

# GC Method Development



# What to Consider

The Sample

Method of injection

Inlet

Detector

Carrier Gas

Column



# COMPOUND REQUIREMENTS FOR GC

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

The compounds must have:

- ✓ Sufficient volatility
- ✓ Thermal stability

NO Inorganic Acids and Bases

Be mindful of salts!

# Sample Considerations

1. Sample matrix
  - residues?
  - dirty samples?
2. Analyte Composition
  1. Isomers?
  2. Polar vs. non-Polar?
  3. Organic Acids?
  4. Light Gases?
  5. Noble Gases?
  6. Halogens?

# Sample Residues

## Semi-volatile residues

Bake out

Back flush

## Non-volatile residues

Guard column

Bake out

Back flush

## Dirty Samples

Sample clean up?

Back flush

# Use What You Know About the Analytes

Complex Mixture?

Few analytes?

Homologous Series?

Mixture of polar and non-polar?

Labile analytes?

Volatility?

Gas or Liquid Sample?



**We have a thought about the sample  
...What's next?**



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# Let's Get the Sample Onto the Column...

Manual Injection

Liquid Injection

Headspace

Purge & Trap

Gas Sampling Valve

SPME

Thermal Desorption

Custom





# The Inlet

Volatiles Interface

Cool-On-Column

Purged Packed

PTV

Split / Splitless

Multi-Mode



# Volatiles Interface

Used for 'volatile' samples

Sample is already a vapor

Headspace

Purge & Trap



# Volatiles Interface

Mode	Sample Concentration	Sample to Column	Comments
Split	High	Very little, most is vented	
Splitless	Low	All	Can switch to split mode electronically
Direct	Low	All	Must physically disconnect split vent, plug the interface, and reconfigure the GC. Maximizes sample recovery and eliminates possibility of contamination to pneumatic system.

# Cool-On-Column

- Good for Labile Samples
  - Sample is deposited “ON” the column
  - Temperature of inlet follows Oven Temperature
- Good for ‘Active’ analytes
  - Minimizes inlet discrimination
  - No inlet Liner\*
- Good for Trace Analysis
- High Boiling Point Compounds
  - No Inlet Discrimination
- Guard Column Highly Recommended

# Purged Packed

Good for HIGH flow applications

Used with Packed columns

Can be used with 0.53 mm and 0.32 mm ID columns

**\*\*Has a minimal capacity for sample expansion**

**\*\*Back Flash\*\***



# PTV

## (Programmable Temperature Vaporization)

Mode	Sample Concentration	Sample to Column	Comments
Split	High	Very Little	
Pulsed Split	High	Very Little	
Splitless	Low	All	
Pulsed Splitless	Low	All	
Solvent Vent	Low	All	Multiple injections concentrate analytes and vent solvent.

# Split / Splitless

Mode	Sample Concentration	Sample to Column	Comments
Split	High	Very Little	
Pulsed Split	High	Very Little	Useful with large injections
Splitless	Low	All	
Pulsed Splitless	Low	All	Useful with large injections. *better transfer of sample to column*

# SPLIT INJECTOR

## Split Ratio

- Too low: Poor peak shape
  - Column overload
- Too high: Poor sensitivity
  - Wastes carrier gas (gas saver)
- Usually non-linear
  - Do not use ratio as a dilution factor



# MINIMUM RECOMMENDED SPLIT RATIO

	mm I.D.	Lowest ratio
Higher flow rates ↓	0.10	1:50 - 1:75
	0.18 - 0.25	1:10 - 1:20
	0.32	1:8 - 1:15
	0.53	1:2 - 1:5

\*Want to have 20 mL/min flow through the inlet\*

# Multimode

Mode	Sample Concentration	Sample to Column	Discussion
Split	High	Low	
Pulsed Split	High	Low	
Splitless	Low	All	
Pulsed Splitless	Low	All	
Solvent Vent	Low	All	Multiple Injections concentrate sample and vent solvent
Direct	Low	All	

# Sample Expansion...Liners?

Split / Splitless Inlet

Multimode Inlet

Packed inlet

PTV



# Inlet Liners - Purpose

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

- the solvent type
- column head pressure
- temperature of inlet

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

Solvent (1 $\mu$ L, ambient)	Volume ( $\mu$ L 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.

