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

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GC Column Specialist
May 11, 2010

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

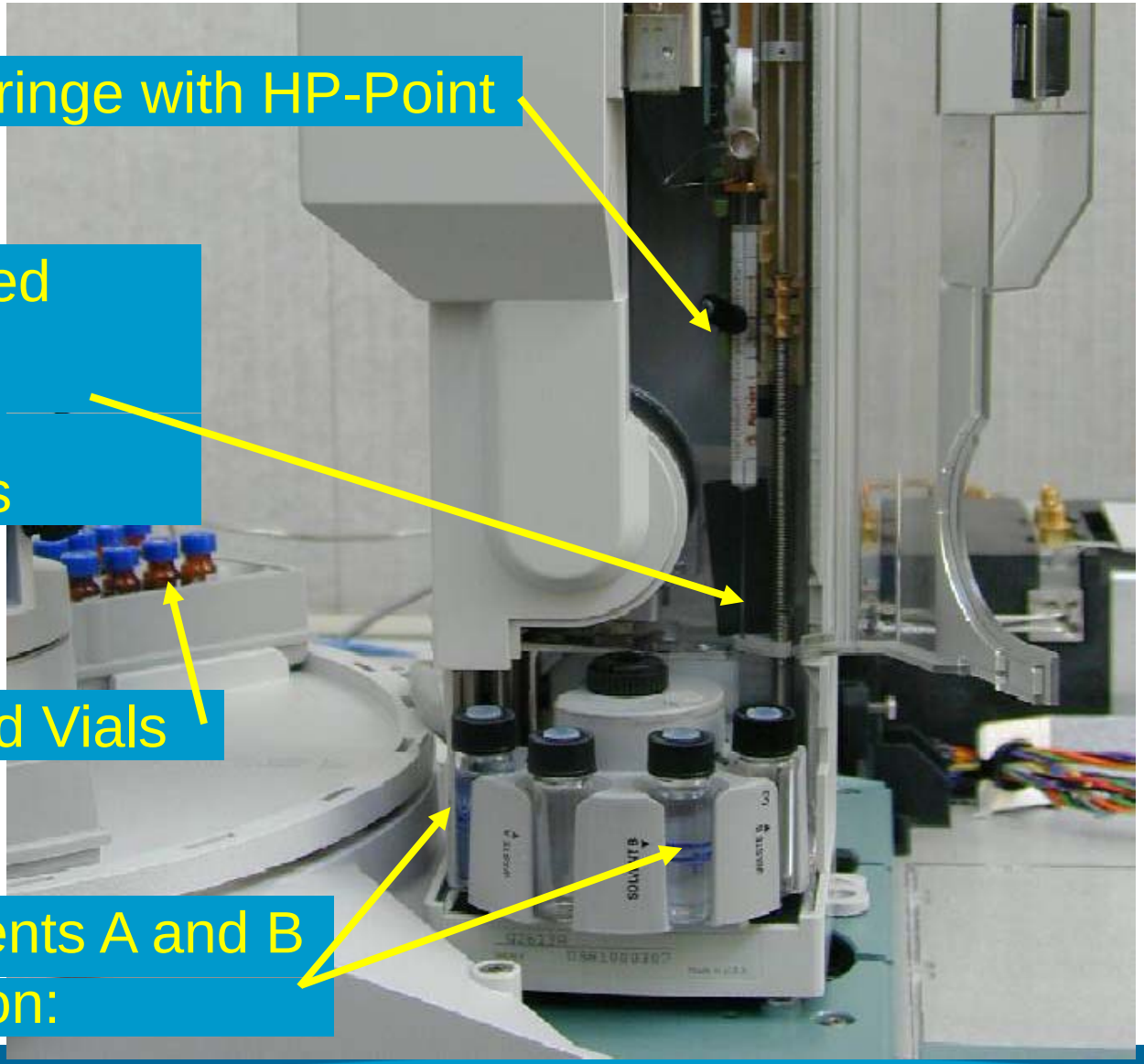
Sample washes before injection

Sample pumps prior to injection

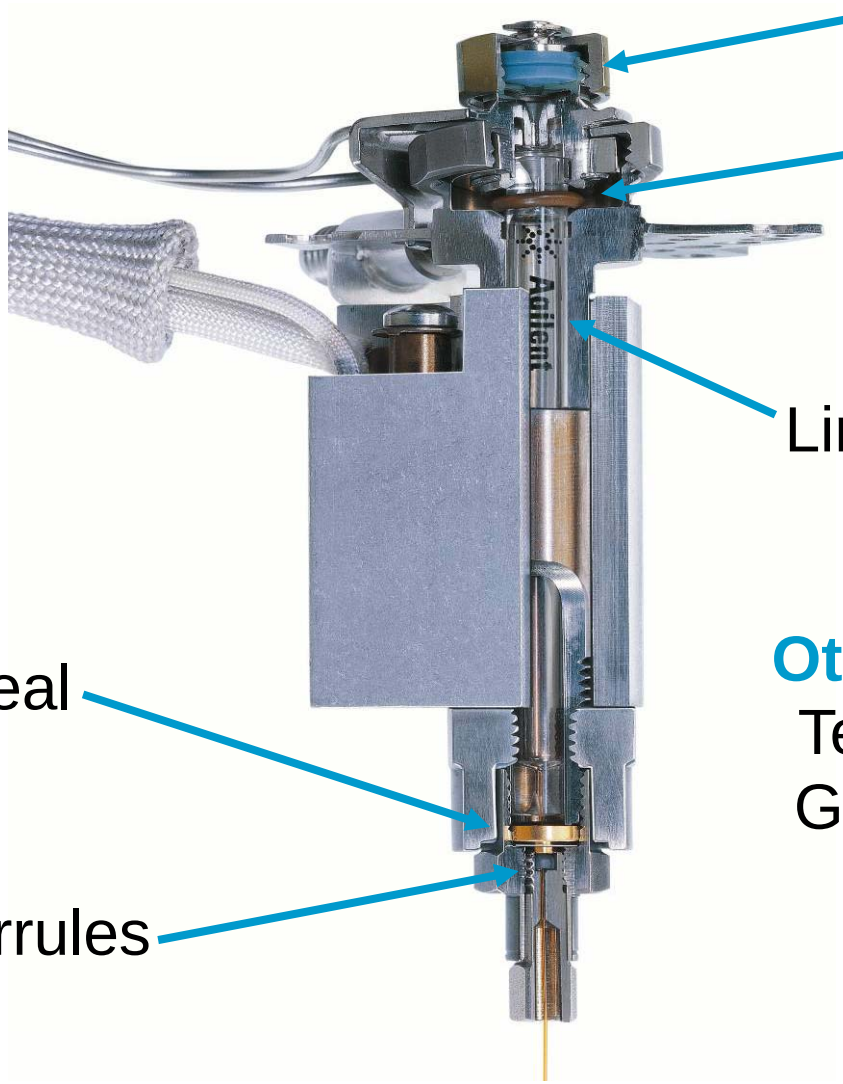
Plunger speed?

Viscosity delay?

Typical Auto-Injector Setup



Inlet Considerations



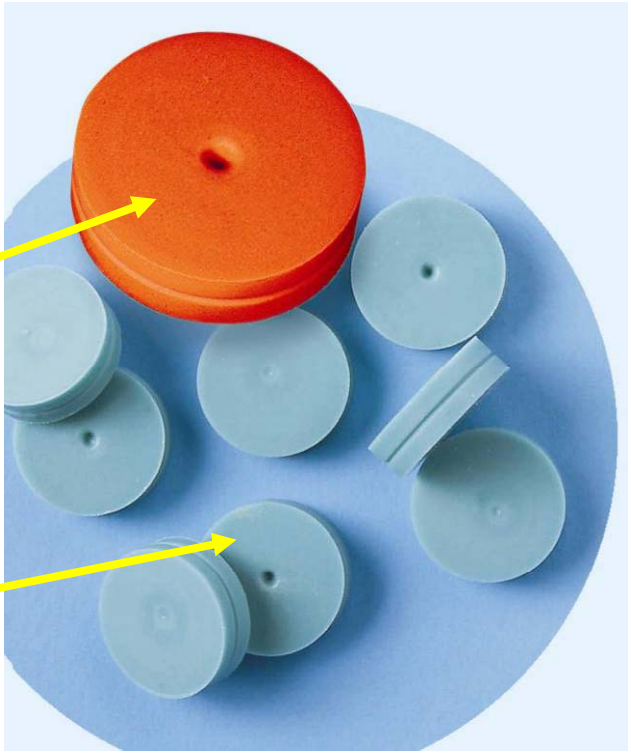
Other Considerations:
Temperature
Gas flow rates

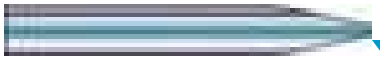
Preferred Inlet Septa

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

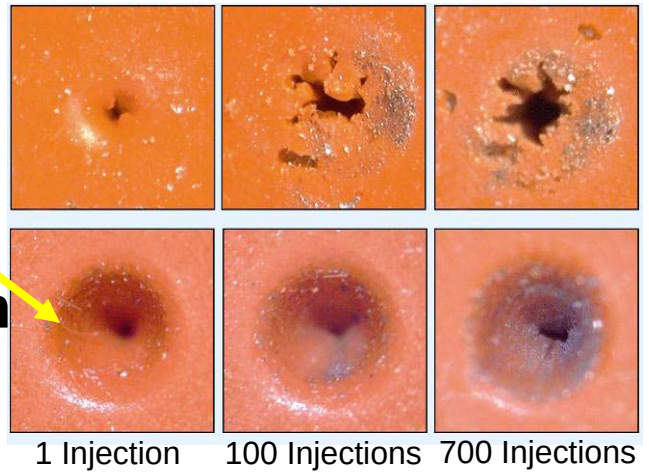
Use Advanced Green septa for inlet temperature up to 350°C

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





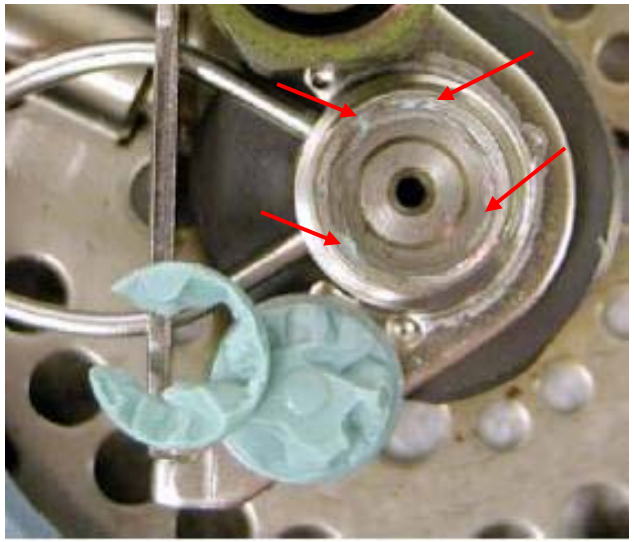
CenterGuide Septum



1 Injection 100 Injections 700 Injections

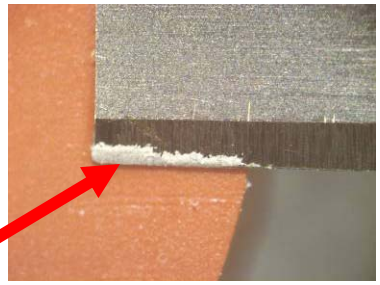
Tips to Maximize Septum Life, Minimize Septum Leaks

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

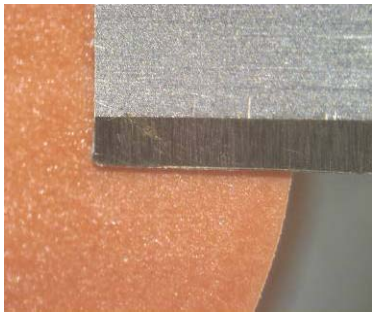


Their's

Talcum Powder!



