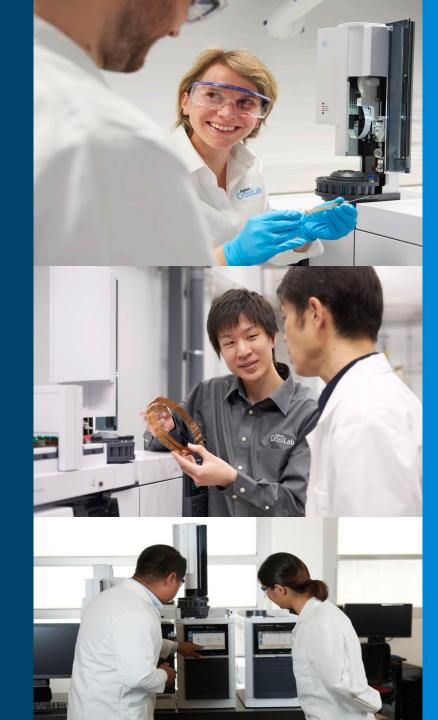
# Building Blocks for a Robust GC Method

Mark Sinnott and Ryan Birney Application Engineers April 23, 2020





### Things to Consider for a Successful Robust Method

The sample

Injection method

Inlet

Carrier gas

Column

Detector

Temperature program



### The Sample

#### Will it fly?

Only 10–20% of all compounds are suitable for GC analysis.

The compounds must have:

- Sufficient volatility
- Thermal stability
- No inorganic acids, bases, or salts.



### The Sample

### **Analyte Composition**

#### What are my analytes?

- Isomers
- Polar versus nonpolar
- Organic acids
- Light gases
- Noble gases
- Halogens



### The Sample

#### Matrix – residue or dirt?

#### Residue

- Semivolatile
  - Bake out
  - Back flush
- Nonvolatile
  - Frequent inlet maintenance
    - Liner, seal, trim column
  - Guard column
  - Back flush

#### Dirty samples

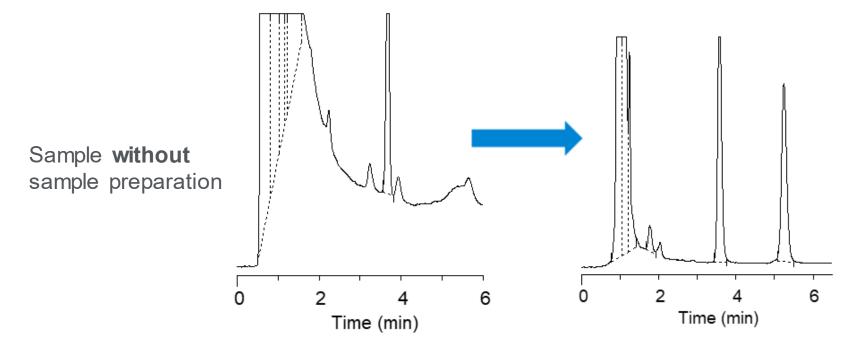
- Sample cleanup
- Back flush



### Importance of the Correct Sample Preparation/Cleanup

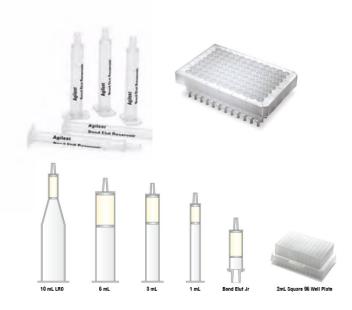
Target analytes are the needle in the haystack of a matrix, sample preparation helps find the needle in the haystack.

- Protect the instrument detection system from contamination
- Improve the detection, method robustness, and reliability
- Reduces frequency of GC maintenance
  - "Pay-me-now or pay-me-later"



Sample with sample preparation

### Offline Options for Sample Matrix Removal



Bond Elut Solid Phase Extraction cartridges and plates



Filter vials – Mini-Uni Prep





SPME (Arrows)



Captiva EMR-Lipid filtration cartridges and plates



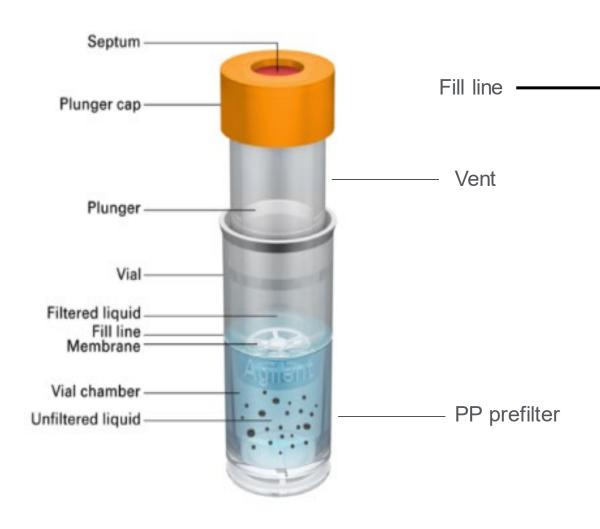
Chem Elut S



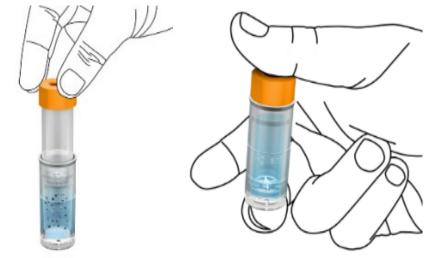
Captiva syringe filters



### Filtration – Captiva Filter Vials



See appendix for solvent compatibility poster request



Description	Part Number
PTFE filter vial, 0.45 µm, 100/pk	5191-5933
PTFE filter vial, 0.20 µm, 100/pk	5191-5934
Nylon filter vial, 0.45 µm, 100/pk	5191-5935
Nylon filter vial, 0.20 µm, 100/pk	5191-5936
RC filter vial, 0.45 µm, 100/pk	5191-5939
RC filter vial, 0.20 µm, 100/pk	5191-5940
PES filter vial, 0.45 µm, 100/pk	5191-5941
PES filter vial, 0.20 µm, 100/pk	5191-5942
Vial closure tool	5191-5943

www.agilent.com/chem/filtervials

Filter vials user guide: 5994-0814EN

### Filtration – Targeted Filtration

#### Captiva EMR-Lipid

- One of the newest Agilent sample cleanup products with the 2-in-1 benefit of removing proteins and lipids.
- It reduces ion suppression, increases analyte sensitivity, improves peak shape, and extends the lifetime of your analytical column.
- Simple pass-through format, 96-well plate, 1 mL, 3 mL, and 6 mL cartridges
- Solvent-retention frit in 1 mL cartridge/96-well plate for in-well protein precipitation
- Unique chemistry and filtration ensure protein and lipid removal
- Depth filtration design allows for smooth elution
- Received the Analytical Scientist Innovation Award (TASIA) of 2017





### SPME Fiber and Arrow Offering from Agilent

#### Solid phase microextraction (SPME)

- Environmental analyses of water samples
- Odor analyses (ppt)
- Flavor analyses of food products
- Forensic analyses of arson/explosives samples
- Toxicology analyses: blood alcohol or drugs in urine/serum
- Surfactants, other industrial applications

- Trace analysis in food
- Drugs and pharmaceuticals
- Herbicides/pesticides
- Medical diagnostics
- Trace impurities in polymers and solid samples
- Solvent residue in raw materials
- **Explosives**