# Liners - 3 Key Aspects Govern Applications

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



# Liner Volume

Choose a liner with enough volume to accommodate the vaporized sample.

Important, especially for polar solvents with large vapor volumes.

If vapor volume of sample exceeds liner volume, samples may back up (backflash) into carrier gas supply lines, causing ghost peaks and reproducibility problems in chromatography.

## Liner Volume (contd.)

Agilent liners are primarily 2mm or 4mm in inner diameter (without tapers and additional features) and 78mm long.

- Thus, 2mm liners hold approx. 0.245 mL or 245  $\mu$ L of vapor  
4mm liners hold approx. 0.972 mL or 972  $\mu$ L of vapor

Recommended injection volumes are 1-2 $\mu$ L or less for organic solvents, 0.5 $\mu$ L for water.

# Liner Volume

How Do we Calculate the Vapor Volume?

Pressure / Flow Calculator

Free download from our Website

[www.chem.agilent.com](http://www.chem.agilent.com)

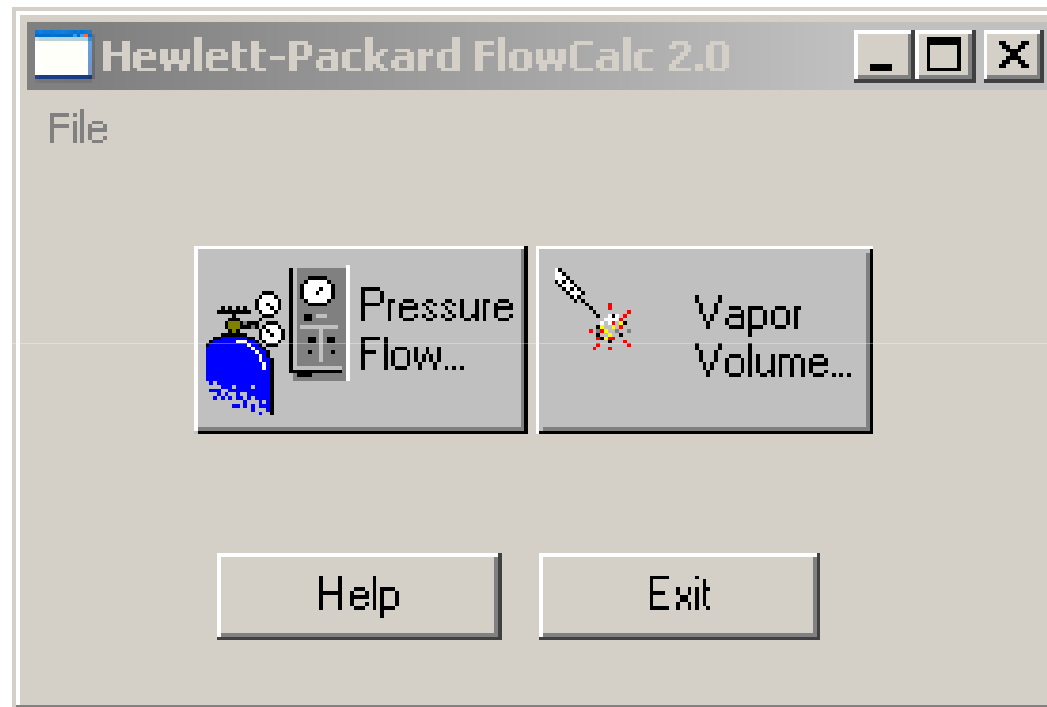
<http://www.chem.agilent.com/en-US/Support/Downloads/Utilities/Pages/GcPressureFlow.aspx>



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# Pressure / Flow Calculator



# Determine what the inlet pressure will be:

**Column Pressure/Flow Calculator**

**Column Parameters**

Length (m)

i.d. (mm)

Temp (C)

**Carrier Gas Parameters**

Inlet Pressure (gauge)

Outlet Flow (mL/min)

Average Velocity (cm/s)

Outlet Pressure (Absolute)