Our's



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

Septa vs GC Column Costs

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

Typical cost of 1 GC Column, 30mx0.25mm 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**
- **Splitless**
- **On-Column**
- **Purge-Packed**
- **Programmable Temperature Vaporization (PTV)**

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**

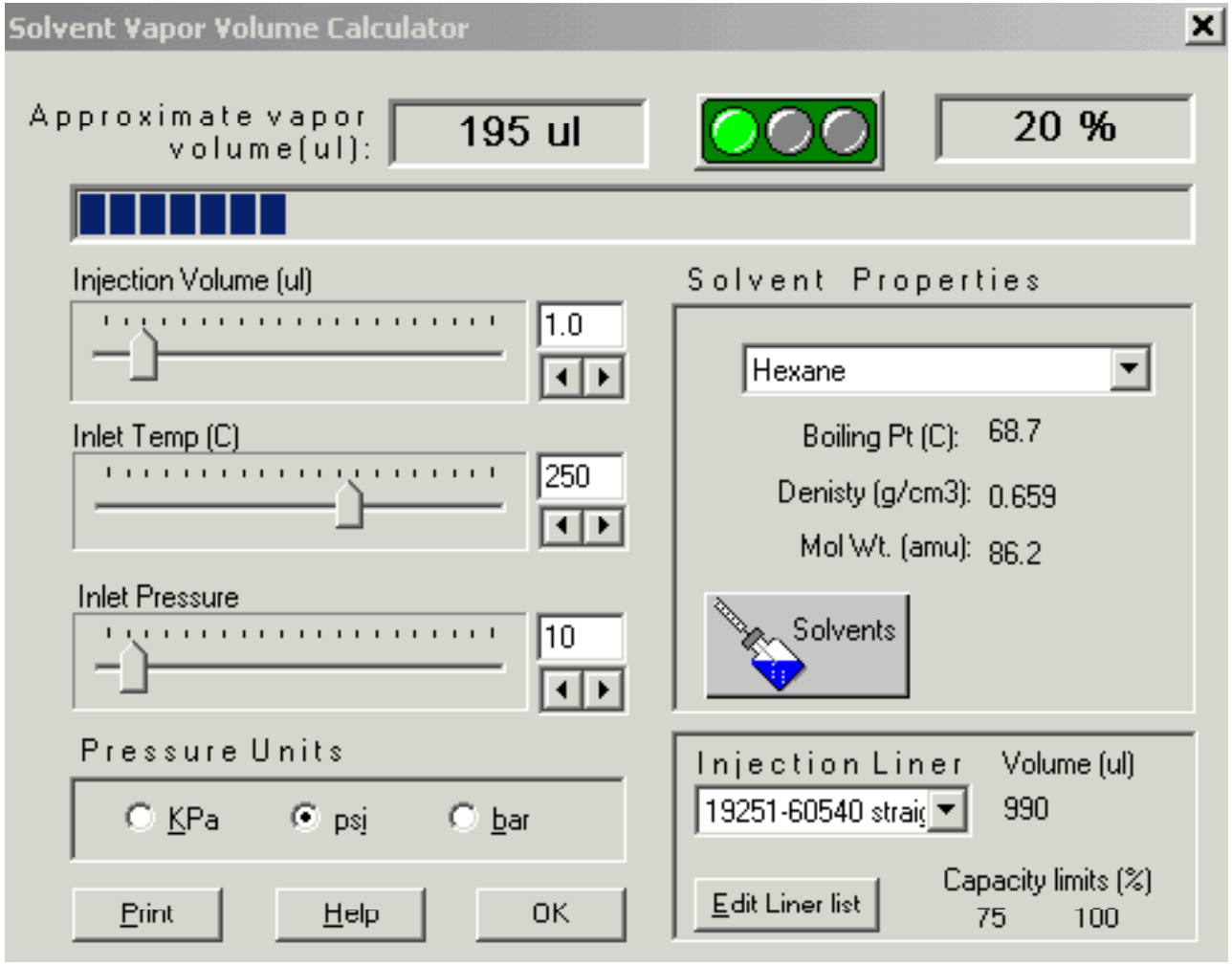
These aspects should be optimized for your sample volume and application.

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.

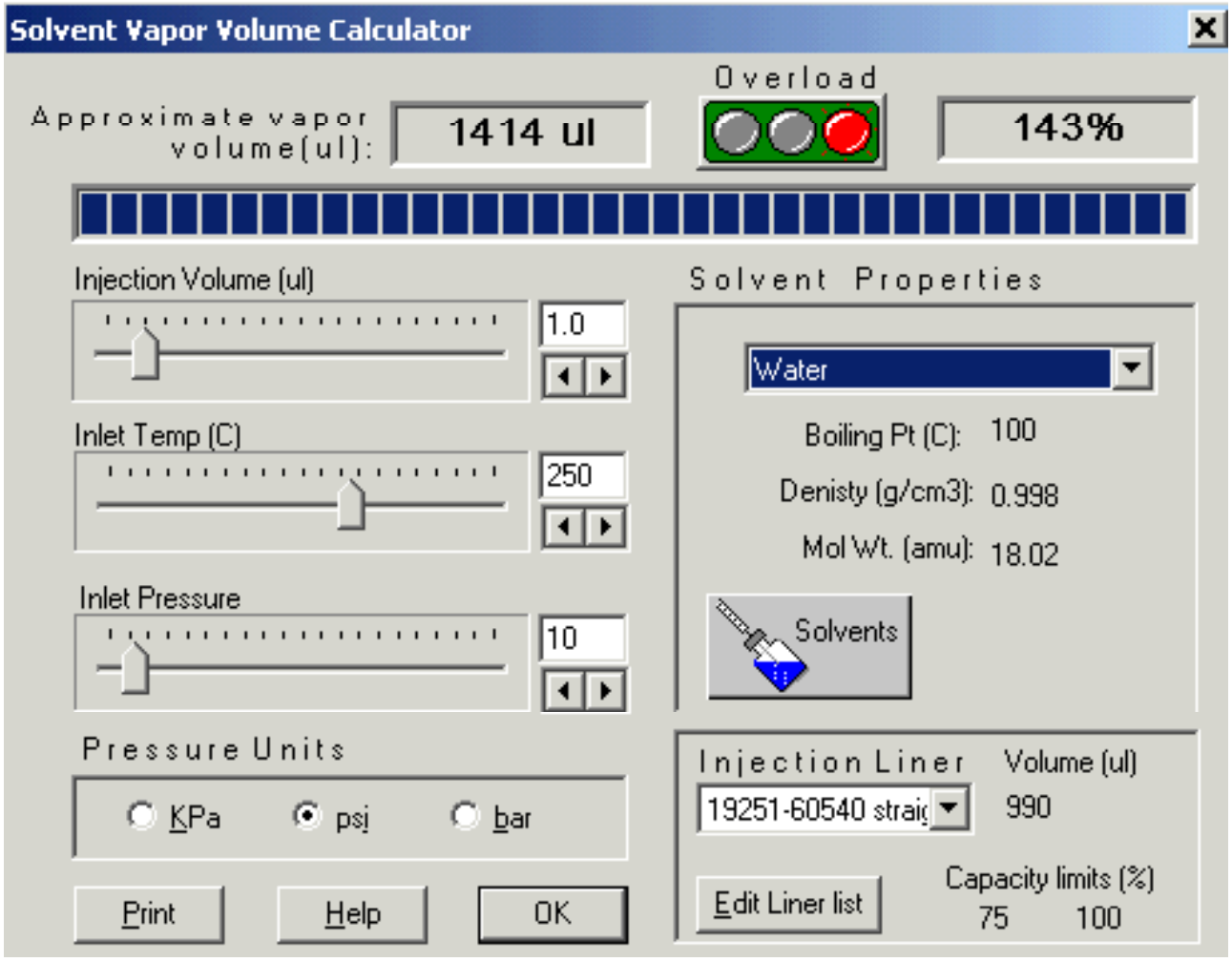
Pressure/Flow Calculator Software

Hexane Looks Good



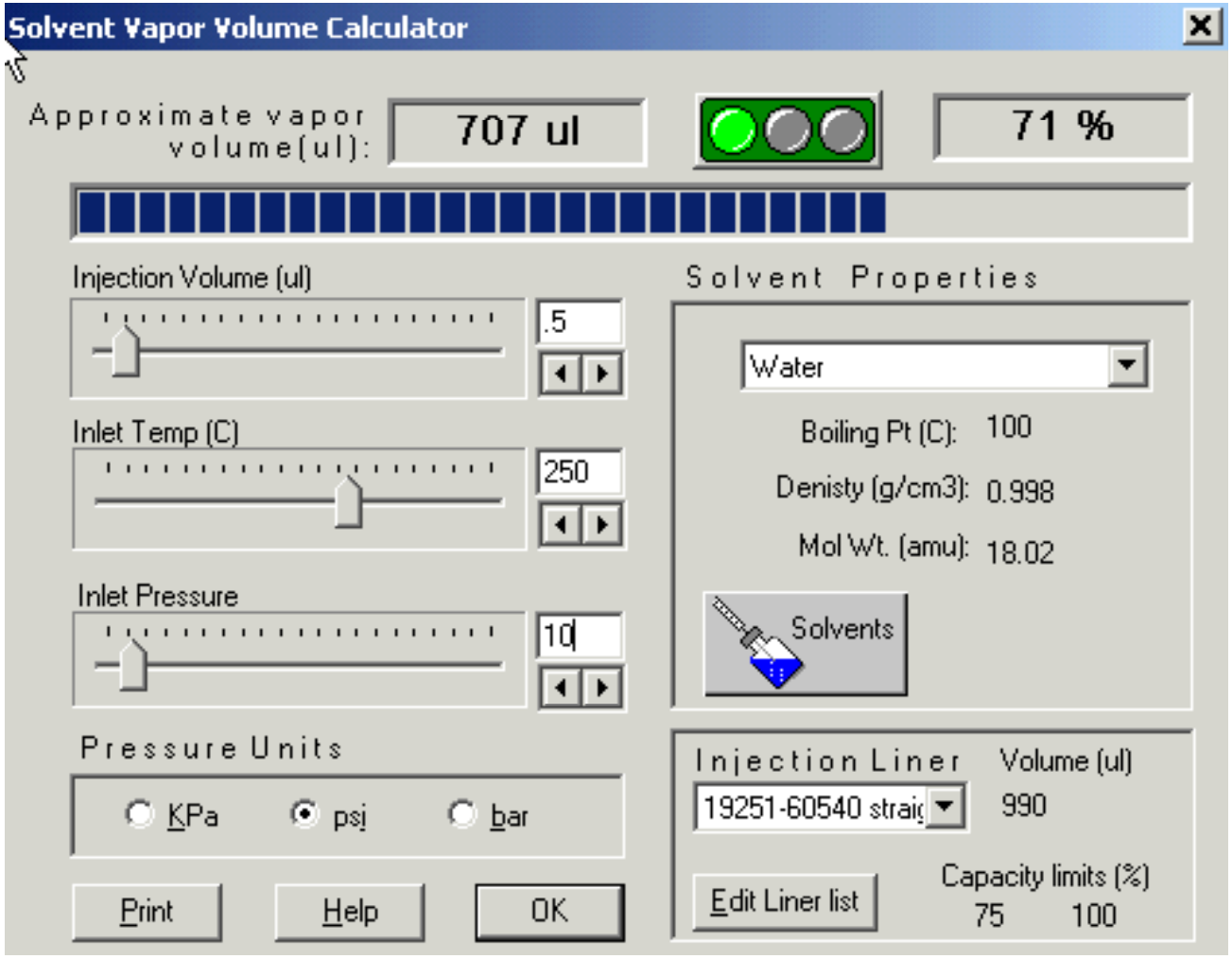
Pressure/Flow Calculator Software

Water Can Be Trouble



Pressure/Flow Calculator Software

Water– Reduce Injection Volume



Liner Treatments or Deactivation

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

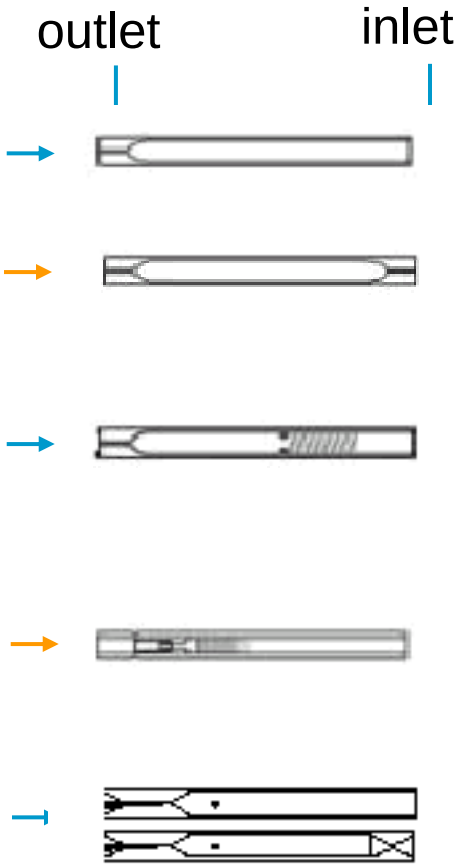
Unwanted sample adsorption leads to tailing peaks and loss of response for active analytes.

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.

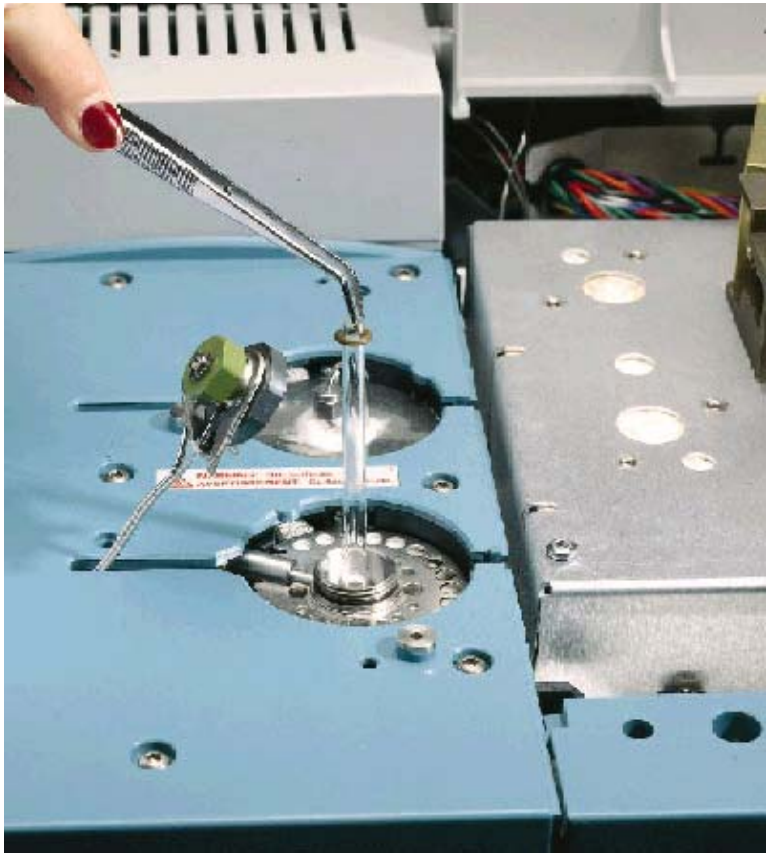
Glass wool and shelf to hold it in place, prevents non-volatiles from reaching column and removes residual sample from needle. Glass wool should be deactivated.