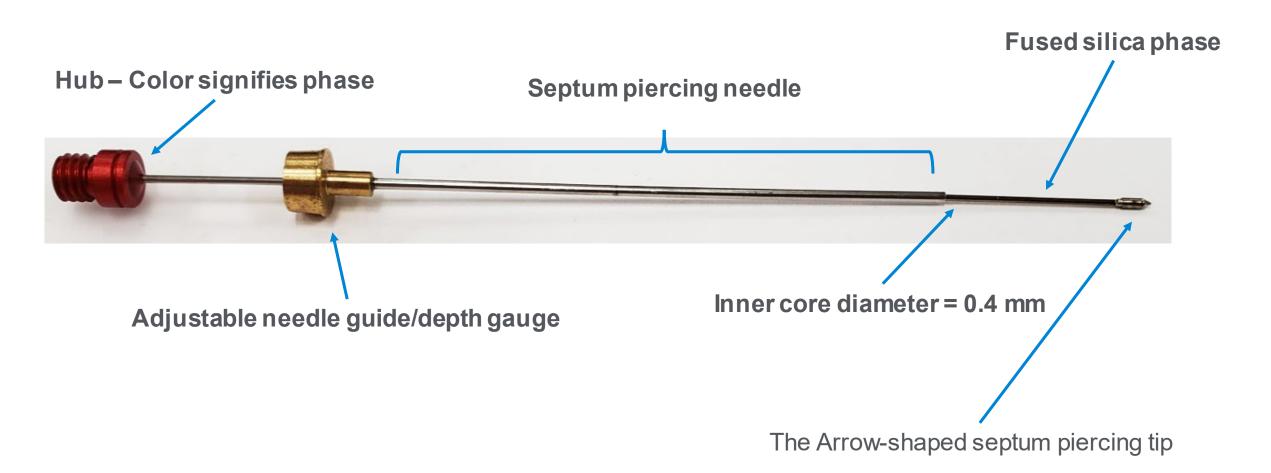


SPME Arrows

#### SPME Fiber & Arrow Characteristics

Solid phase microextraction (SPME)

Parts of the SPME Arrow:



### Injections

### Method of Injection

Manual injection

Liquid injection

Headspace

Purge & trap

Gas sampling valve

SPME

Thermal desorption



#### Liners

#### Purpose of liners

• Provide an "inert" space for liquid samples to vaporize

#### Key aspects

- Liner Volume
- Treatment or deactivation
- Special Characteristics (glass wool or frit, cup, taper, etc.)
- Type of Injection



#### Liners - volume

Choose a liner with enough volume to accommodate the vaporized sample

- Especially important for polar solvents with large vapor volumes
- If vapor volume exceeds liner volume, samples may backflash.
  - May cause ghost peaks and reproducibility issues.

Agilent liners are primarily 2 or 4 mm in inner diameter and 78 mm long.

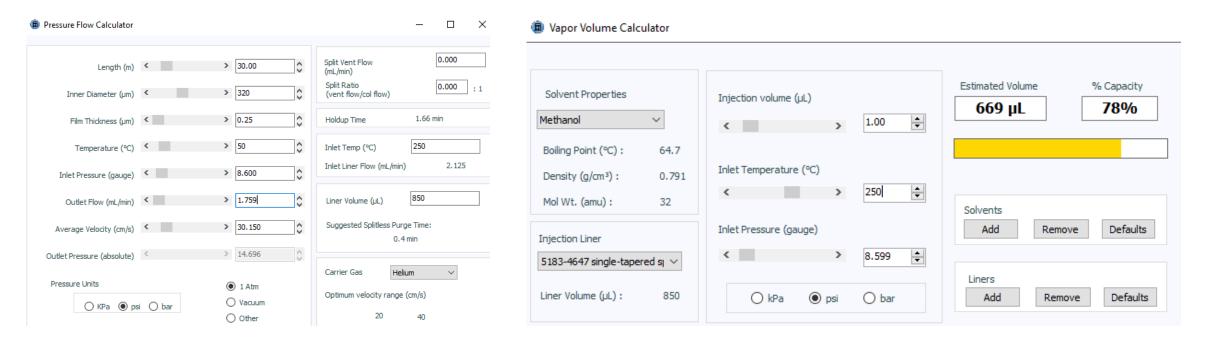
- Therefore:
  - 2 mm liners hold ~ 0.245 mL or 245 µL of vapor
  - 4 mm liners hold ~ 0.972 mL or 972 µL of vapor

Recommended injection volumes are 1–2 µL or less for organic solvents and 0.5 µL for water.

#### Liners - volume

How do we calculate the vapor volume?

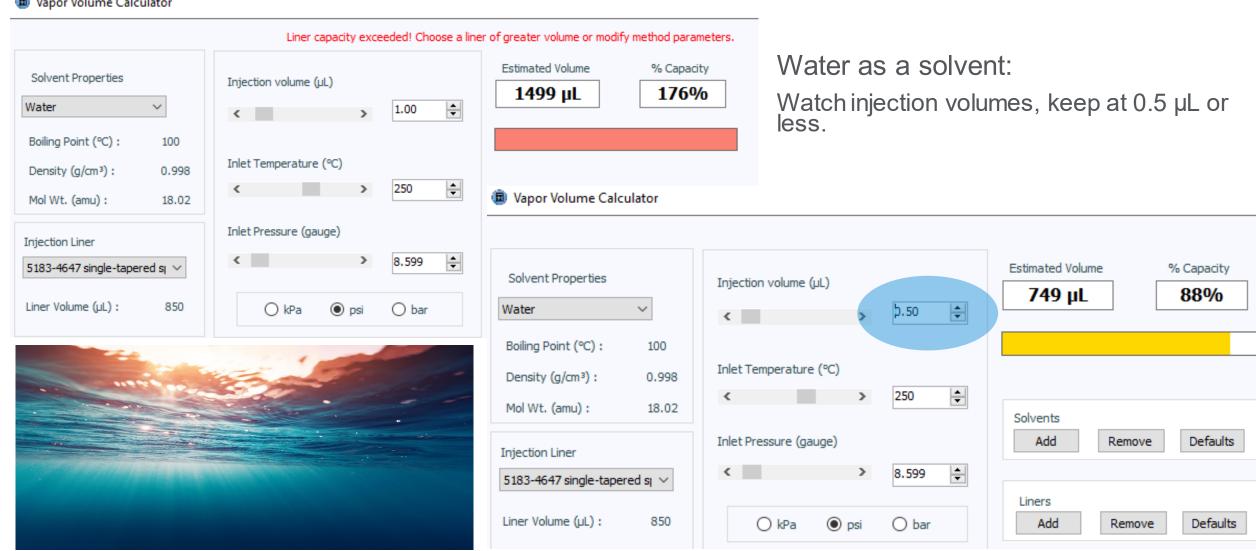
- Pressure / Flow calculator
  - Free download from https://www.agilent.com/en-us/support/gas-chromatography/gccalculators
  - Also built in to recent versions of Agilent GC software





#### Backflash

Vapor Volume Calculator



#### Liners - deactivation

Minimize adsorption of active compounds to surfaces

- Unwanted adsorption can lead to poor peak shape and lower response
  - Deactivated liners are usually treated with a silylating reagent

Agilent has a few different deactivation options:

- Ultra inert
- Original
- None





#### Liners – Special Characteristics

Some liners have special features required for different injection techniques

- Taper (gooseneck) minimize sample contact with gold seal
- Dual taper minimizes contact with gold sea, inlet weldment, and reduces potential for backflash
- Glass wool/frit Prevents nonvolatiles from reaching column, helps with vaporization of heavier compounds, and can help to remove residual sample from needle (split liners)
- Jennings cup Used for sample mixing in split inlets, reduces sample discrimination, prevents nonvolatiles from reaching the column. For clean samples
- Press fit (direct) connection Bottom is designed to hold capillary column firmly (almost all sample) goes onto the column). Side hole required for EPC with Direct Connect liners.
- Others
  - Baffles, spiral paths, laminar cups, column packings with stationary phase
    - All provide a turbulent sample flow path for mixing, a way to collect high molecular weight sample components or particles, surface area to allow efficient vaporization of sample components.