1 Atm    Vacuum    Other

**Split Ratio**

Split vent flow

Split Ratio(vent flow/col flow)  :1

**Holdup time**

**1.67 minutes**

**Inlet**

Inlet Temperature (C)

Inlet Flow (mL/min)


**Carrier gas**


Helium  Opt. Vel. range 20 40

Pressure Units  KPa    psi    bar

# Test Inlet Conditions For Solvent Expansion

**Solvent Vapor Volume Calculator**

Approximate vapor volume(ul): **669 ul**  **79 %**




**Injection Volume (ul)**  
Slider:

**Inlet Temp (C)**  
Slider:

**Inlet Pressure**  
Slider:

**Pressure Units**  
 KPa  psi  bar

**Solvent Properties**  
Methanol  
Boiling Pt (C): 64.7  
Denisty (g/cm3): 0.791  
Mol Wt. (amu): 32  


**Injection Liner** Volume (ul)  
5183-4647 single-t 850  
Capacity limits (%)  
75 100

# Water as Solvent

The screenshot shows the 'Solvent Vapor Volume Calculator' window. At the top, it displays 'Approximate vapor volume(ul): 1499 ul' and an 'Overload' indicator with three lights (two green, one red) and '176%'. Below this is a progress bar. The main interface is divided into several sections: 'Injection Volume (ul)' with a slider set to 1.0; 'Inlet Temp (C)' with a slider set to 250; 'Inlet Pressure' with a slider set to 8.6; 'Pressure Units' with radio buttons for KPa, psi (selected), and bar; 'Solvent Properties' with a dropdown menu set to 'Water' and values for Boiling Pt (C): 100, Density (g/cm3): 0.998, and Mol Wt. (amu): 18.02; and 'Injection Liner' with a dropdown menu set to '5183-4647 single-t' and a volume of 850 ul. At the bottom, there are buttons for 'Print', 'Help', 'OK', and 'Edit Liner list', along with 'Capacity limits (%)' of 75 and 100.

**Solvent Vapor Volume Calculator**

Approximate vapor volume(ul): **1499 ul**    Overload: **176%**

Injection Volume (ul): **1.0**

Inlet Temp (C): **250**

Inlet Pressure: **8.6**

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: **5183-4647 single-t**    Volume (ul): **850**


Edit Liner list    Capacity limits (%): 75    100

Print    Help    OK

# Water as Solvent

## Cut Injection Volume in Half

**Solvent Vapor Volume Calculator**


Approximate vapor volume(ul): **750 ul**  **88 %**

**Injection Volume (ul)**  
Slider: 0.5

**Inlet Temp (C)**  
Slider: 250

**Inlet Pressure**  
Slider: 8.6

**Pressure Units**  
 KPa  psi  bar

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



**Injection Liner** Volume (ul)  
5183-4647 single-t 850


**Capacity limits (%)**  
75 100

**Buttons:** Print, Help, OK, Edit Liner list

# Water as Solvent Pulsed Injection

**Solvent Vapor Volume Calculator**

Approximate vapor volume(ul): **750 ul**  **88 %**




**Injection Volume (ul)**  
Slider: 1.0

**Inlet Temp (C)**  
Slider: 250

**Inlet Pressure**  
Slider: 31.9

**Pressure Units**  
 KPa  psi  bar

**Solvent Properties**  
Water  
Boiling Pt (C): 100  
Denisty (g/cm3): 0.998  
Mol Wt. (amu): 18.02  


**Injection Liner** Volume (ul)  
5183-4647 single-t 850

**Capacity limits (%)**  
75 100

Print Help OK Edit Liner list

# 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 polar compounds.

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)