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

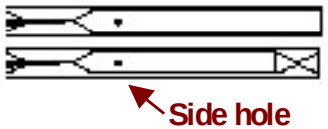
Liner	Part No.	Comments
	19251-60540	Simplest split liner, glass wool, no-deactivation, large volume, 990μL volume. Use for general purpose applications for compounds with low glass adsorption activity. Also used for Splitless mode.
	5183-4647	Glass wool (held near needle entrance to remove residual sample on needle), deactivated, 870μL 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, 800μL 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, 800μL 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

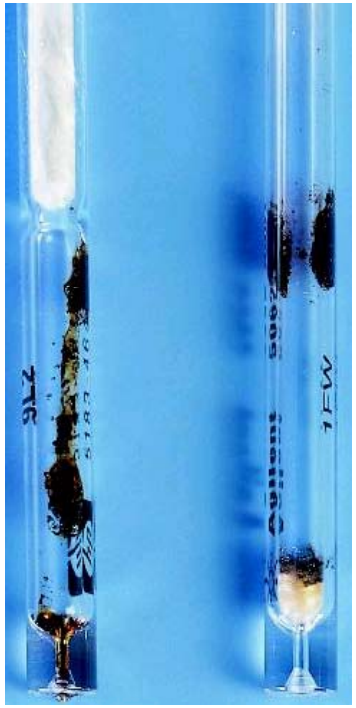
For Trace Level Analysis

- 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, 900μL 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, 900μL volume. Glass wool aides volatilization and protects column. For trace (dirty) samples.
	5181-3315	Double taper, deactivated, 800μL volume. Taper on inlet reduces chance for backflash into carrier gas lines. High efficiency liner for trace, active samples.
	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

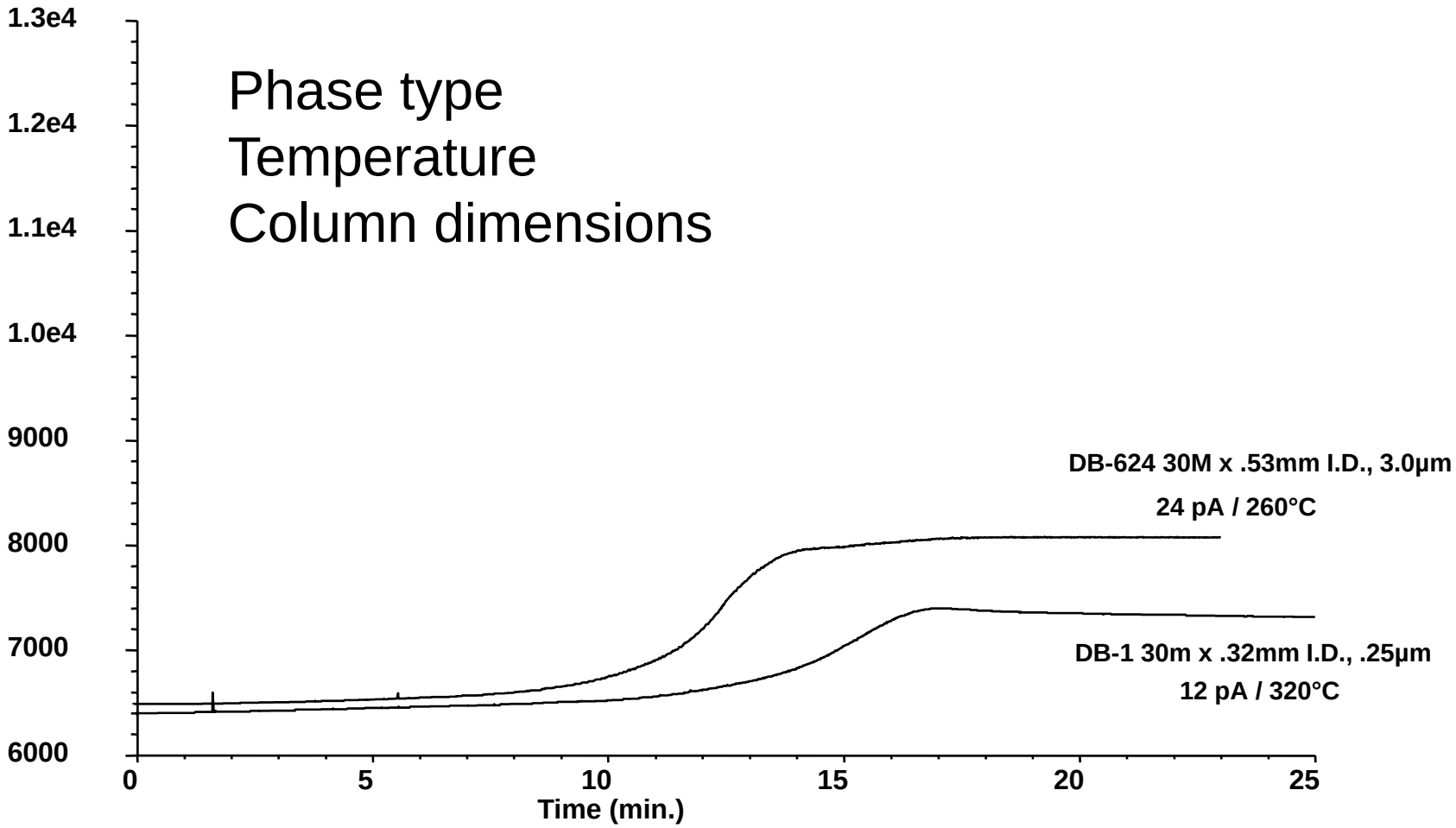
Last several years have seen modest advances in GC column technology

- 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:



Phase type
Temperature
Column dimensions

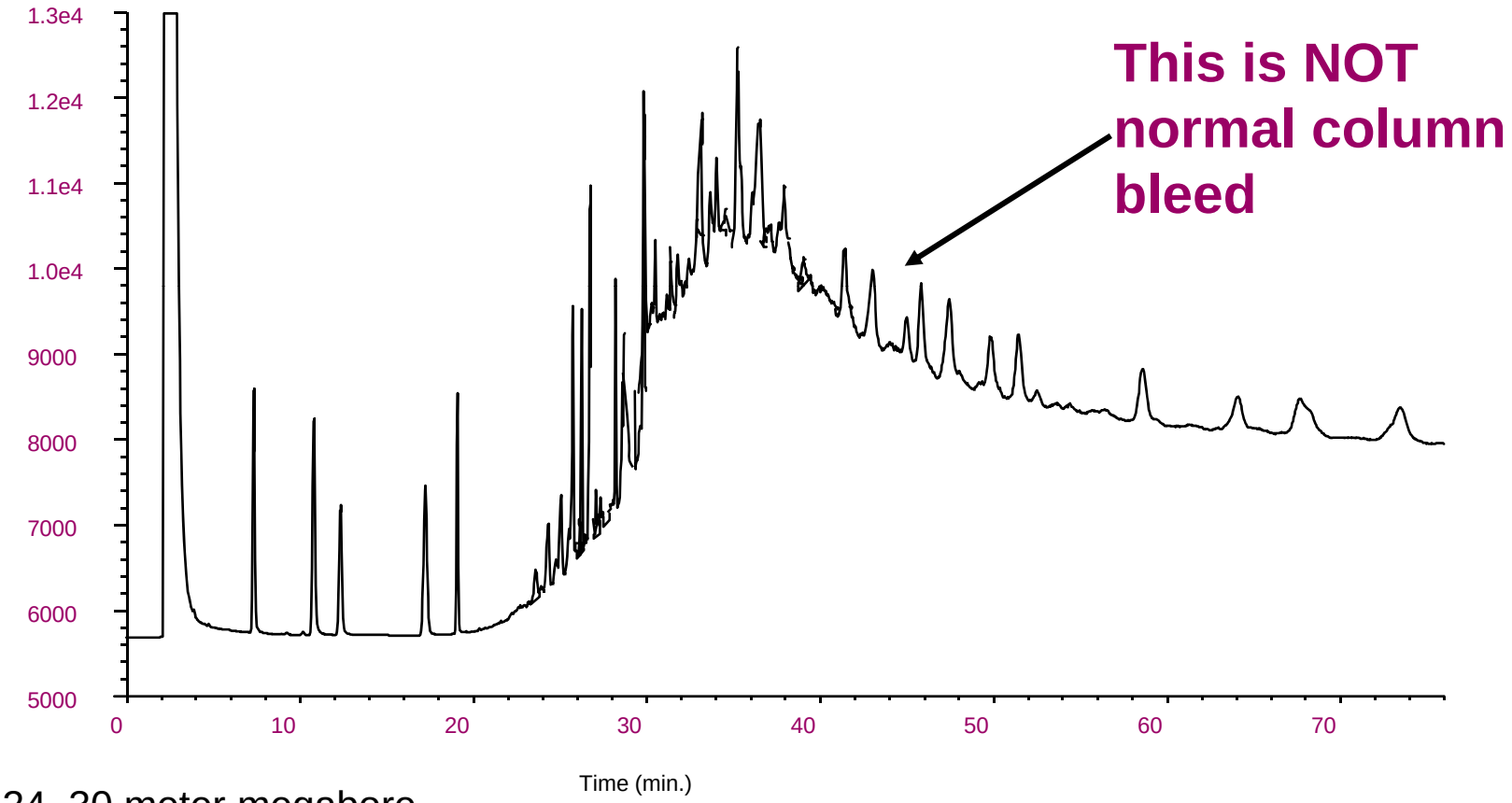
What Is A Bleed Problem?

An abnormal elevated baseline at high temperature

IT IS NOT

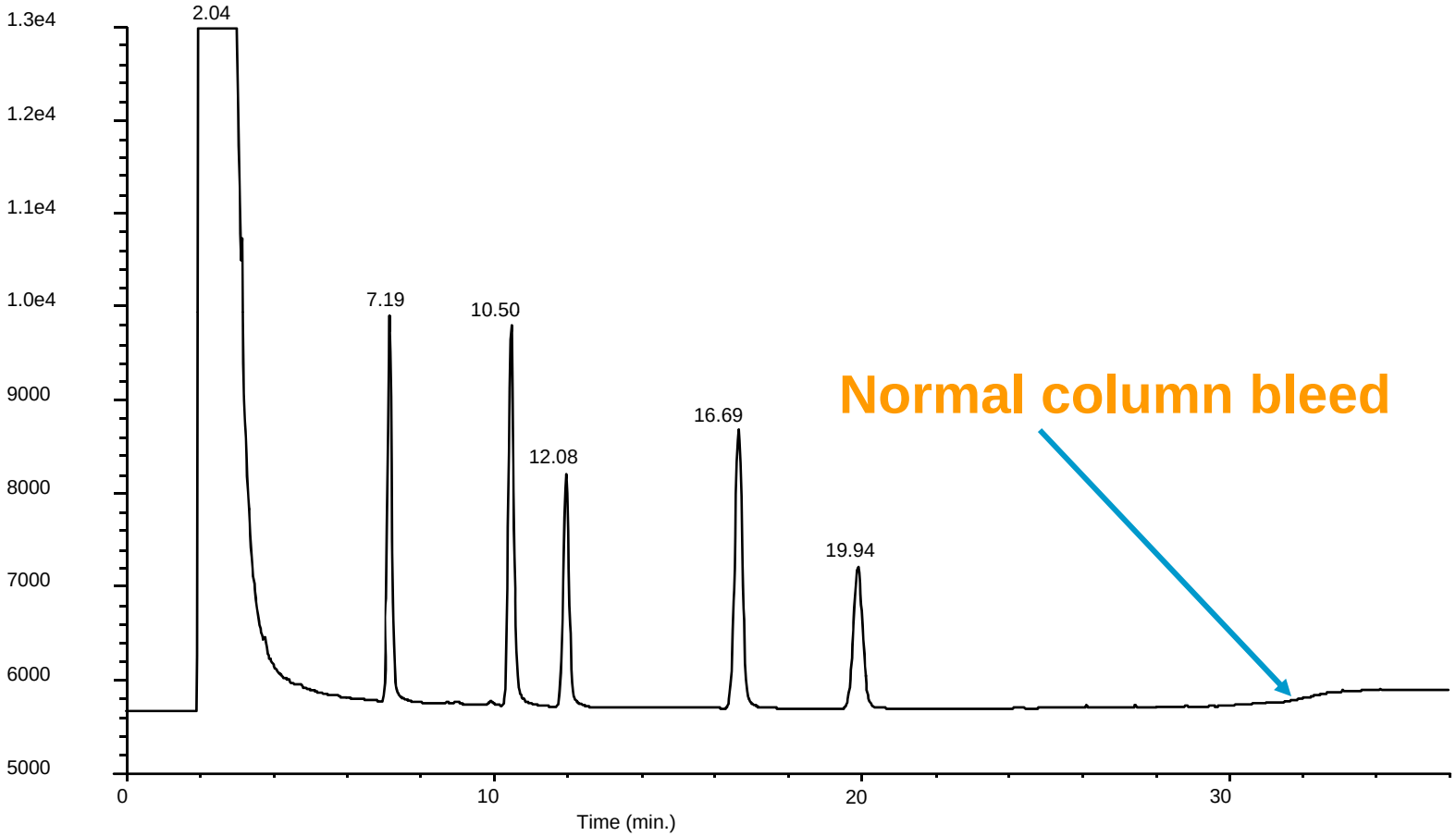
- ✓ A high baseline at low temperature
- ✓ Wandering or drifting baseline at any temperature
- ✓ Discrete peaks

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

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

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.