#### Liners – wool/glass frit

#### Placement

- Near the top
  - Wipes syringe needle
  - Can improve injector precision
  - Helps to prevent backflash
  - Assists in volatilization of heavy compounds in split injections
- Near the bottom
  - Helps in volatilization of heavy compounds in splitless injections
  - Increases mixing
- Both
  - Prevent particulates from getting onto the column head

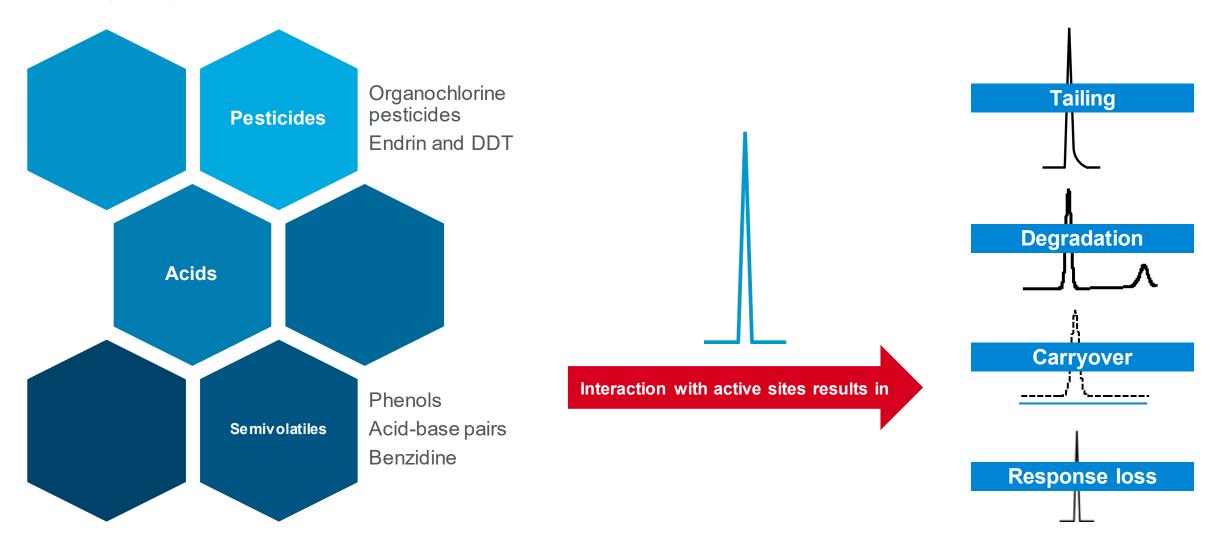






#### The Benefits of the Glass Frit

Dislodged glass wool fibers expose active sites that interact with sensitive analytes





Inlet	Column	Mode	Sample Concentration	Comments	Sample to Column
Split/ Splitless	Capillary	Split Purged split Splitless Purged splitless	High High Low Low	Most commonly used inlet. Very flexible	Very little Very little All All
Cool-on-column	Capillary	N/A	Low or labile	Minimal discrimination and decomposition	All
Packed	Packed large capillary	N/A N/A	Any Any	OK if resolution is not critical	All All
Programmed temperature vaporization	Capillary	Split Pulsed split Splitless Pulsed splitless Solvent vent	High High Low Low	Not great for hot injections  Can concentrate analytes and vent solvent	Very little Very little All All Most
Volatiles interface	Capillary	Direct Split Splitless	Low High Low	Purge and Trap/Headspace	All Very little All
Multimode	Capillary	Split Pulsed split Splitless Pulsed splitless Solvent vent	High High Low Low Low	Flexibility of standard SSL inlet and PTV	Very little Very little All All Most

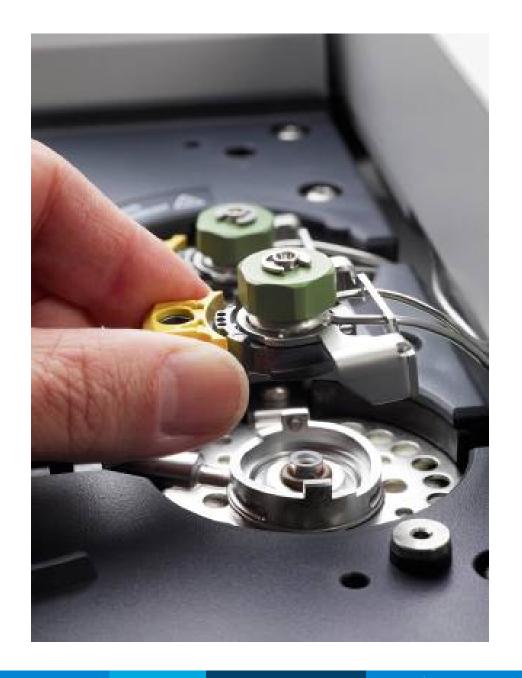


#### Overview

Small Fraction of the sample is introduced into the column

Used for high concentration samples

Superior injection efficiency = narrow peaks = high resolution



#### Major variables

**Split ratio** – determines the fraction of sample on-column and efficiency of injection (sensitivity versus peak width)

**Liner** – influences efficiency of vaporization/discrimination

**Temperature** – hot enough to vaporize sample without degradation or causing backflash

**Injection volume** – typically 0.2–2 µL, increasing it does not have as much of an effect as one might think (smaller is usually always better provided you can meet RSD requirements)

#### Split ratios

- Too low
  - Poor peak shape
  - Column overload
  - Inlet shut down\*
- Too high
  - Poor sensitivity
  - Wastes carrier gas (use gas saver!)
- Usually nonlinear
  - Cannot use split ratio as a "dilution factor"

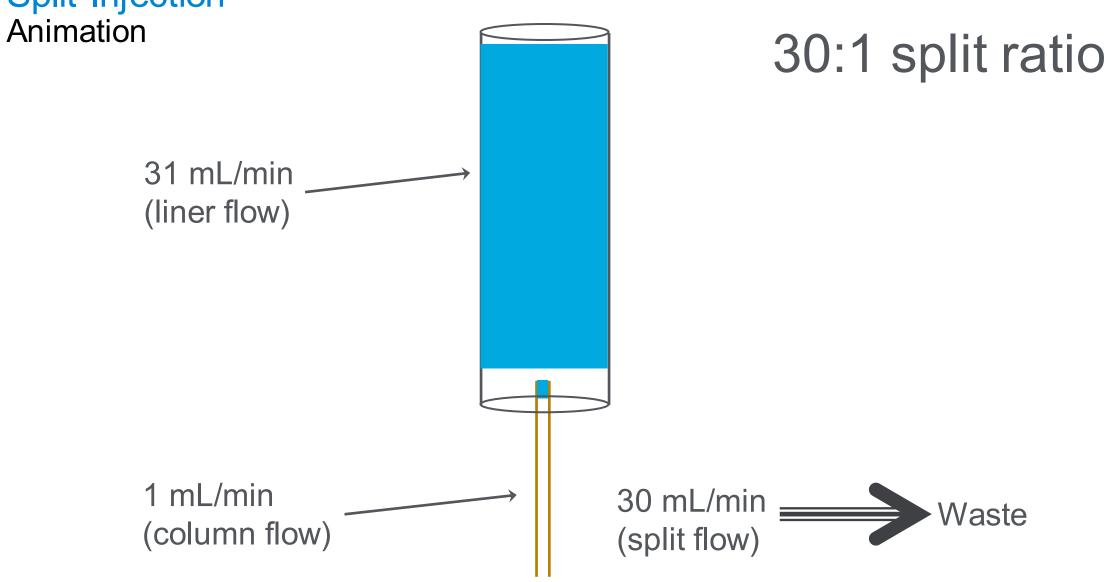
	ld (mm)	Lowest ratio*
High	0.10	1:50 - 1:75
Higher flow rates	0.18 - 0.25	1:10 - 1:20
rates□	0.32	1:8 - 1:15
	0.53	1:2 - 1:5



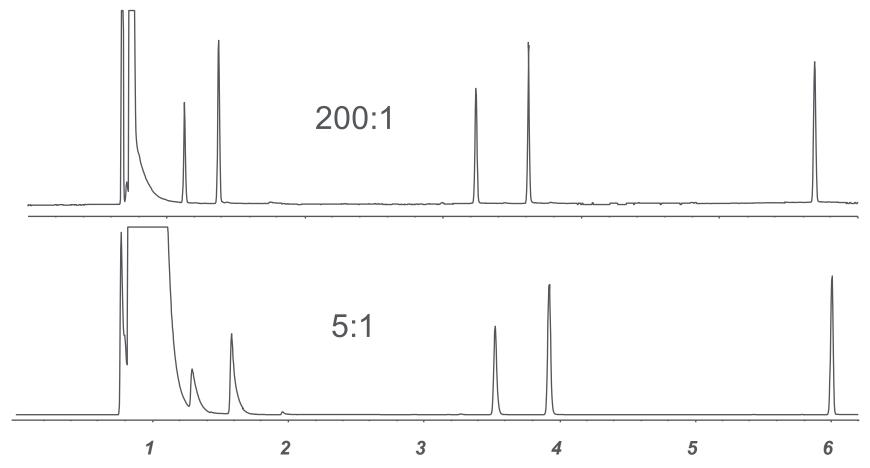
<sup>\*</sup>keep total inlet flow ≥ ~20 ml/min to prevent inlet shut-down

