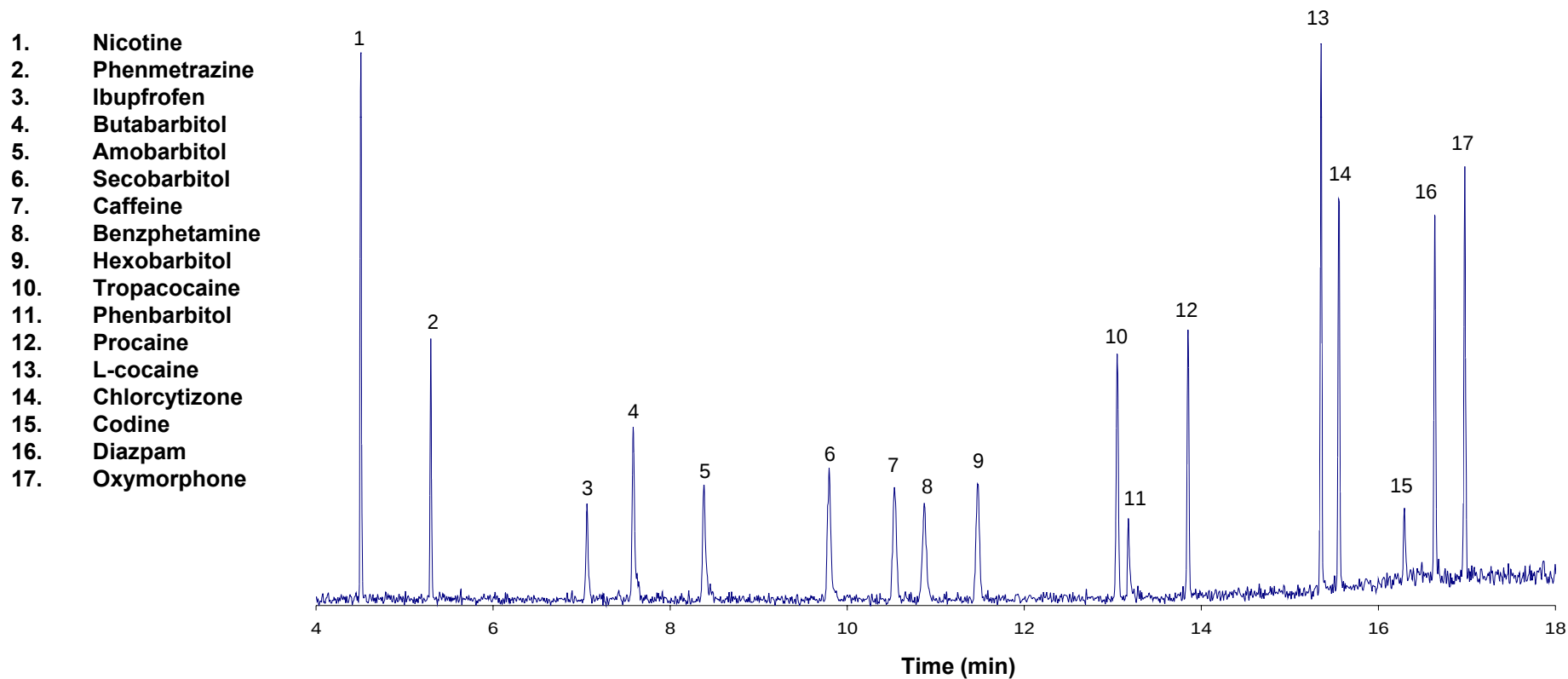
Application Examples

- Semi Volatile Analysis
- Brominated Fire Retardants
- Drugs of abuse
- Pesticides in Orange Oil
- PAHs
- PBDEs



Drugs of Abuse

Column: DB-5ms Ultra Inert 30 m x 0.25 mm x 0.25 μ m (Agilent part # 122-5532UI)
Carrier: Helium 43.8 cm/sec constant flow
Oven: 120% C (2min) 20 % C/min to 180 % C (6 min hold), 18 % C /min to 270% C (2min),
25 % C/min to 325% C (2 min)
Inlet: split 30:1, ~ 1 ng on column 250 %C, single taper liner (Agilent # 5181-3316)
MSD:



Agilent Technologies

Agilent Restricted

Benzodiazepines

Column: DB-5ms Ultra Inert

122-5532UI

30m x 0.25 mm x 0.25 µm

Carrier: Hydrogen, 53 cm/sec, constant flow

Flow 1.6 for 11 min

Program (mL/min): 1.6 to 2.4 at 60 mL/min² hold 2 min

Oven: 170 2.4 to 5.0 at 50 mL/min² hold 9 min
°C for 3.2 min

170-250 °C at 24.7 °C/min, hold 5.3 min

250-280 °C at 18.6 °C/min, hold 4.0 min

Injection: 280-325 °C at 50.0 °C/min, hold 4 min
Pulsed Splitless, 280 °C

20 psi pulse pressure for 0.38 min

50 mL/min purge at 0.40 min

Detector: Direct Connect liner G1544-80730
FID, 350 °C

Sample: 5-10 ng on column

1 Medazepam

2 Halazepam

3 Oxazepam

4 Lorazepam

5 Diazepam

6 Desalkyl

7 Nordazepam

8 Clobazam

9 Oxazolam

10 Temazepam

11 Flumitrazepam

12 Bromozepam

13 Prazam

14 Lormetazepam

15 Nitrazepam

16 Chlordiazepoxide

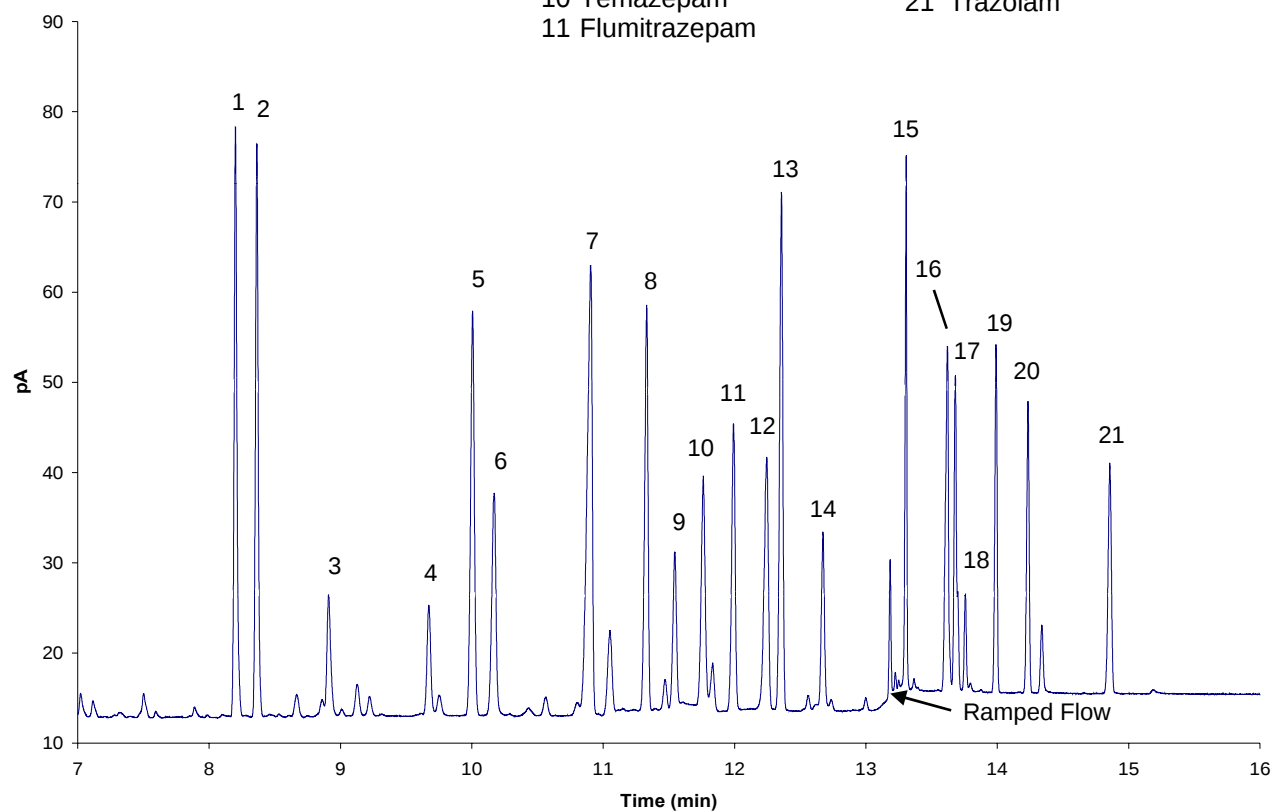
17 Clonazepam

18 Demoxepam

19 Estazolam

20 Alprazolam

21 Trazolam



Agilent Technologies

Agilent Restricted

Conclusion

- ✓ **Maximize consistency of sample stability by minimizing handling variance**
- ✓ **Develop methods using the correct inlet and auto-injector parts, including septa, syringes, ferrules, O-Rings and most importantly, inlet liners**
- ✓ **Follow a regular routine of inlet, column and detector preventative maintenance**
- ✓ **Keep an accurate instrument record with all settings documented and all maintenance logged for future reference**
- ✓ **Choose capillary GC columns based on performance and true quality testing**

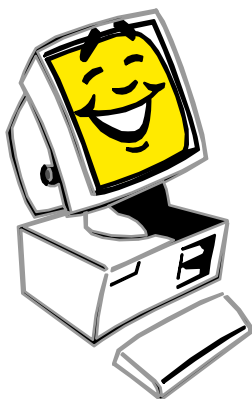


Agilent J&W Scientific Technical Support

800-227-9770 (phone: US & Canada)*

** Select option 4, then option 1.*

www.agilent.com/chem



Agilent Technologies

Agilent Restricted

References

1. US EPA Method 8270D Revision 4 February 2007 “Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)”
2. K. Grob Jr., G. Grob, and K. Grob “Comprehensive, Standardized Quality Test for Glass Capillary Columns,” *Journal of Chromatography A*, Vol. 156, Issue 1, 21 August 1978, Pages 1-20
3. Mitch Hastings, Allen K. Vickers, and Cameron George “Inertness Comparison of Sample of 5% Phenyl dimethyl polysiloxane Columns,” Poster Presentation, 54th Annual Pittsburg Conference, Orlando, FL March 2003
4. Jim Luong, Ronda Gras, and Walter Jennings “An Advanced Solventless Column Test for Capillary GC Columns,” *J. Sep. Sci.*, 2007, 30, 2480-2492
5. Mike Szelewski and Bill Wilson, “Improvements in the Agilent 6890/5973 GC/MSD System for Use with USEPA Method 8270,” Application Note 5988-3072EN, November 7, 2001