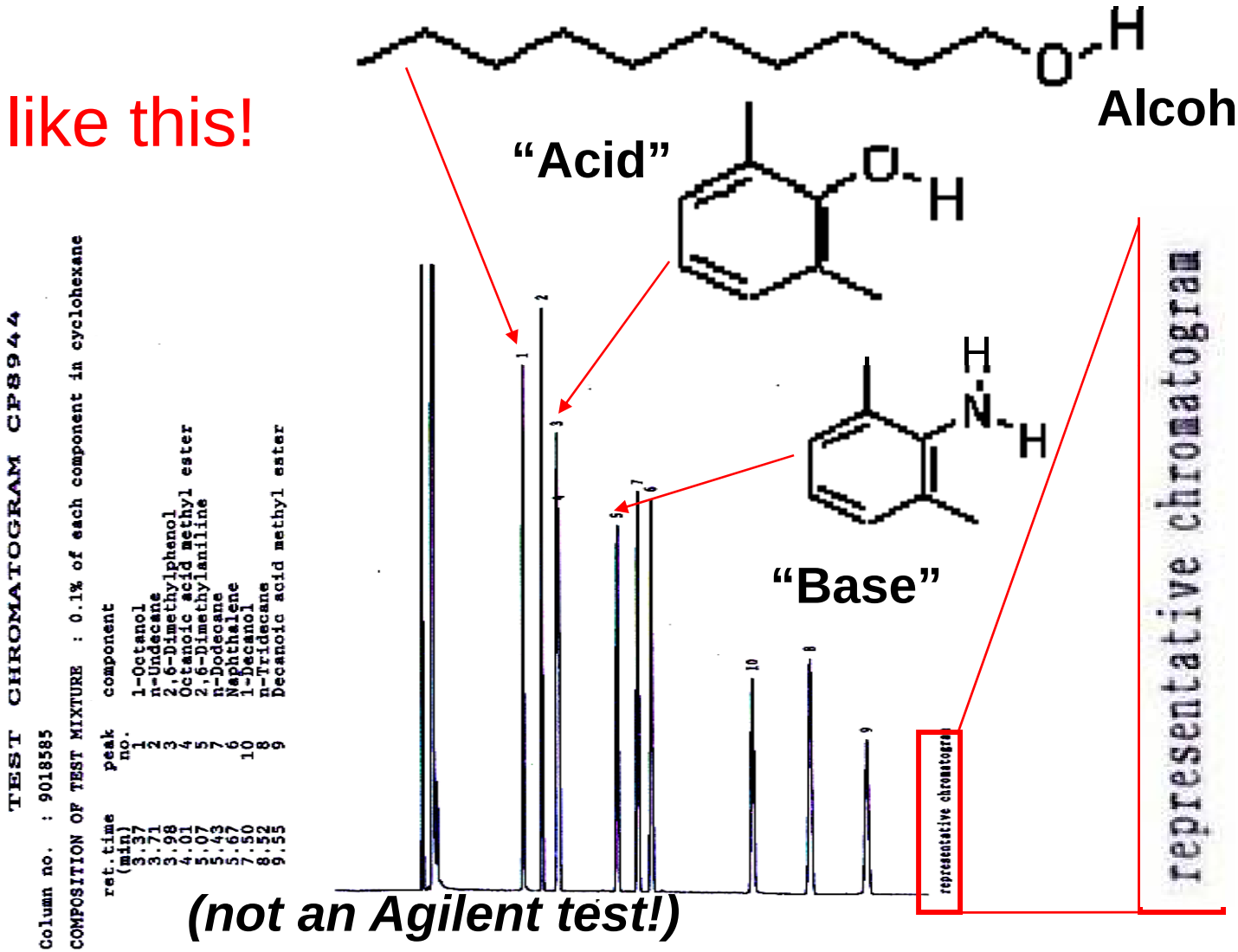
Everything looks the same “from the cheap seats”, so take a close up look at small pictures in brochures and advertisements

QC Test Mixture Components

Compounds	Purpose
Hydrocarbons	Efficiency
	Retention
Alcohols	Activity
FAME’s, PAH’s	Retention
Acids	Acidic Character
Bases	Basic Character

How is High GC column Inertness Assured?

Not like this!



(not an Agilent test!)

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, TRIDECANE	1.43
4-CHLOROPHENOL, TRIDECANE	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. TRIDECANE	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

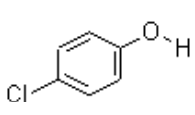
Inlet: Split (275°C) **Detector:** FID (325°C)

Carrier Gas: Hydrogen **Flow:** 33.9 cm/sec (1.0 ml/min)

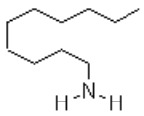
Holdup Compound: Pentane (1.477-min)

Temperature Progra Isothermal at 135°C

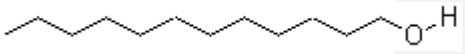
Acid

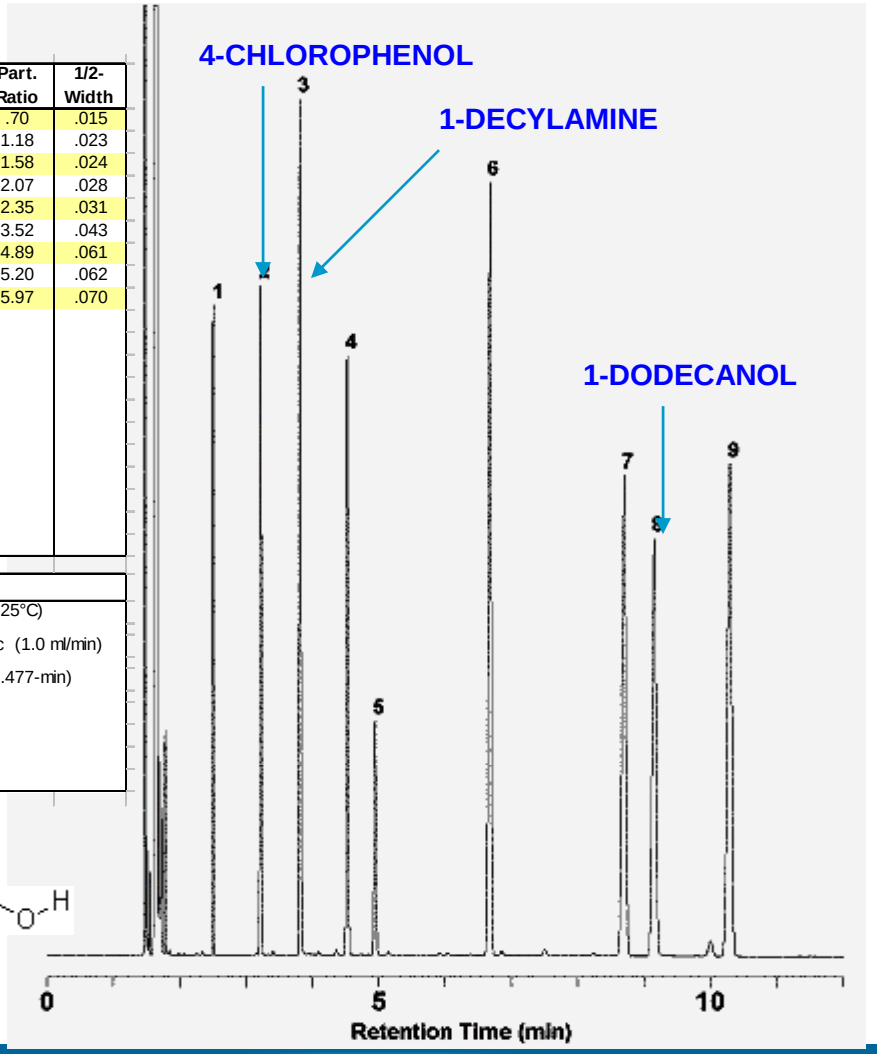


Base



Alcohol





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

Performance Results

Theoretical Plates/Meter:

TETRADECANE	4165

UTE%:

TETRADECANE	89.9%

Retention Index:

1-METHYLNAPHTHALENE	1323.7
1-UNDECANOL	1370.7

Compound Identification	Retent. Time	Part. Ratio	1/2-Width
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. TRIDECANE	5.627	3.34	.037
5. 1-METHYLNAPHTHALENE	6.283	3.84	.043
6. 1-UNDECANOL	7.820	5.03	.051
7. TETRADECANE	8.963	5.91	.060
8. DICYCLOHEXYLAMINE	9.877	6.62	.064

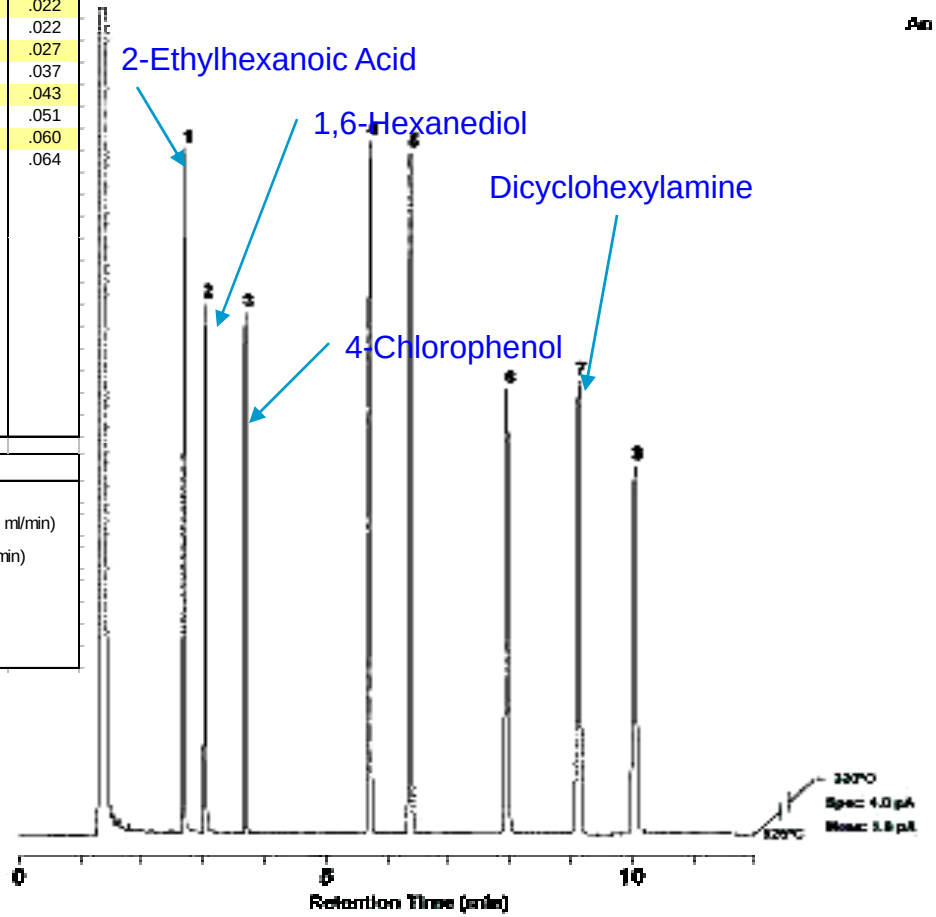
Test Conditions

Inlet: Split (250°C) **Detector:** FID (320°C)

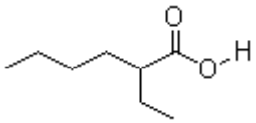
Carrier Gas: Hydrogen **Flow:** 38.6 cm/sec (1.2 ml/min)

Holdup Compound: Methane (1.297-min)

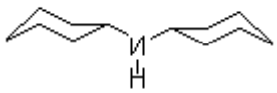
Temperature Program: Isothermal at 125°C



Acid



Base



Diol

Comprehensive Testing--Demanding Criteria

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)