### Liners – split injection

Liner	Part Number Each 5/pk 25/pk	Comments
	5190-2294 5190-3164 5190-3168	Simplest split liner, glass wool, UI deactivation, large volume (990 $\mu$ L). Use for general purpose, can be used in splitless mode
Glass nub	5190-2295 5190-3165 5190-3169	Glass wool, UI deactivation, 870 µL volume. Glass nub ensures that a gap remains below liner for split injection. Efficient for most applications
	5190-5105 5190-5105-005 5190-5105-025	Sintered glass frit, UI deactivation. Ideal for basic drugs analysis. Sintered glass frit more reproducible than glass wool.
	18740-80190	Liner with Jennings cup, no wool. 800 µL volume. Reduces inlet discrimination.



### Split Injection - 200:1 versus 5:1



DB-1, 15 m x 0.25 mm id, 0.25  $\mu$ m 60 °C for 1 min, 60–180 °C at 20°/min; Helium at 30 cm/sec 1. n-heptane 2. toluene 3. n-decane 4. n-butylbenzene 5. n-tridecane

More challenging the SPLIT

Most of the sample is introduced into the column

Used for low concentration samples

Poor injection efficiency = wider peaks = less resolution

Sample refocusing may be necessary

#### 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 most of the analytes and solvent are introduced to the column
- Later, the split vent is opened and residual solvent is vented (purge time/flow)
- Timing, carrier/split vent flows, and the 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
- Typical splitless parameters:
  - Purge flow of 50 mL/min
  - Purge time of 0.5–2.0 minutes



#### Major variables

**Purge activation time** – determines amount of sample onto column and efficiency of injection (sensitivity versus peak shape)

**Liner** – preventing backflash more critical than vaporization properties (liner volume, tapers, and wool are less important...)

**Injection volume** – typically 1 μL or less (backflash: 0.5 μL max for water!)

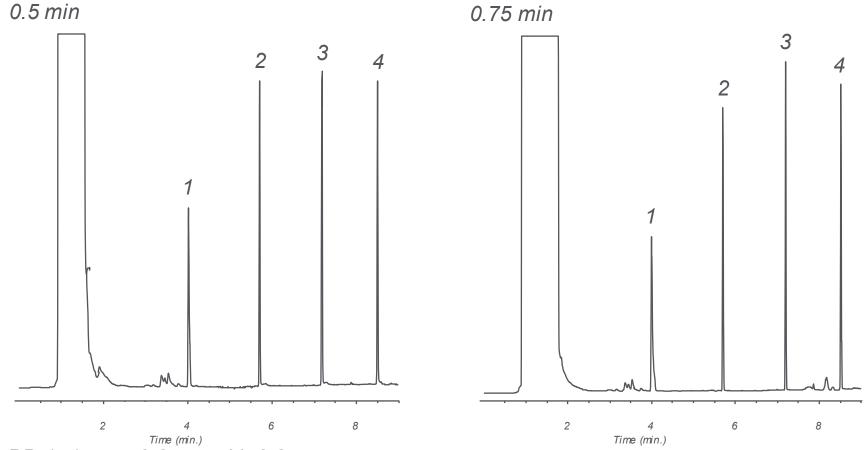
**Temperature** – long residence times allow for lower temperatures

### Liners – splitless injection

Liner	Part Number Each 5/pk 25/pk	Comments
	5190-2292 5190-3162 5190-3166	Single taper, UI deactivated, 900 µL volume. Taper isolates sample form gold seal, reducing breakdown of active compounds. Trace samples, general applications.
	5190-2293 5190-3163 5190-3167	Single taper, UI deactivated, glass wool, 900 $\mu$ L volume. Glass wool aides with volatilization of heavier compounds and protects the column. Trace, dirty samples.
	5190-5112 5190-5112-005 190-5112-025	Singer taper, UI deactivated, sintered glass frit. Glass frit acts like glass wool but is more reproducible.
	5190-3983 5190-4007 ****_****	Double taper, UI deactivated, 800 µL volume. Taper on inlet reduces backflash. High efficiency for trace, active samples.
•	5190-7011 (5/pk) 5190-7012 (5/pk) 5190-7013 (5/pk) 5190-7014 (5/pk) 5190-7020 (5/pk)	Direct Connect liners, single and dual taper, original deactivation. Column press fits into liner. Focuses almost all sample onto column and reduces exposure to inlet. Ultimate for trace, active samples. Various hole placements for use with EPC

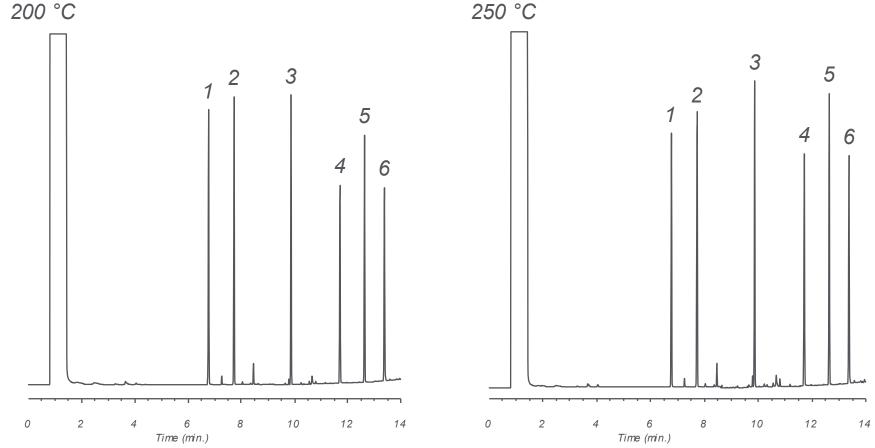
## Splitless Injection Purge flow 30 mL/min Animation Purge Time 0.5 min 1 mL/min (liner flow) 30 mL/min (split flow) 1 mL/min Waste (column flow)

### Purge activation time



DB-1, 15 m x 0.25 mm id, 0.25 μm 60 °C for 1 min, 60-180 °C at 20°/min; Helium at 30 cm/sec 1. n-decane 2. n-dodecane 3. n-tetradecane 4. n-hexadecane

#### Injector temperature



DB-1, 15 m x 0.25 mm id, 0.25 μm 50 °C for 0.5 min, 50-325 °C at 20°/min; Helium at 30 cm/sec

Phthalates: 1. dimethyl 2. diethyl 3. dibutyl 4. benzyl butyl 5.bis(2-ethylhexyl) 6. dioctyl



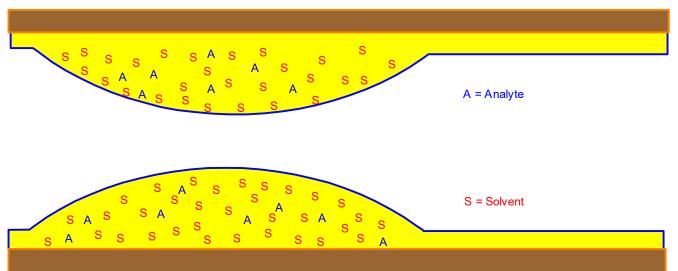
#### Sample refocusing

Sample refocusing improves efficiency

Use low column temperature to refocus solvent - called the *solvent effect* 

Use cold trapping

### The Splitless "Solvent" Effect



The initial oven temperature is set below the boiling point of the solvent.

The solvent condenses at the head of the column swelling the stationary phase and trapping the analyte.