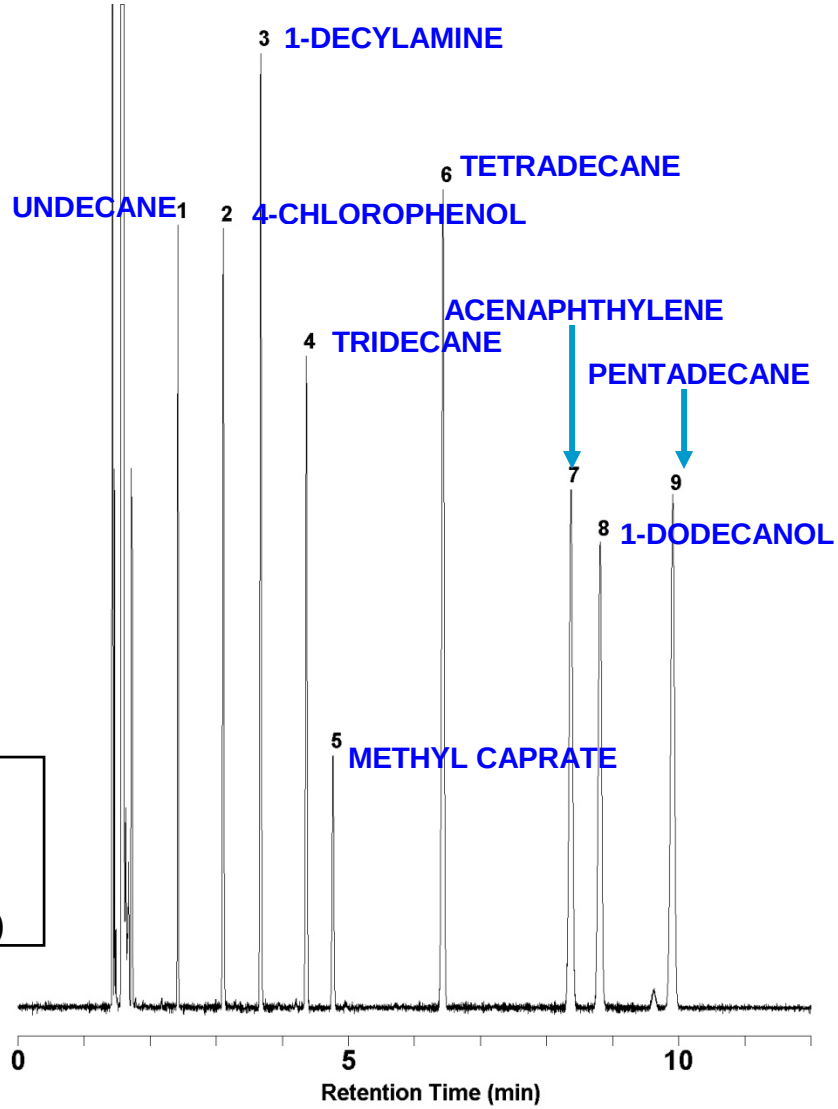
Holdup Compound: Pentane (1.428-min)

Temperature Progra Isothermal at 135°C

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)**



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

Performance Results

Theoretical Plates/Meter:

Pentadecane 4326

ACENAPHTHYLENE	1462.0
1-DODECANOL	1473.7

Peak Height Ratio:

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4-CHLOROPHENOL, TRIDECANE	1.19

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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

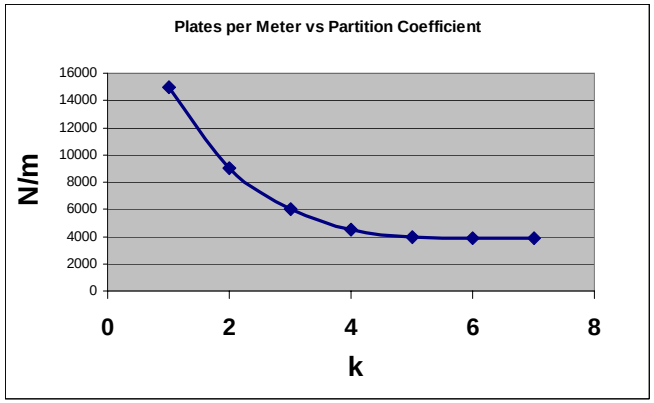
Test Conditions

Inlet: Split (275°C) **Detector:** FID (325°C)

Carrier Gas: Hydrogen **Flow:** 35.0 cm/sec (1.0 ml/min)

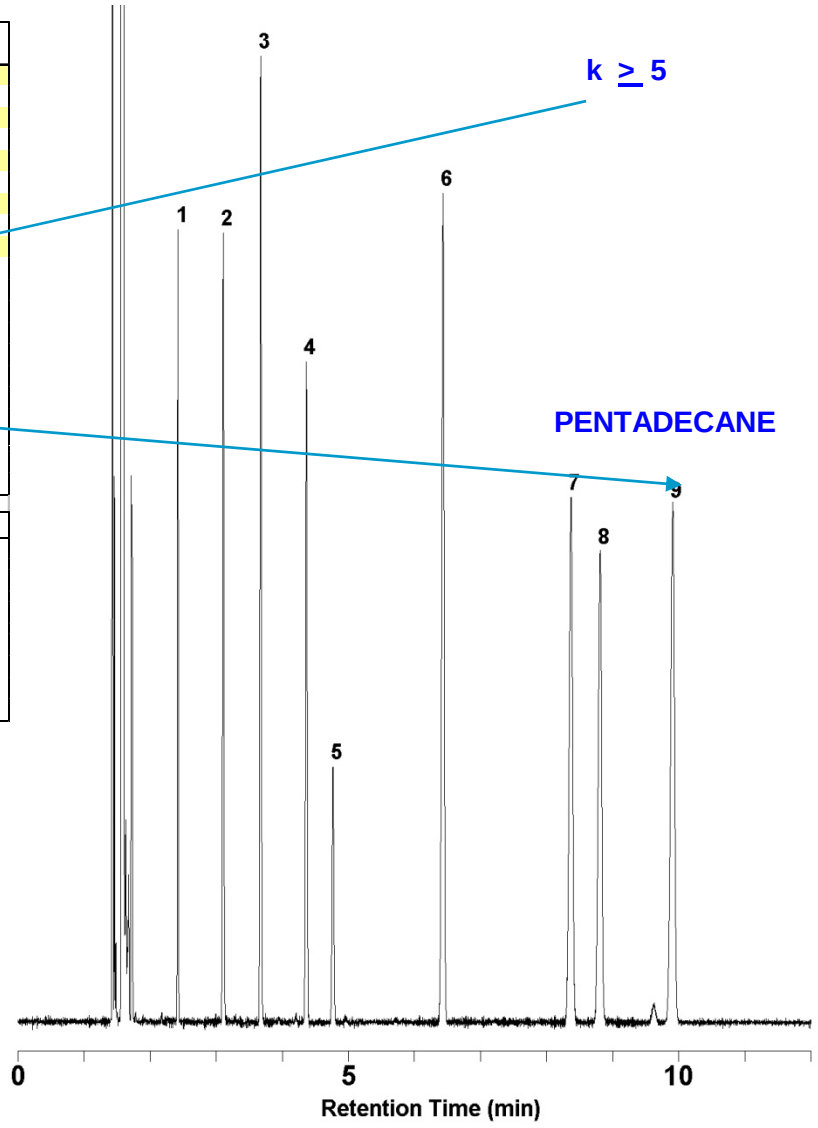
Holdup Compound: Pentane (1.428-min)

Temperature Progra Isothermal at 135°C



Measured Isothermally

Probe k ≥ 5



Demanding Criteria--Selectivity (RI)

Performance Results

Retention Index:

Methyl Caprate	1324.1
Acenaphthylene	1462.0
1-Dodecanol	1473.7

3 probes, 3 functional groups

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

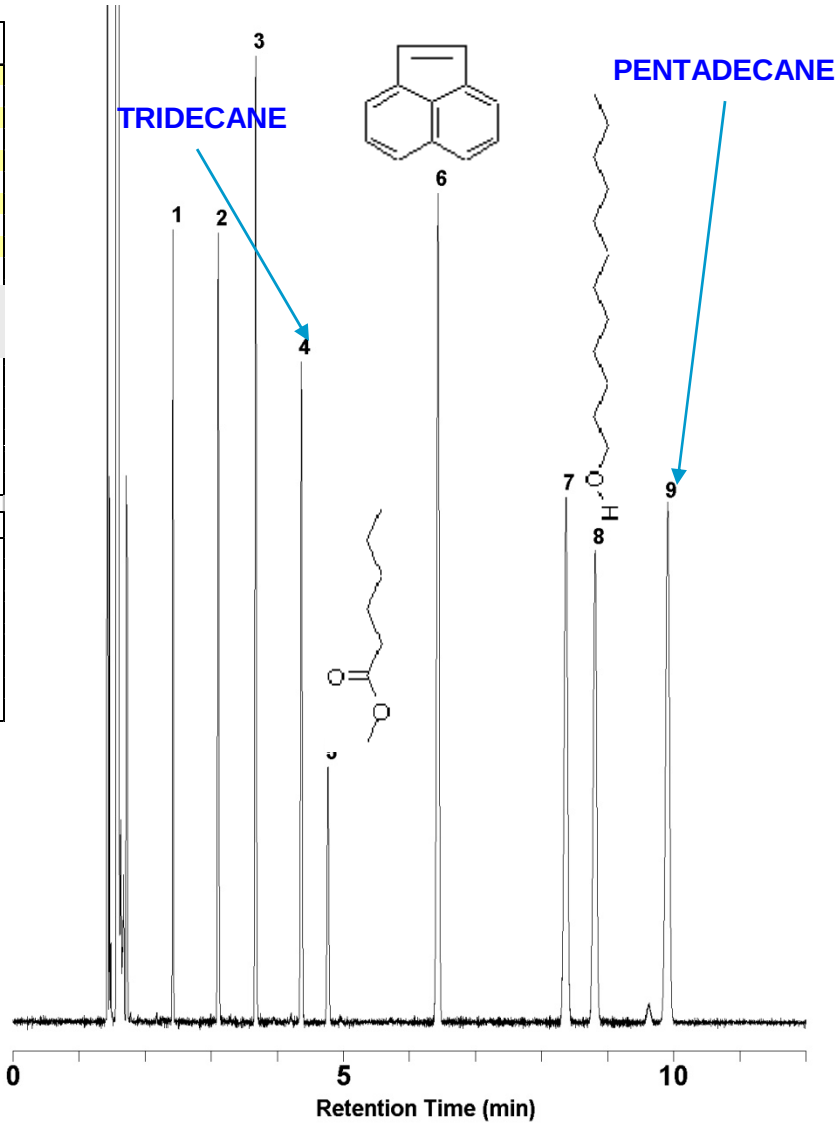
Test Conditions

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 Progra Isothermal at 135°C



Demanding Criteria--Inertness

Performance Results

Theoretical Plates/Meter:

PENTADECANE	4326

Retention Index:

METHYL CAPRATE	1324.1
ACENAPHTHYLENE	1462.0
1-DODECANOL	1473.7

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

Peak Height Ratio:

1-Dodecanol Tetradecane	0.57
1-Decylamine Tridecane	1.47
4-Chlorophenol Tridecane	1.19

Test Conditions

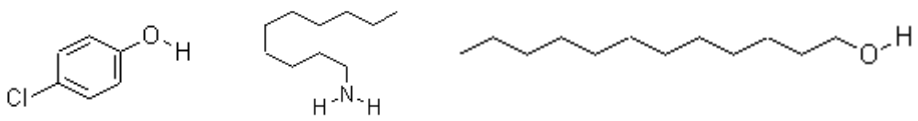
Inlet: Split (275°C) **Detector:** FID (325°C)

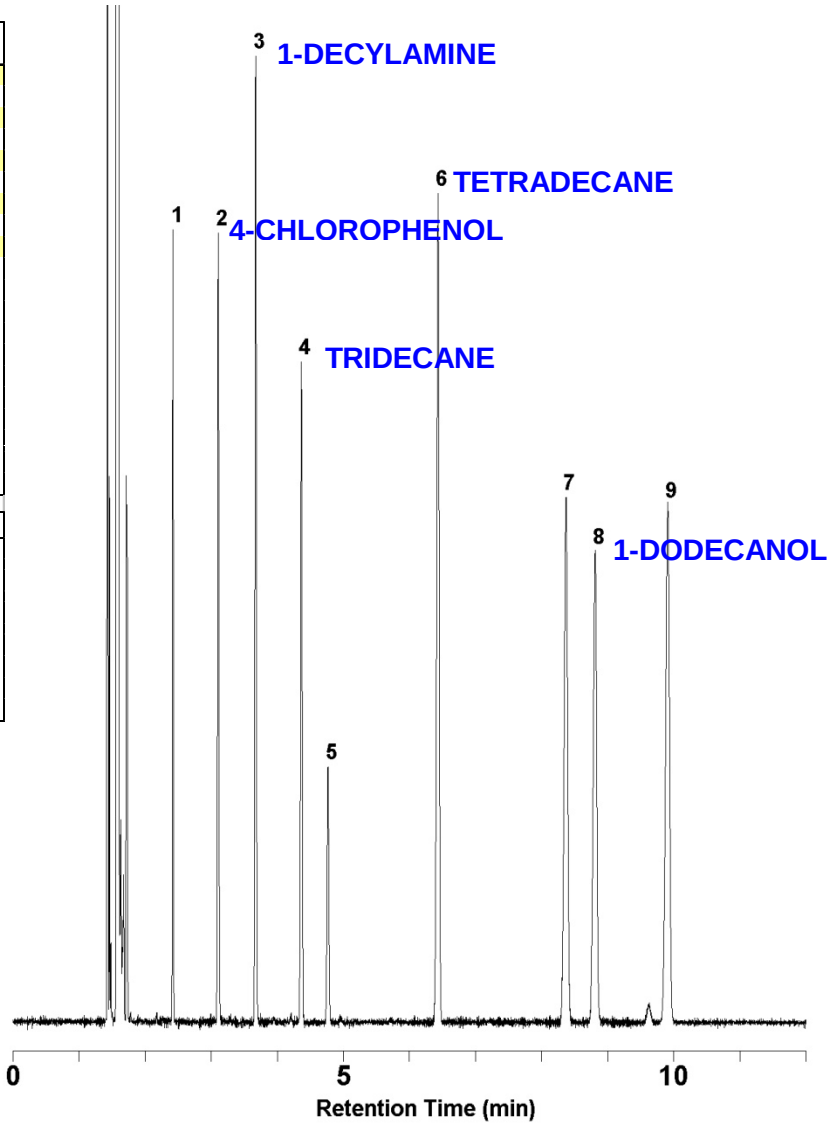
Carrier Gas: Hydrogen **Flow:** 35.0 cm/sec (1.0 ml/min)

Holdup Compound: Pentane (1.428-min)

Temperature Progra Isothermal at 135°C

Acid **Base** **Alcohol**





Demanding Criteria--Inertness: A Closer Look

Performance Results

Theoretical Plates/Meter:

PENTADECANE	4326

Retention Index:

METHYL CAPRATE	1324.1
ACENAPHTHYLENE	1462.0
1-DODECANOL	1473.7

Compound Identification	Retent. Time	Part. Ratio	1/2-Width
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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

Peak Height Ratio:

1-Dodecanol Tetradecane	0.57
1-Decylamine Tridecane	1.47
4-Chlorophenol Tridecane	1.19

Test Conditions

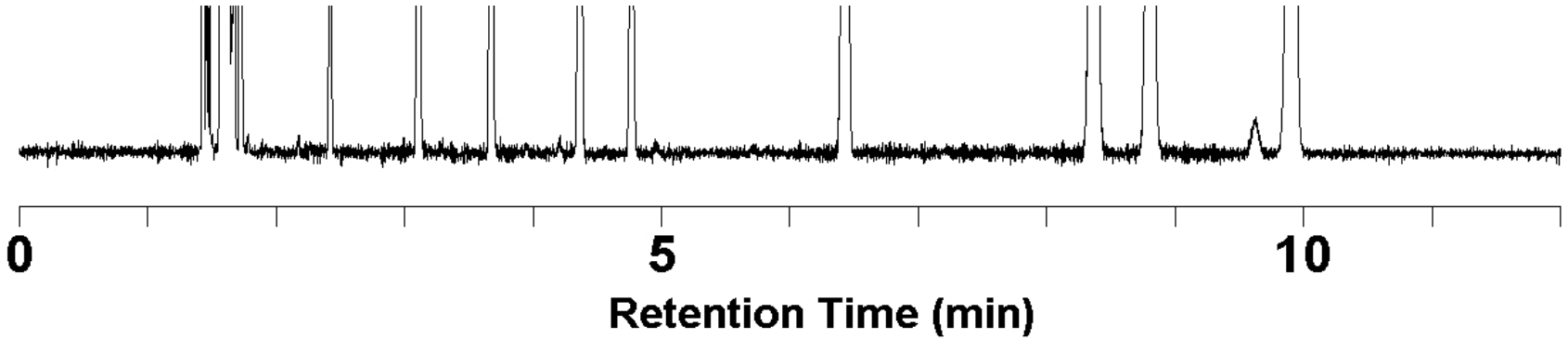
Inlet: Split (275°C) **Detector:** FID (325°C)

Carrier Gas: Hydrogen **Flow:** 35.0 cm/sec (1.0 ml/min)

Holdup Compound: Pentane (1.428-min)

Temperature Progra Isothermal at 135°C

Can you tell which the active probes are?



Demanding Criteria--Inertness: A Closer Look

Performance Results

Theoretical Plates/Meter:

PENTADECANE	4326

Retention Index:

METHYL CAPRATE	1324.1
ACENAPHTHYLENE	1462.0
1-DODECANOL	1473.7

Compound Identification	Retent. Time	Part. Ratio	1/2-Width
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Peak Height Ratio:

1-Dodecanol Tetradecane	0.57
1-Decylamine Tridecane	1.47
4-Chlorophenol Tridecane	1.19

Test Conditions

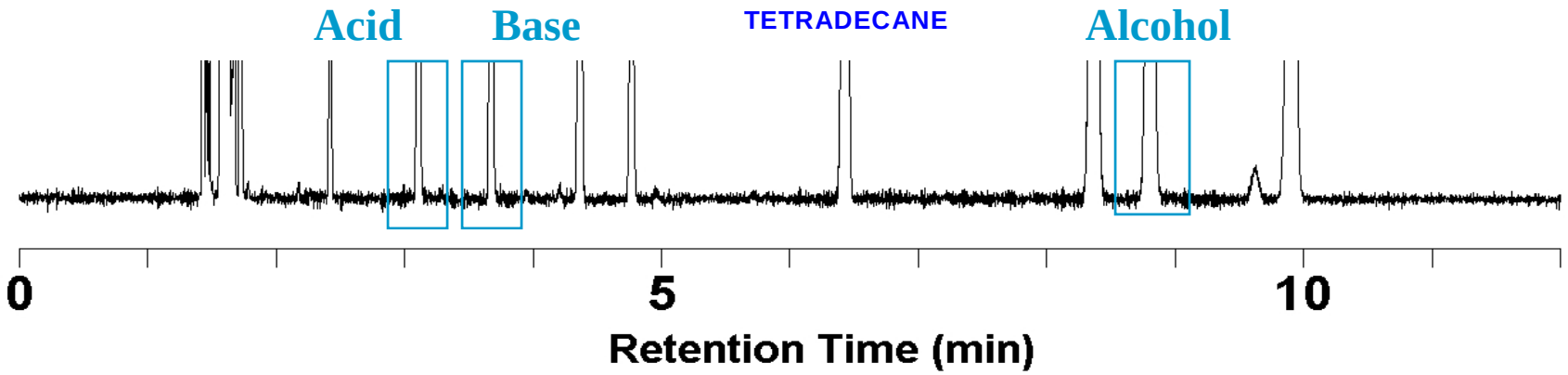
Inlet: Split (275°C) **Detector:** FID (325°C)

Carrier Gas: Hydrogen **Flow:** 35.0 cm/sec (1.0 ml/min)

Holdup Compound: Pentane (1.428-min)

Temperature Progra Isothermal at 135°C

Can't ID by peak tailing!



Demanding Criteria--Looks Flawless!

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

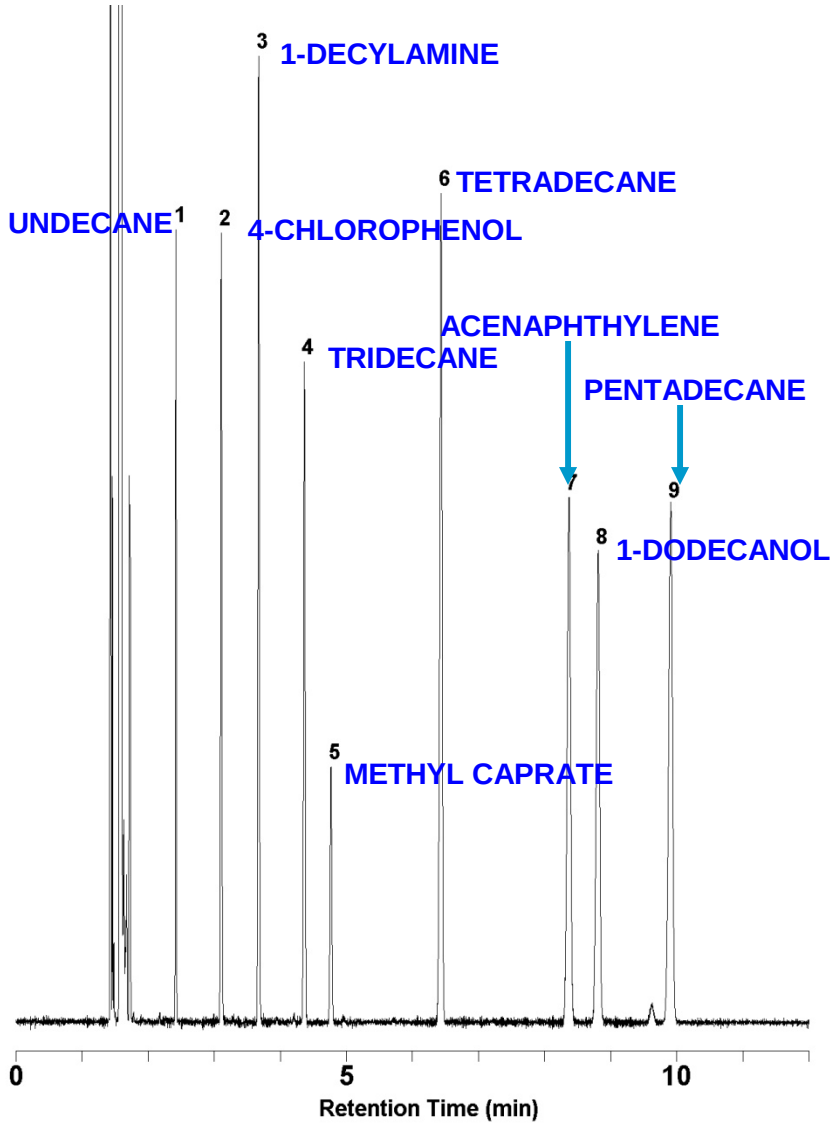
Test Conditions

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 Progra Isothermal at 135°C



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

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

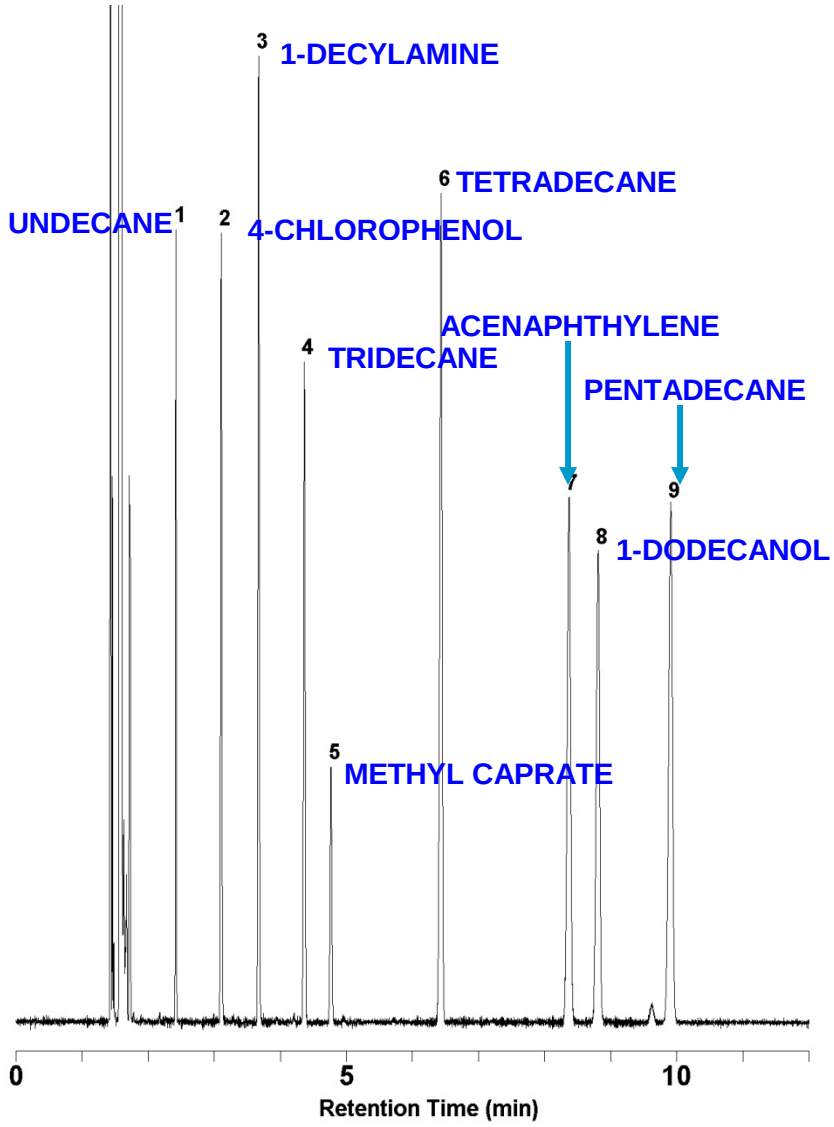
Test Conditions

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 Progra Isothermal at 135°C



Catalog: 19091S-433 **Stationary Phase:** HP-5MS

Serial: US2469141H **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

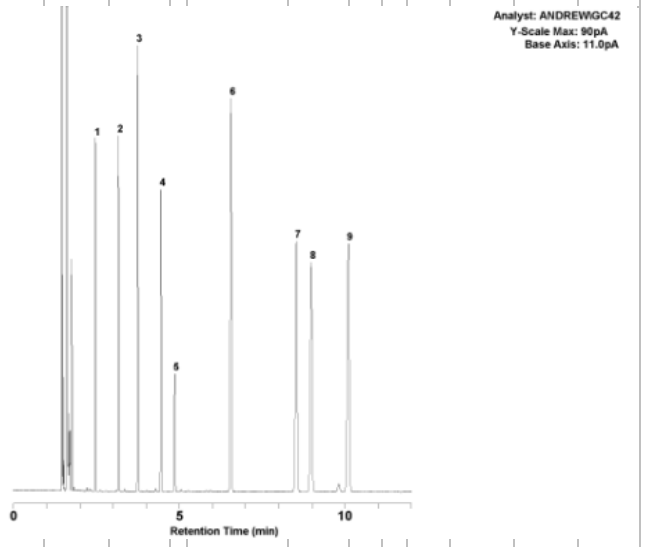
Test Conditions

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 **Stationary Phase:** HP-5MS