Solvent	Boiling point (°C)	Initial oven temperature (°C)
Dichloromethane	40	10–30
Chloroform	61	25-50
Carbon disulfide	46	10-35
Diethyl ether	35	10–25
Pentane	36	10–25
Hexane	69	40–60
lso-octane	99	70–90

Solvent and stationary phase must be compatible

## Splitless injection

### Solvent effect

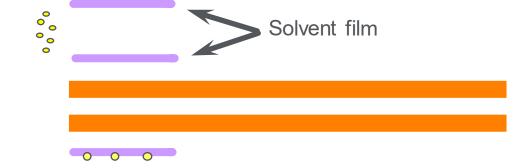
Initial column temperature at least 10 to 20 **°C below** sample solvent boiling point



Required to obtain good peak shapes unless cold trapping occurs

Rule of thumb, if solute boiling point > 150 °C above initial column temperature, the solute will cold trap

Cold trapping has greater efficiency than solvent effect



3.

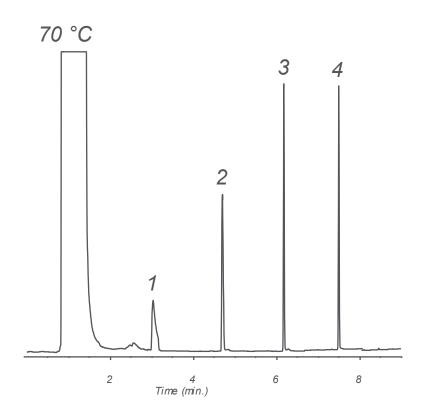


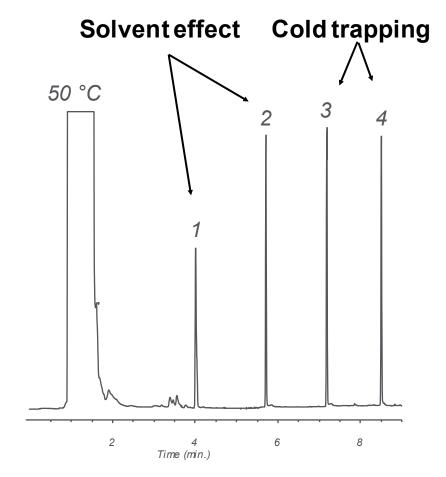
0 0 0

## Splitless Injection

### Initial column temperature

Hexane Solvent (boiling point = 68–69 °C)





DB-1, 15 m x 0.25 mm id, 0.25 μm 50 °C or 70 °C for 0.5 min, to 210 °C at 20 °C/min; Helium at 30 cm/sec 1. n-decane 2. n-dodecane 3. n-tetradecane 4. n-hexadecane

## GC Column - Selectivity

### Selecting the correct column

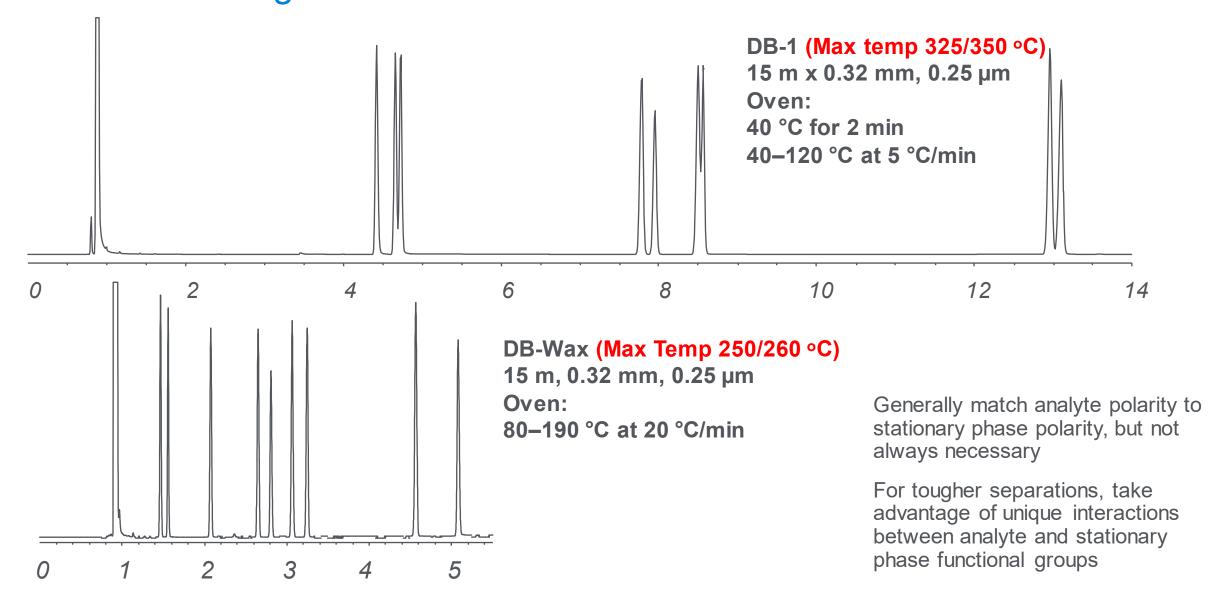
- Use pre-existing methods as a reference
  - Application notes, posters, established methods
- Column temperature limits are a good indicator of over-all column stability (non-polar are more stable than polar)
- Choose the most non-polar/stable column available that will still resolve your analytes
- For more complex mixtures, match analyte polarity to column polarity (i.e. "like dissolves like.")
- For even tougher separations, look for unique interactions that analytes may have with a phase

#### Use the Agilent GC Application Support Team

- gc-column-support@agilent.com
- 800-227-9770; options 3, 3, 1



## Start with the Right Phase – "Like Dissolves Like"



## "New" J&W DB-HeavyWAX

### The WAX Column You've Been Waiting For!

- ✓ Increased temperature range
  - √ 280°C Isothermal
  - √ 290 °C Programmed
- ✓ Increased Thermal stability
- ✓ Lower Bleed



www.agilent.com/chem/db-heavywax



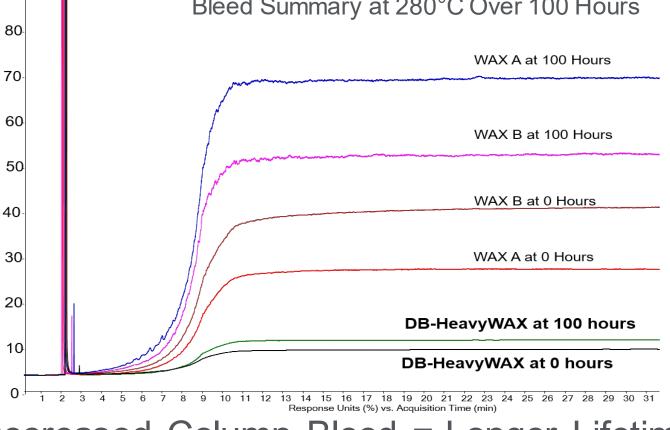
## Benefits of the J&W DB-HeavyWAX

- Increased Thermal Stability
  - Stable Retention Times
  - Consistent Peak Order
- Deceased Column Bleed
  - Greater sensitivity for "heavier" compounds 70
  - Increase analyte range
  - Decrease analysis time
  - Safely bake out column
    - ➤ Up to 290°C
- Behaves like a WAX because it is a WAX
  - Simpler method translation

## **Increased Temperature Range**

- √ 280°C Isothermal
- ✓ 290 °C Programmed

Bleed Summary at 280°C Over 100 Hours



Increased Thermal Stability + Decreased Column Bleed = Longer Lifetime



90

### GC Column - Dimensions

### Selecting the correct column

Larger diameters columns have more capacity, but they are less efficient (wider peaks = less resolution)

• Longer columns are more efficient, but result in longer run-times

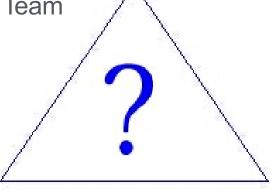
Narrower columns are more efficient, but have less capacity

Thicker film columns will help resolve volatiles/early eluting analytes

Again, use the Agilent GC Application Support Team

gc-column-support@agilent.com

• 800-227-9770; options 3, 3, 1.