Serial: US2469142H **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
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Test Conditions

Inlet: Split (275°C) **Detector:** FID (325°C)

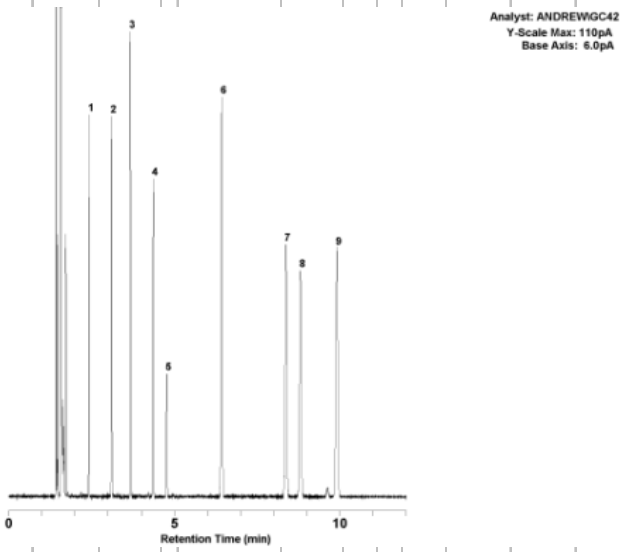
Carrier Gas: Hydrogen **Flow:** 35.0 cm/sec (1.0 ml/min)

Holdup Compound: Pentane (1.428-min)

Temperature Program: Isothermal at 135°C

Failed

High Bleed High: 14.4 Low: 8.9

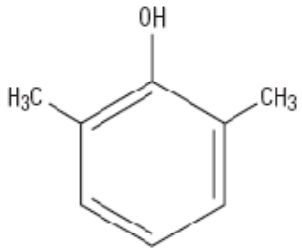


Column Manufacturing-Failed Columns

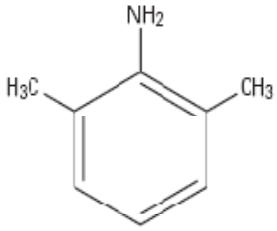
Even more demanding probes

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

Chemical Structures

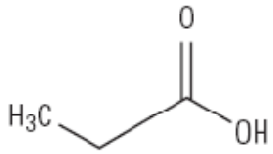


2, 6-Dimethylphenol

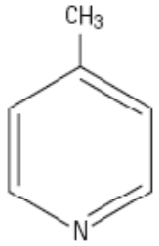


2, 6-Dimethylaniline

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.*

New Agilent J&W Ultra Inert Capillary GC Columns

Raising the Bar for CONSISTENT Column Inertness Performance

High Inertness Advantages

Low Bleed is only Half the Story.

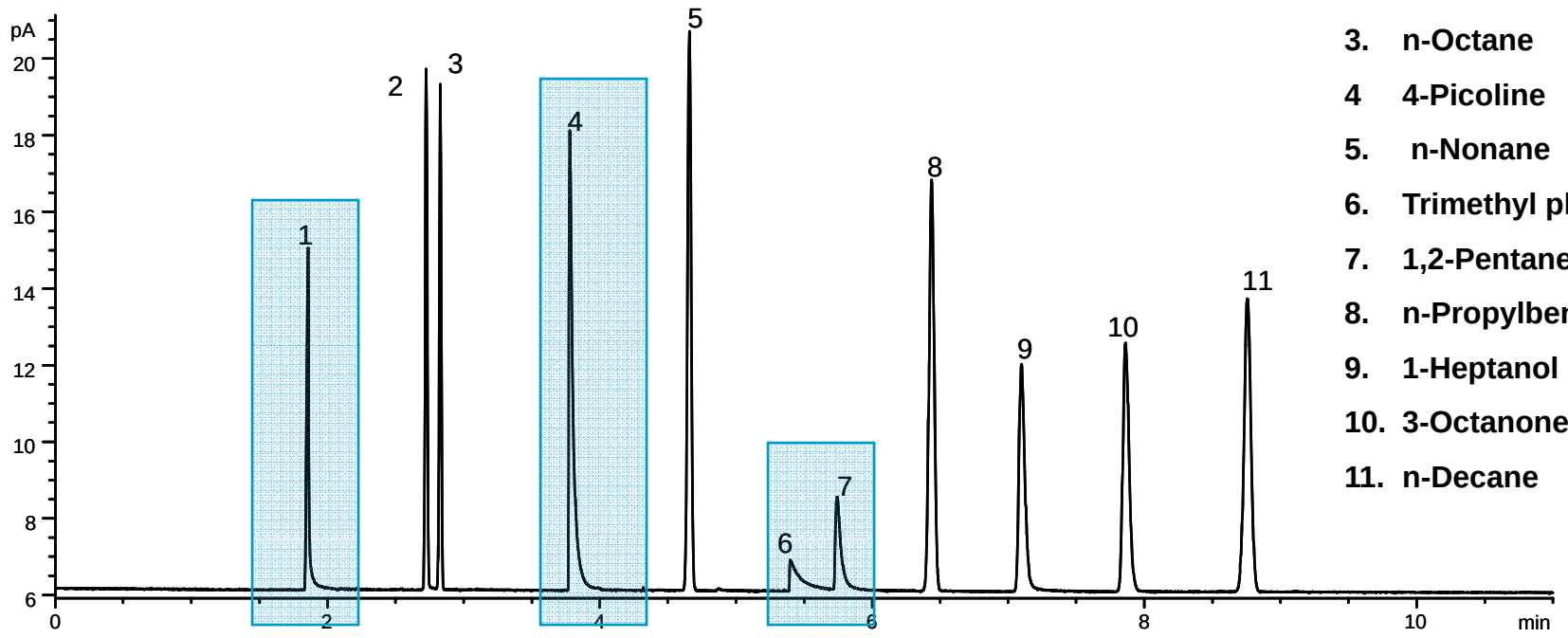
Inertness…"What goes in, comes out," or the lack of activity.

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**

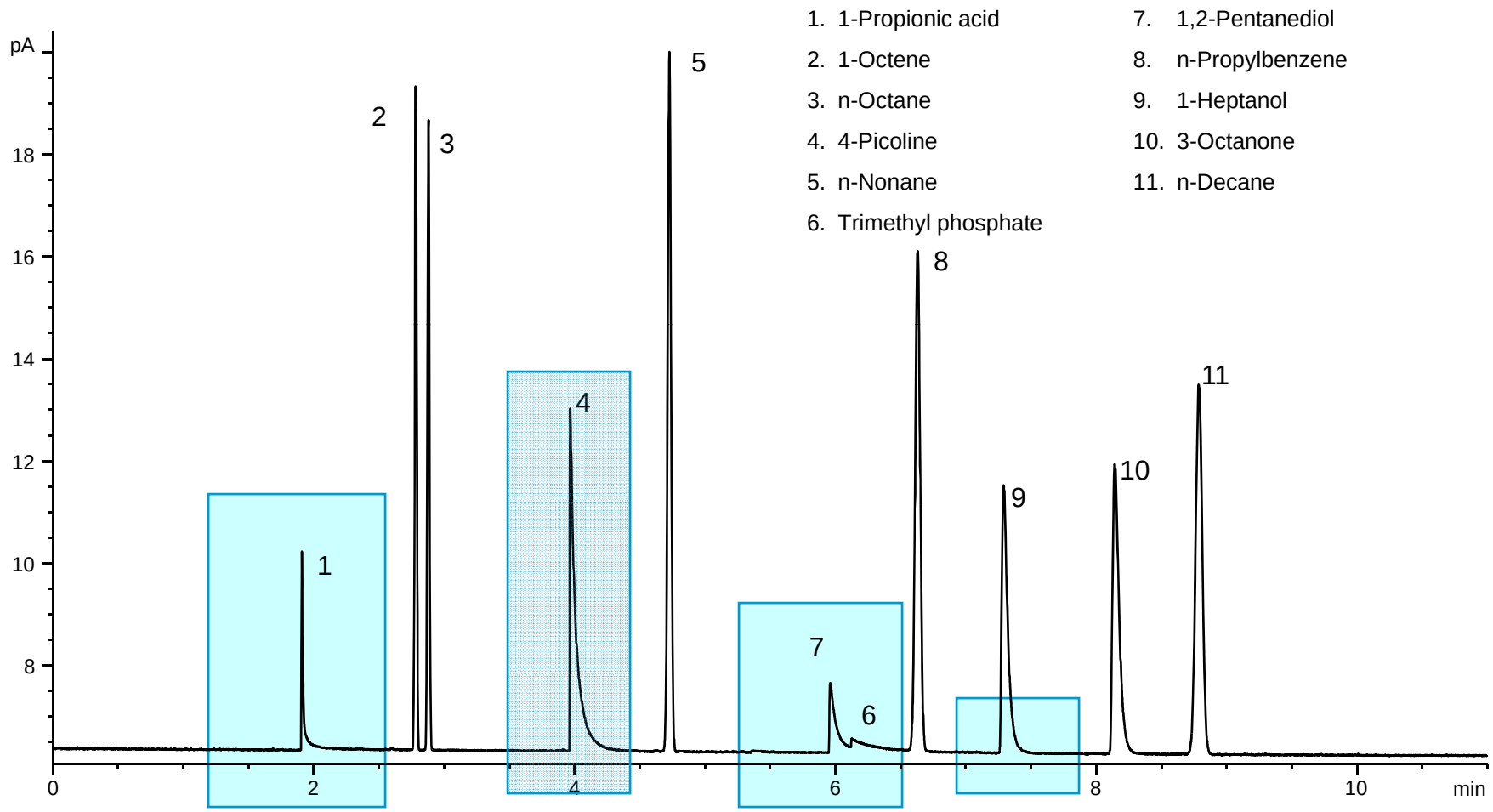
Über test results on a competitor's "good column"



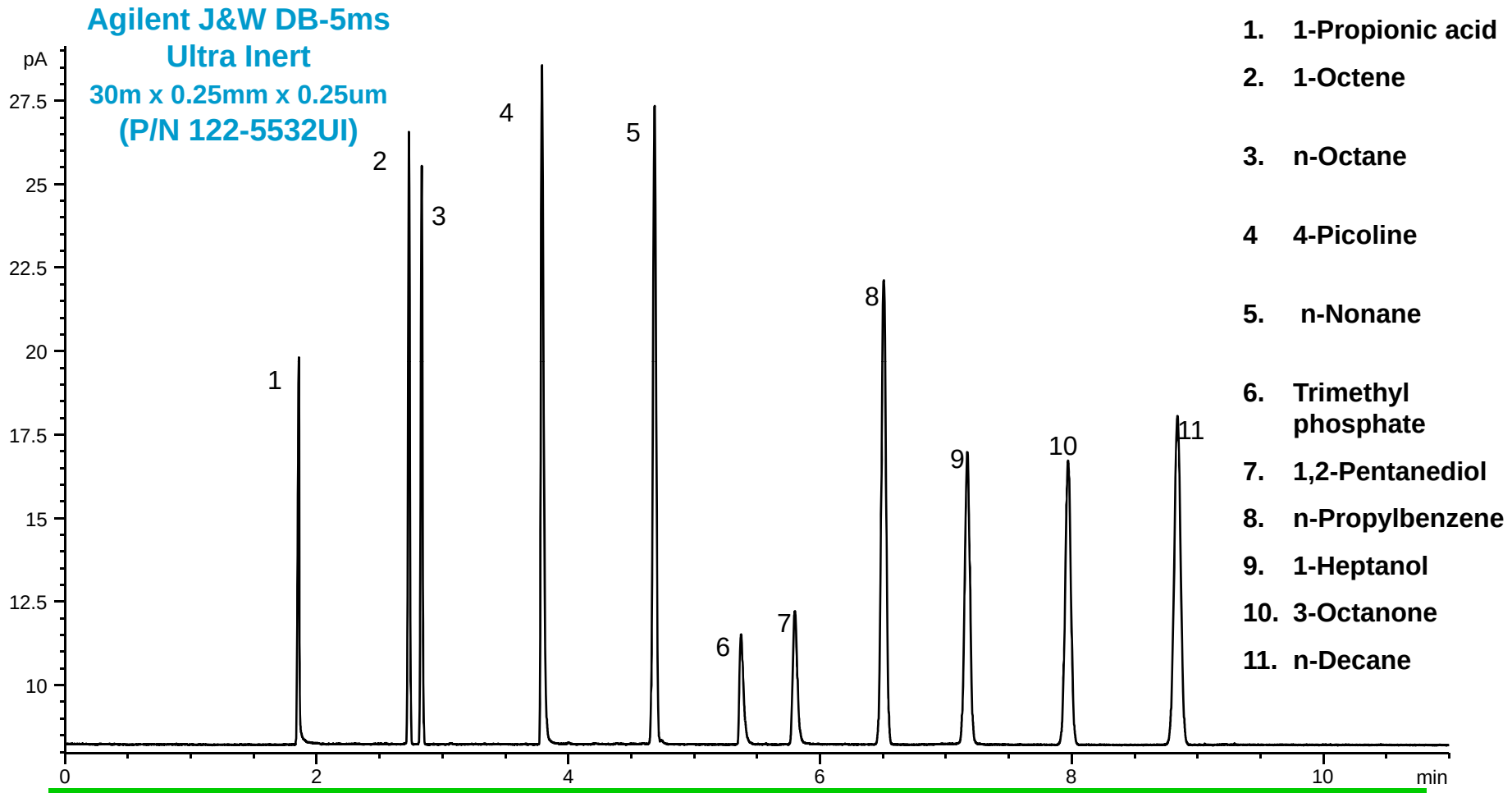
1. 1-Propionic acid
2. 1-Octene
3. n-Octane
4. 4-Picoline
5. n-Nonane
6. Trimethyl phosphate
7. 1,2-Pentanediol
8. n-Propylbenzene
9. 1-Heptanol
10. 3-Octanone
11. n-Decane