Speed



Capacity

Resolution

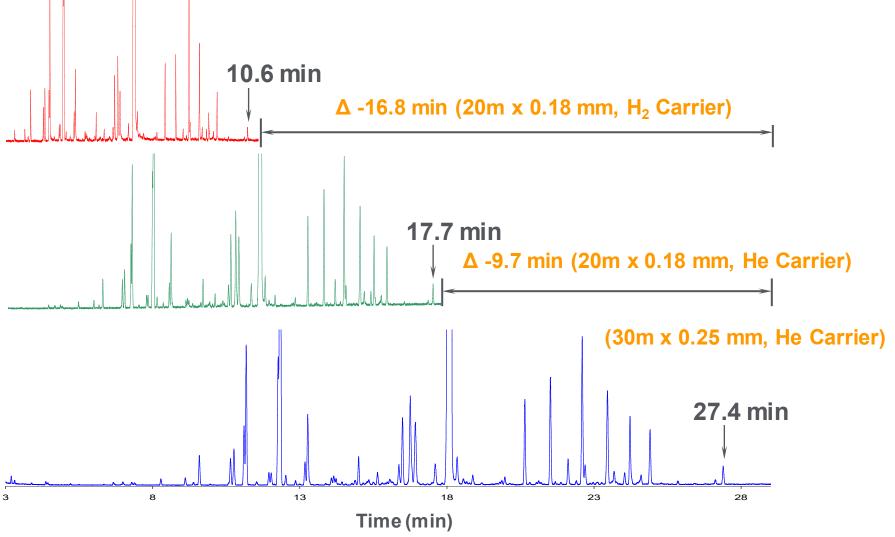
#### Column dimensions webinar:

https://agilenteseminar.webex.com/agilenteseminar/lsr.php?RCID=b21639d8d4abf46753fe3a1af3d2743b

#### **Fast GC Webinar:**

https://agilenteseminar.webex.com/agilenteseminar/lsr.php?RCID=bbe62a3a03e5d36464af090a64e4db61

## The power of manipulating column dimensions/carrier gas



https://agilenteseminar.webex.com/agilenteseminar/onstage/playback.php?RCID=87b891d4848654caf821c246b26918fd https://agilenteseminar.webex.com/agilenteseminar/onstage/playback.php?RCID=2e666bb9c62e4f623b44061a3480f748

## Carrier Gas Considerations

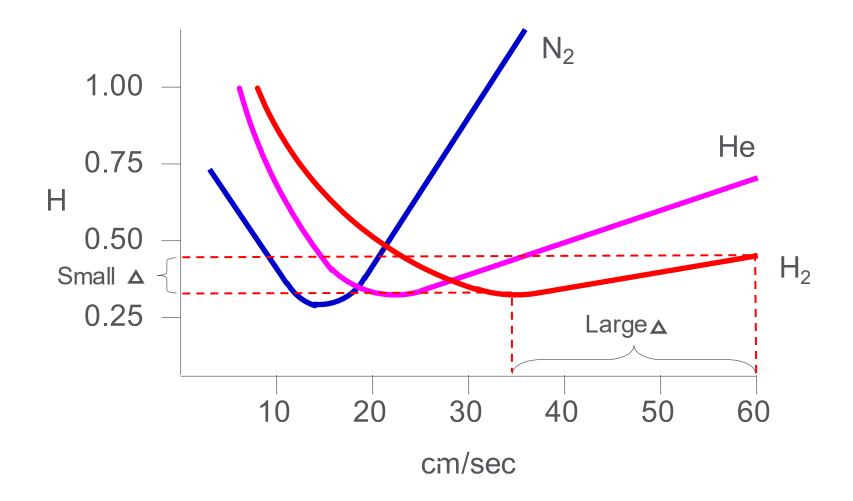
Carries the solutes down the column





Selection and velocity influences efficiency and retention time

## Van Deemter Curves



### Carrier Gas

#### Helium

- Reasonably fast analysis
- Good sensitivity with mass spec
- Relatively expensive

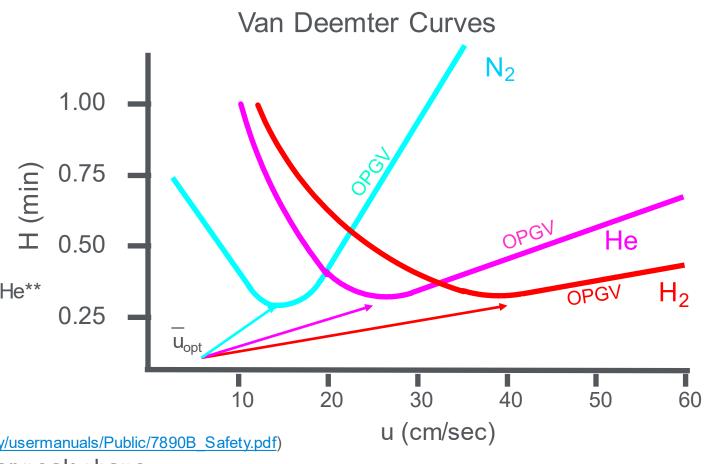
#### Nitrogen

- Cheapest carrier gas
- Can use nitrogen generator
- Slowest speed of analysis
- Smaller Van-Deemter "sweet-spot"
- Can't use with MSD analysis
  - Can be used for periods of non-use to conserve He\*\*

#### Hydrogen

- Fastest speeds possible
- Can use hydrogen generator
- Fairly inexpensive
- $\bullet \ \ Safety\ concerns\ in\ labs\ (\underline{\tt https://www.agilent.com/cs/library/usermanuals/Public/7890B\_Safety.pdf})$
- Lower sensitivity with mass spec, possible poor peak shape
- Can be used with MSD, but need to be very careful and generally not recommended\*\*

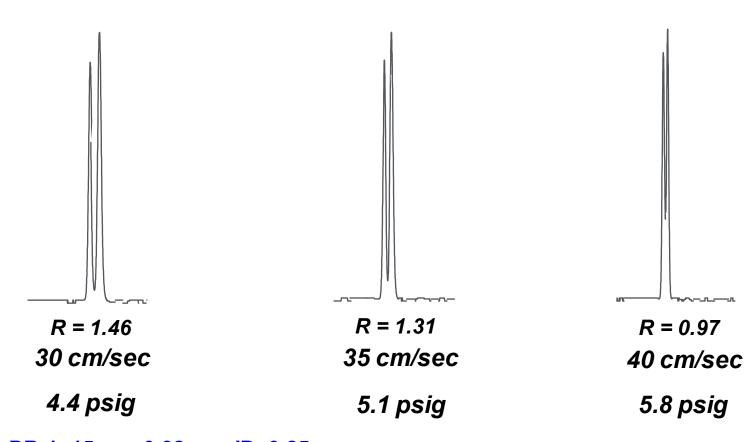




## Resolution versus Linear Velocity

### Helium

Resolution of 1.5 = baseline resolution



DB-1, 15 m x 0.32 mm ID, 0.25 um 60 °C isothermal 1,3- and 1,4-Dichlorobenzene

## **Detectors**

## A quick overview

Detector	Dynamic Range	Type of Detection	MDL
TCD	10 <sup>5</sup>	Universal	400 pg Tridecane
FID *	10 <sup>7</sup>	Responds to C-H bonds	1.8 pg Tridecane
ECD	5x10 <sup>5</sup>	Responds to free electrons	6 fg/mL Lindane
NPD	10 <sup>5</sup>	Specific to N or P	0.4 pgN/s 0.06 pg P/s
FPD	10 <sup>3</sup> S, 10 <sup>4</sup> P	Specific to S or P	60 fg P/s 3.6 pg S/s
SCD	104	Specific & Selective to S	0.5 pg S/s
NCD	104	Specific & Selective to N	3 pg N/s
MSD		Universal	S/N 400:1 1 pg/µL OFN