- 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 competitor's "good column"



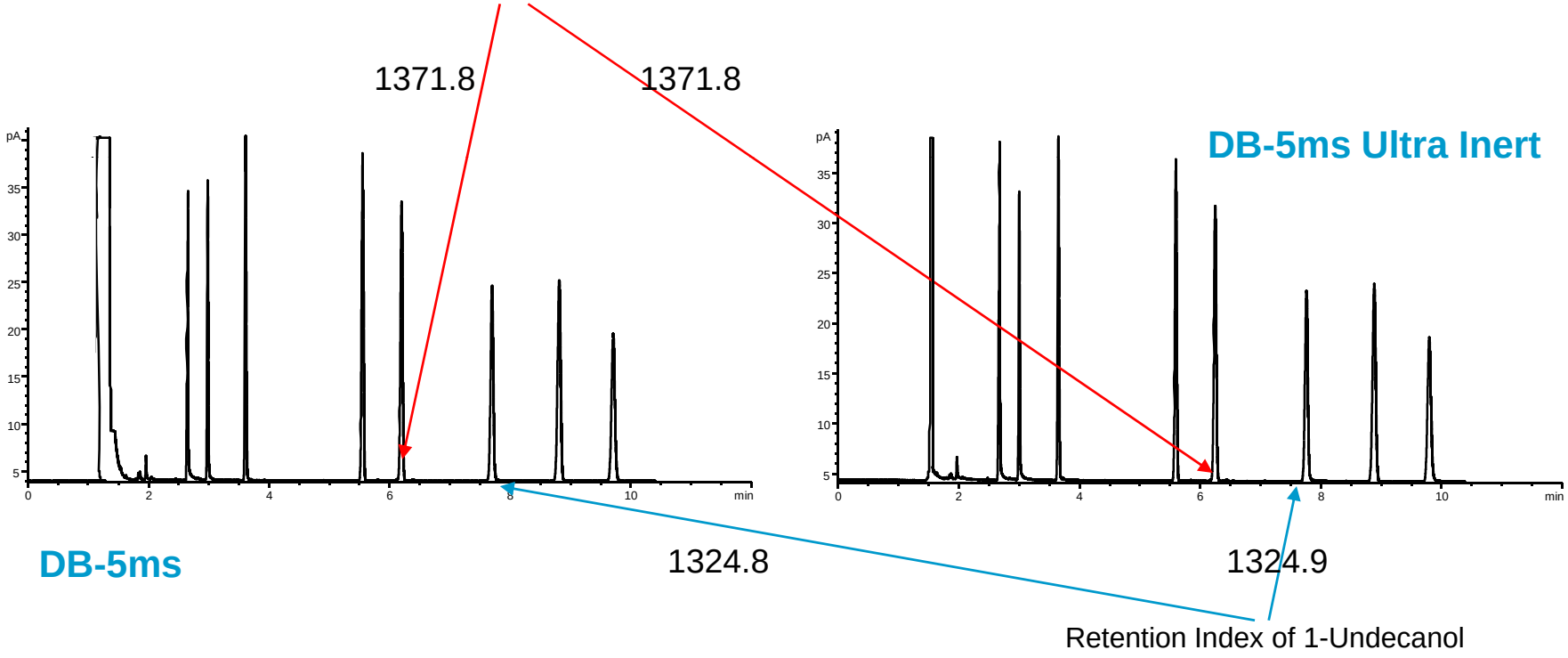
Ultra Inert Test Mix on Agilent J&W DB-5ms Ultra Inert



- 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



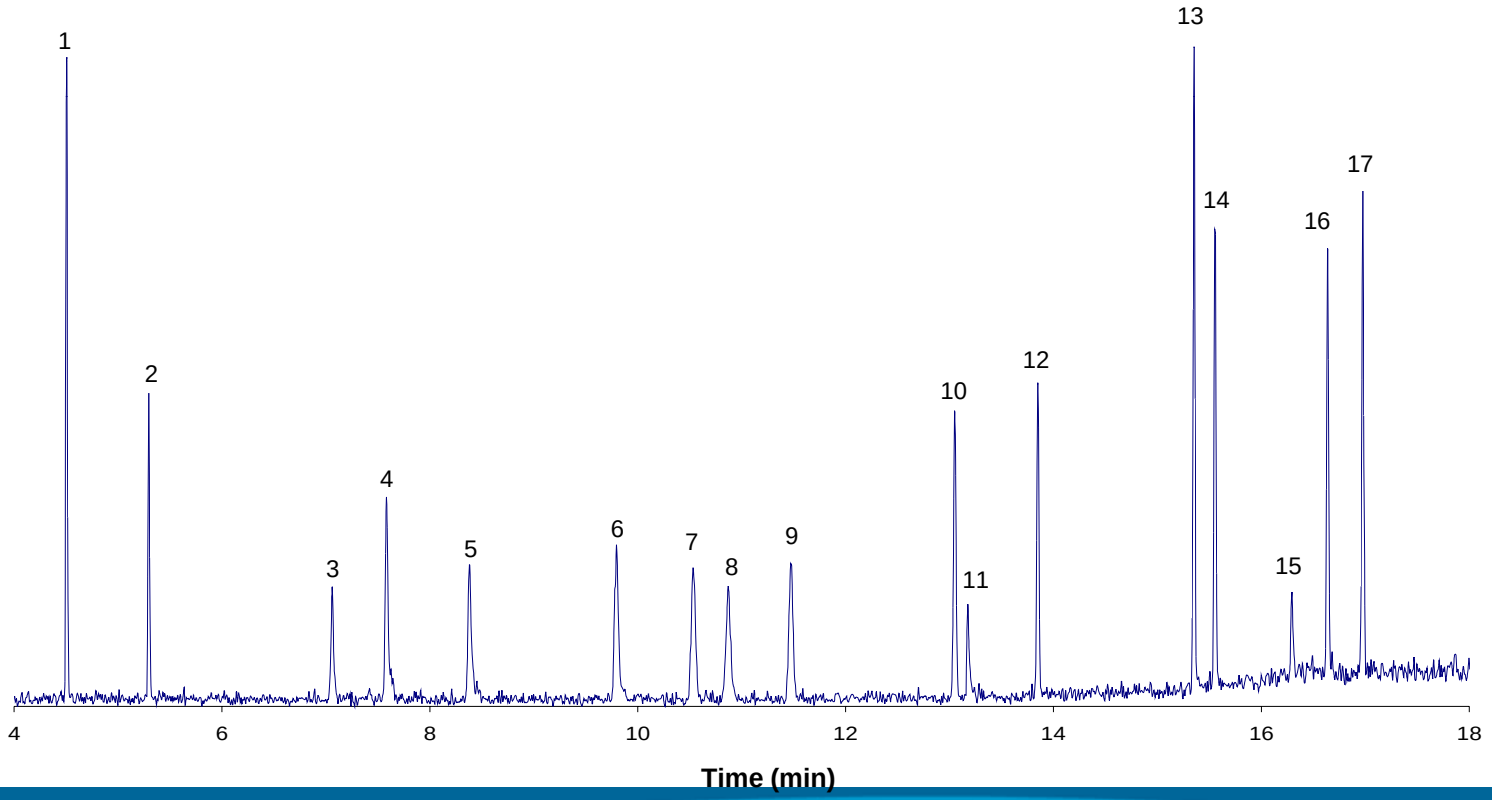
Application Examples

- 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 µm (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:	**transfer line 300 % C, source 280 % C, quad 200 % C, full scan m/z 50-450**

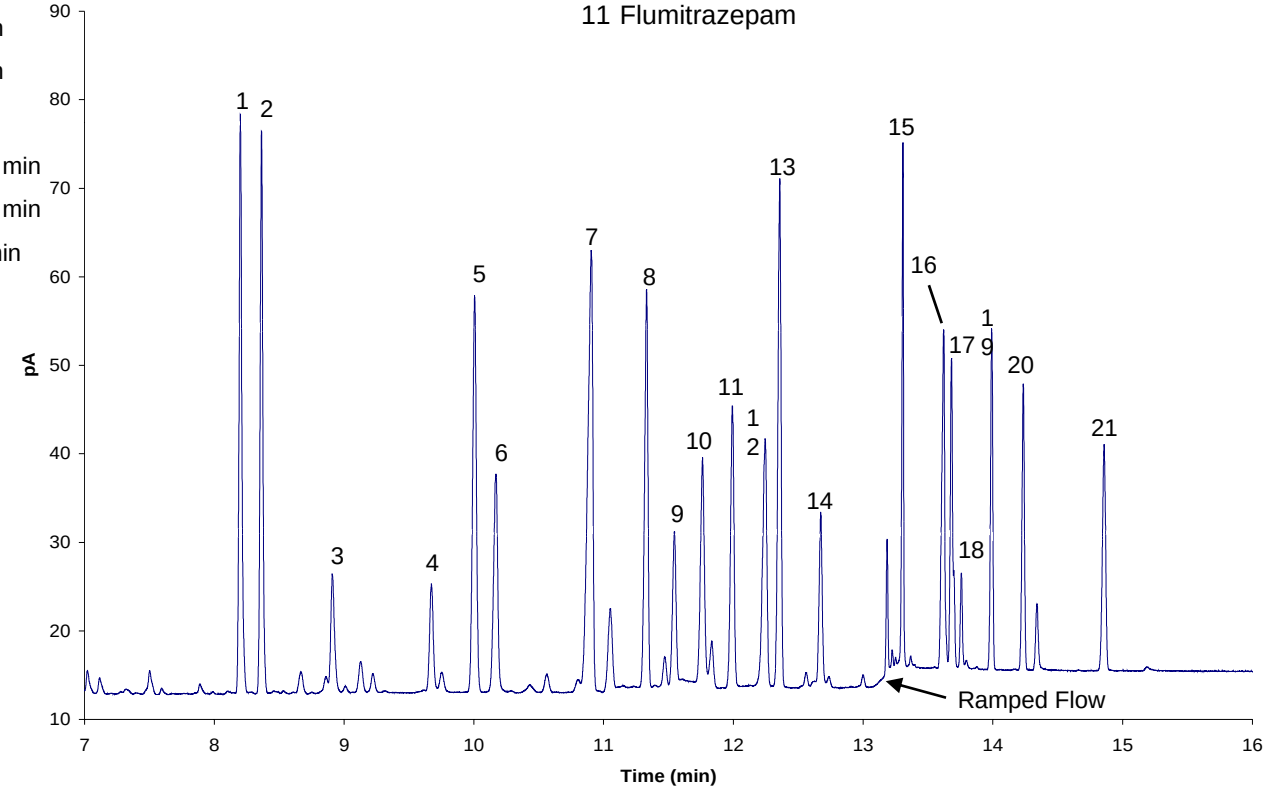
1. **Nicotine**
2. **Phenmetrazine**
3. **Ibupfrofen**
4. **Butabarbitol**
5. **Amobarbitol**
6. **Secobarbitol**
7. **Caffeine**
8. **Benzphetamine**
9. **Hexobarbitol**
10. **Tropacocaine**
11. **Phenbarbitol**
12. **Procaine**
13. **L-cocaine**
14. **Chlorcytizone**
15. **Codine**
16. **Diazpam**
17. **Oxymorphone**



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 Program (mL/min):	1.6 for 11 min
	1.6 to 2.4 at 60 mL/min^2 hold 2 min
	2.4 to 5.0 at 50 mL/min^2 hold 9 min
Oven:	170 °C for 3.2 min
	170-250 °C at 24.7 °C/min, hold 5.3 min
Injection:	250-280 °C at 18.6 °C/min, hold 4.0 min
	280-325 °C at 50.0 °C/min, hold 4 min
Detector:	FID, 350 °C
Sample:	5-10 ng on column
	Pulsed Splitless, 280 °C
	20 psi pulse pressure for 0.38 min
	50 mL/min purge at 0.40 min
	Direct Connect liner G1544-80730

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 Albrazolam
21 Trazolam



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.***

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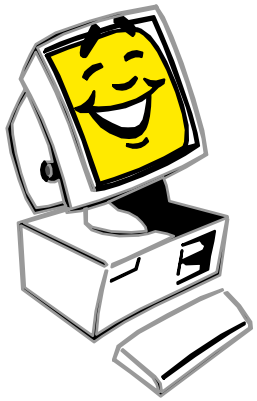

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