<sup>\*</sup> Better sensitivity with N<sub>2</sub> make-up over He

## Developing a Temperature Program





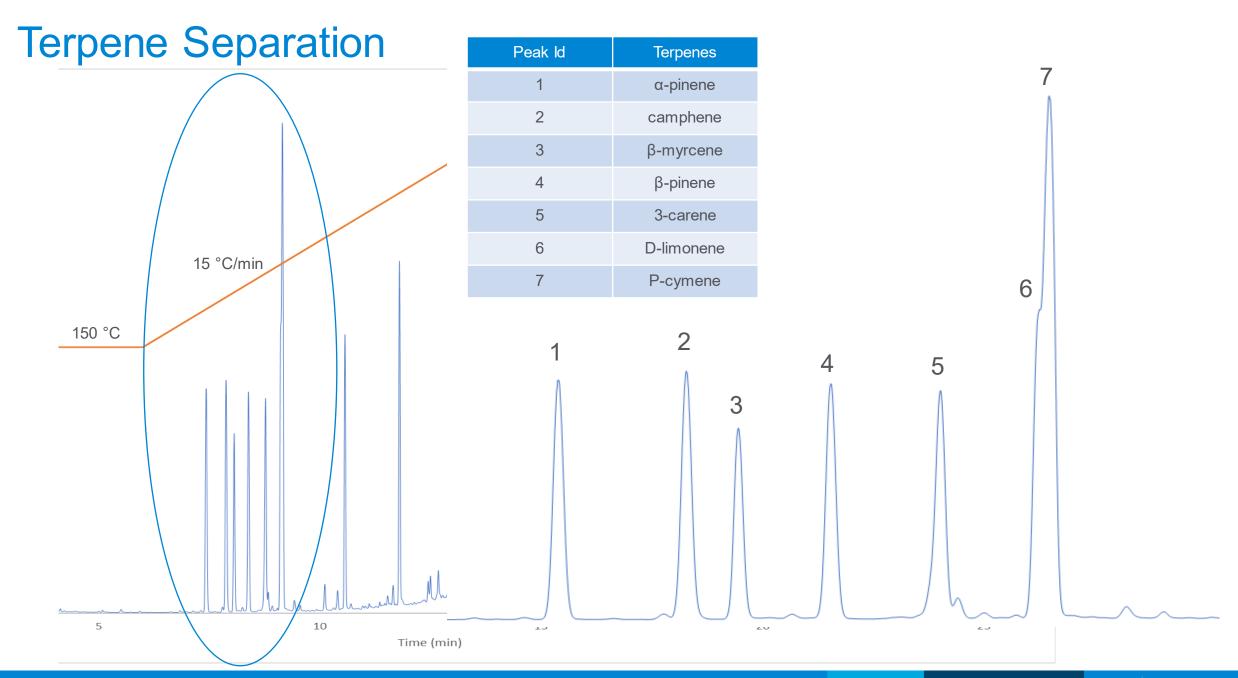
52

# Compound List

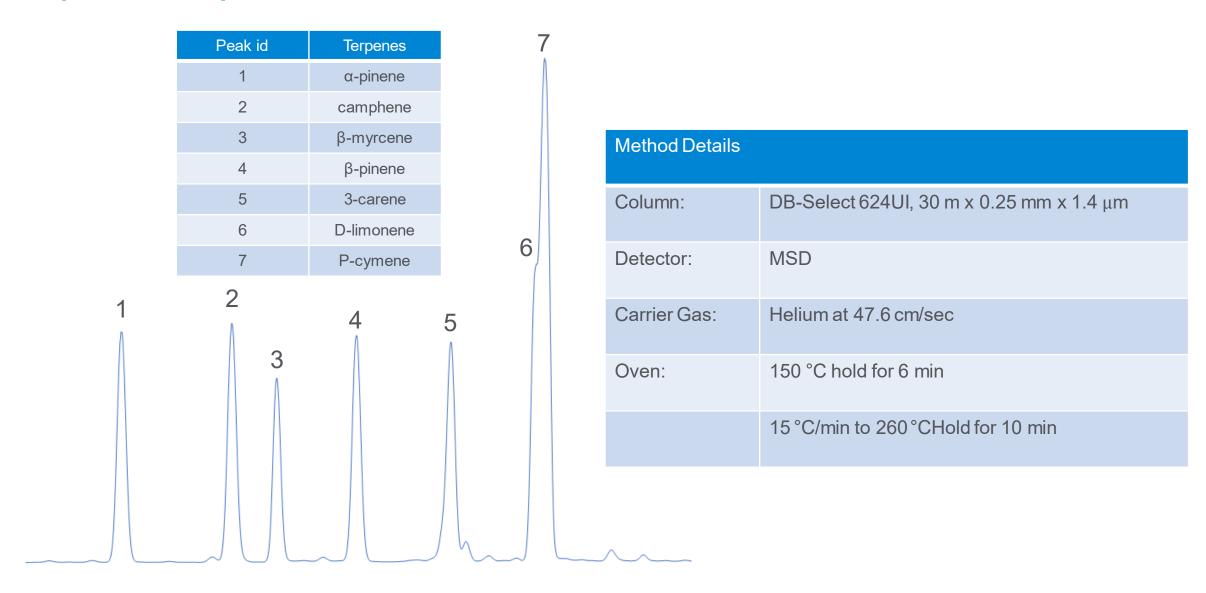


Peak Id	Terpenes
1	α-pinene
2	camphene
3	β-myrcene
4	β-pinene
5	3-carene
6	D-limonene
7	P-cymene

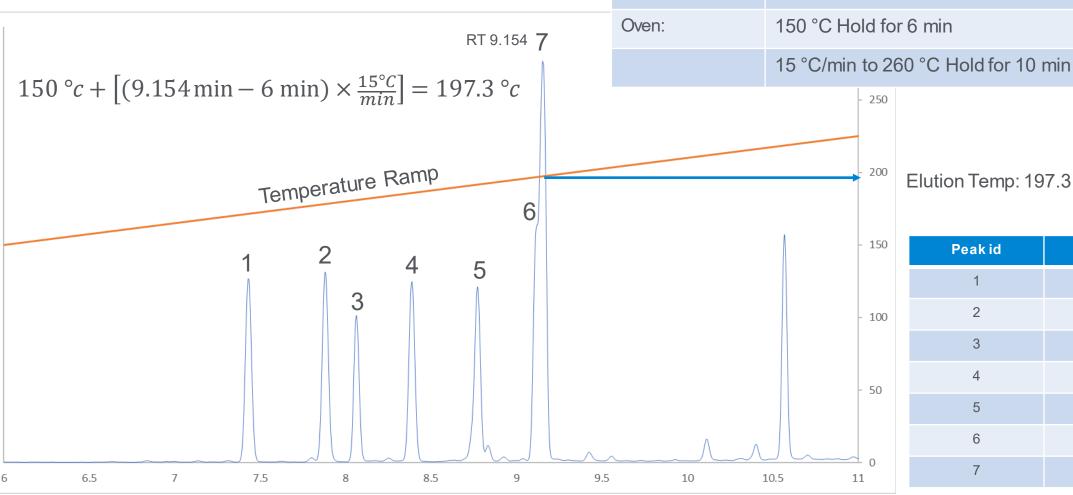




# Terpene Separation



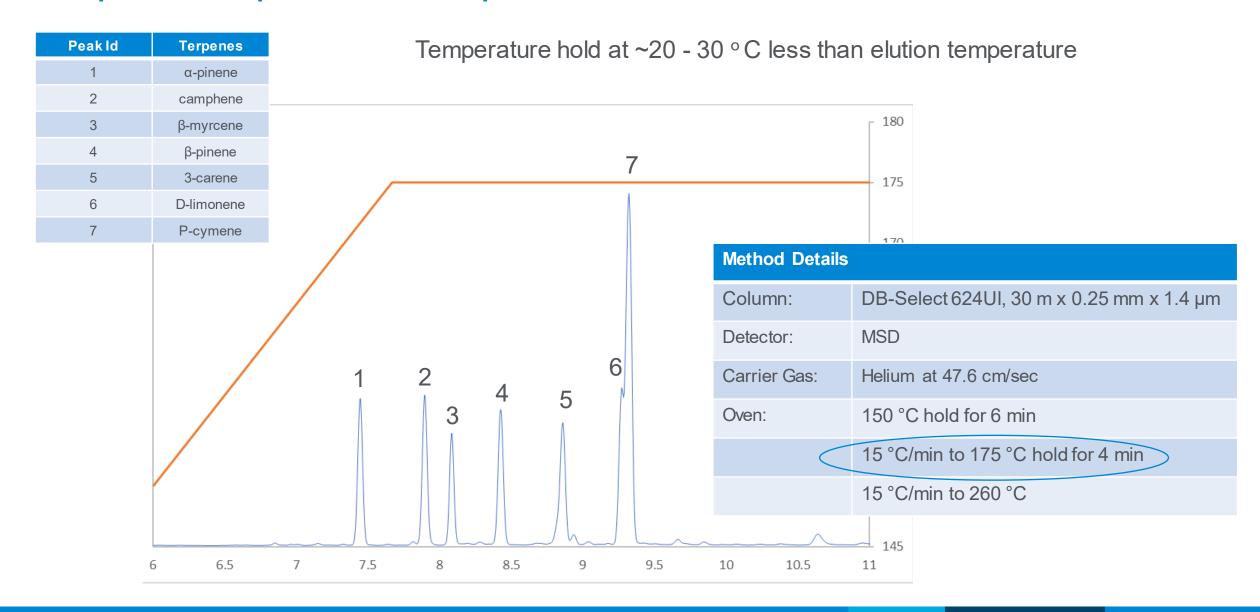
# Terpene Separation

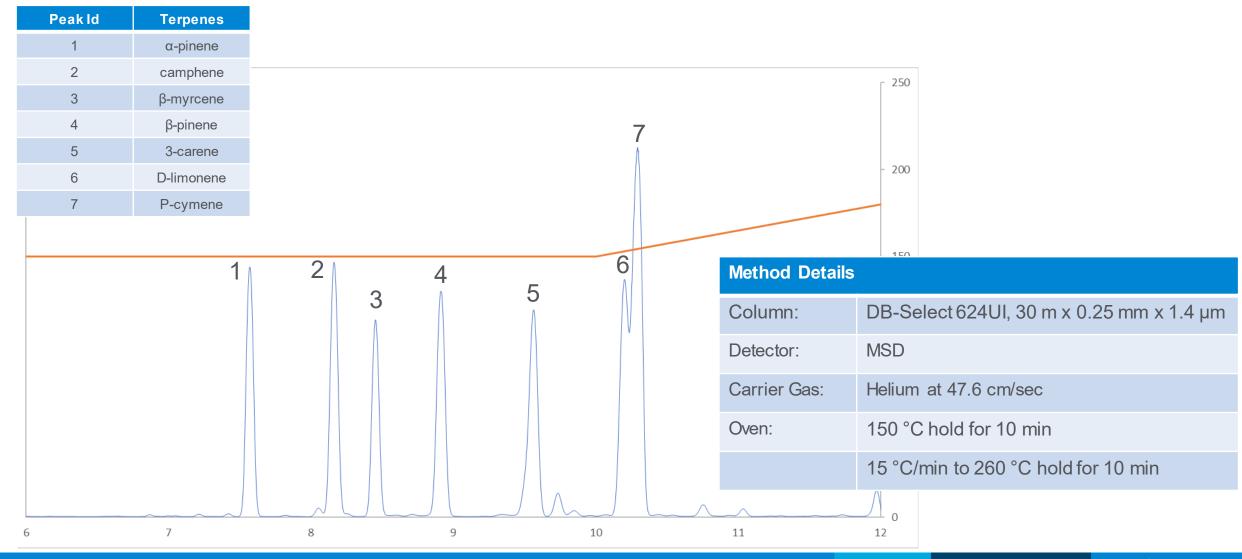


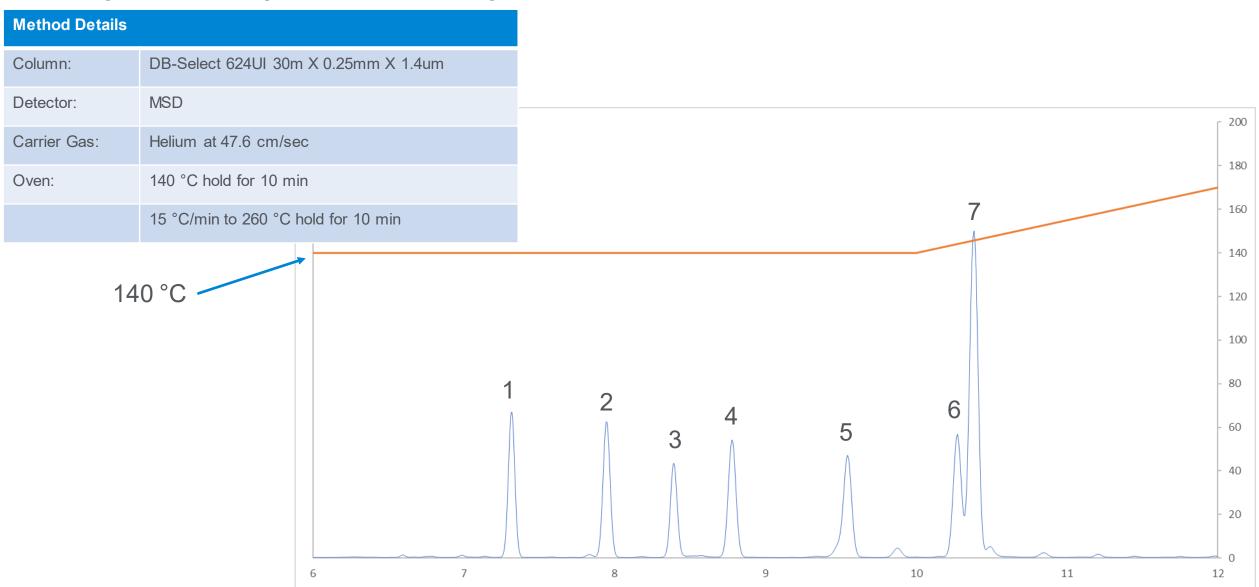


Elution Temp: 197.3 ° C

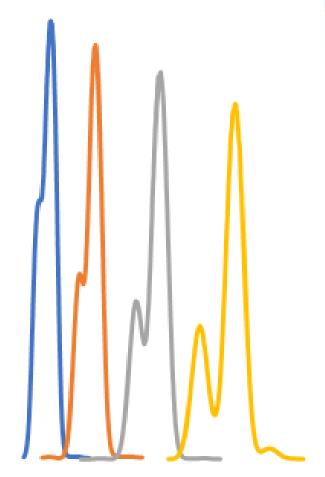
Peak id	Terpenes
1	α-pinene
2	camphene
3	β-myrcene
4	β-pinene
5	3-carene
6	D-limonene
7	P-cymene











Method Details	
Oven:	150 °C hold for 6 min
	15 °C/min to 260 °C hold for 10 min

	Method Details
Oven:	150 °C hold for 6 min
	15 °C/min to 175 °C hold for 4 min
	15 °C/min to 260 °C

Method Details		
Oven:	150 °C hold for 10 min	
	15 °C/min to 260 °C hold for 10 min	

Method Details		
Oven:	140 °C hold for 10 min	
	15 °C/min to 260 °C hold for 10 min	

## Conclusions

Think about the sample first

- Is it "GC-able"?
  - Volatile? Stable?
- Sample composition
- Sample matrix/cleanup: "Pay-me-now or pay-me-later"

Choose inlet parameters based on detection limit requirements

Use information sources first when choosing a column

Choose the most stable column that will still resolve your analytes

Use appropriate carrier gas and optimum velocity

Calculate elution temperature and adjust oven program with appropriate ramps/holds

Make use of Technical Support...we are here to help!!



## Contact Agilent Chemistries and Supplies Technical Support



1-800-227-9770 option 3, option 3: webinars

Option 1 for GC and GC/MS columns and supplies

Option 2 for LC and LC/MS columns and supplies

Option 3 for sample preparation, filtration, and QuEChERS

Option 4 for spectroscopy supplies

Option 5 for chemical standards

Available in the USA and Canada 8–5, all time zones



lc-column-support@agilent.com

spp-support@agilent.com

spectro-supplies-support@agilent.com

chem-standards-support@agilent.com

#### All webinars:

https://www.agilent.com/en/training-events/eseminars/gc-gc-ms-webinars

#### ScanView:

https://community.agilent.com/docs/DOC-2118-software-supported-method-development-the-scanviewprogram