# Special Characteristics

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

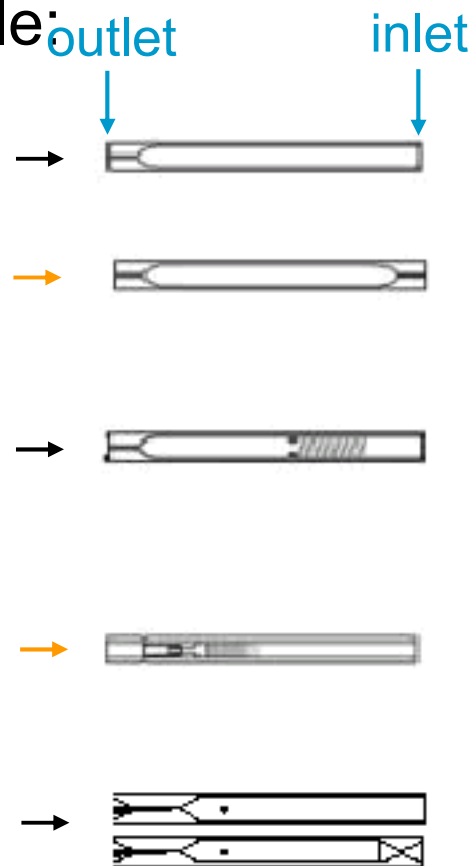
Taper (gooseneck), minimizes sample contact with gold seal.

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.





## Special Characteristics (contd.)





Other special characteristics include:

- Baffles
- Spiral paths
- Glass or ceramic frits or beads
- Laminar cups (elongated version of Jennings cups)
- Column packings with stationary phases




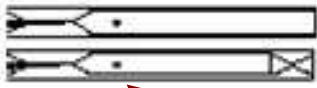
All designed to provide:

- a turbulent sample flow path for sample mixing
- protrusions, barriers, or adsorbents to collect high molecular weight sample components or particles
- surfaces for efficient vaporization of sample components.

# 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.
 <p>Glass nub</p>	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. For <b>manual injection only</b> . 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 <b>manual injection only</b> . For dirty samples, traps non-volatiles and particulates well. For high and low MW compounds. Not recommended for use with EPC.

# 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.
 <p>Side hole</p>	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.

# GLASS WOOL

## Liner Packing Recommendations

Amount, size and placement must be consistent for consistent results

Can be broken upon installation into the liner, exposing active sites

Liner deactivation with glass wool plug in place is ideal

# GLASS WOOL

## Placement in Liner

Near top of liner:

- Wipes syringe needle of sample
- Can improve injector precision
- Helps to prevent backflash

Near bottom of liner:

- Helps in volatilization of high MW components
- Increases mixing

Both positions help retain some non-volatile residues from reaching the column

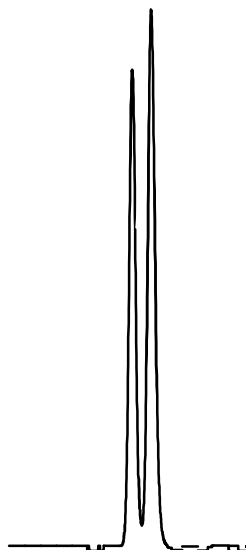
# Carrier Gas Considerations

- Carries the solutes down the column
- Selection and velocity influences efficiency and retention time

# RESOLUTION VS. LINEAR VELOCITY

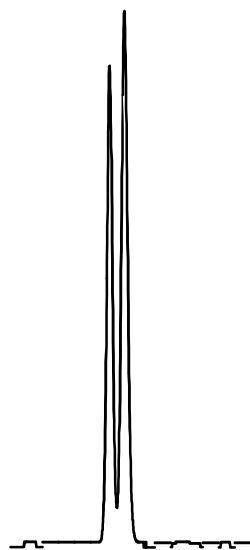
Helium

Resolution of 1.5 = baseline resolution



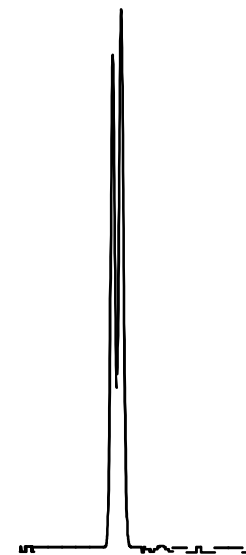
**$R = 1.46$**   
**30 cm/sec**

**4.4 psig**



**$R = 1.31$**   
**35 cm/sec**

**5.1 psig**



**$R = 0.97$**   
**40 cm/sec**

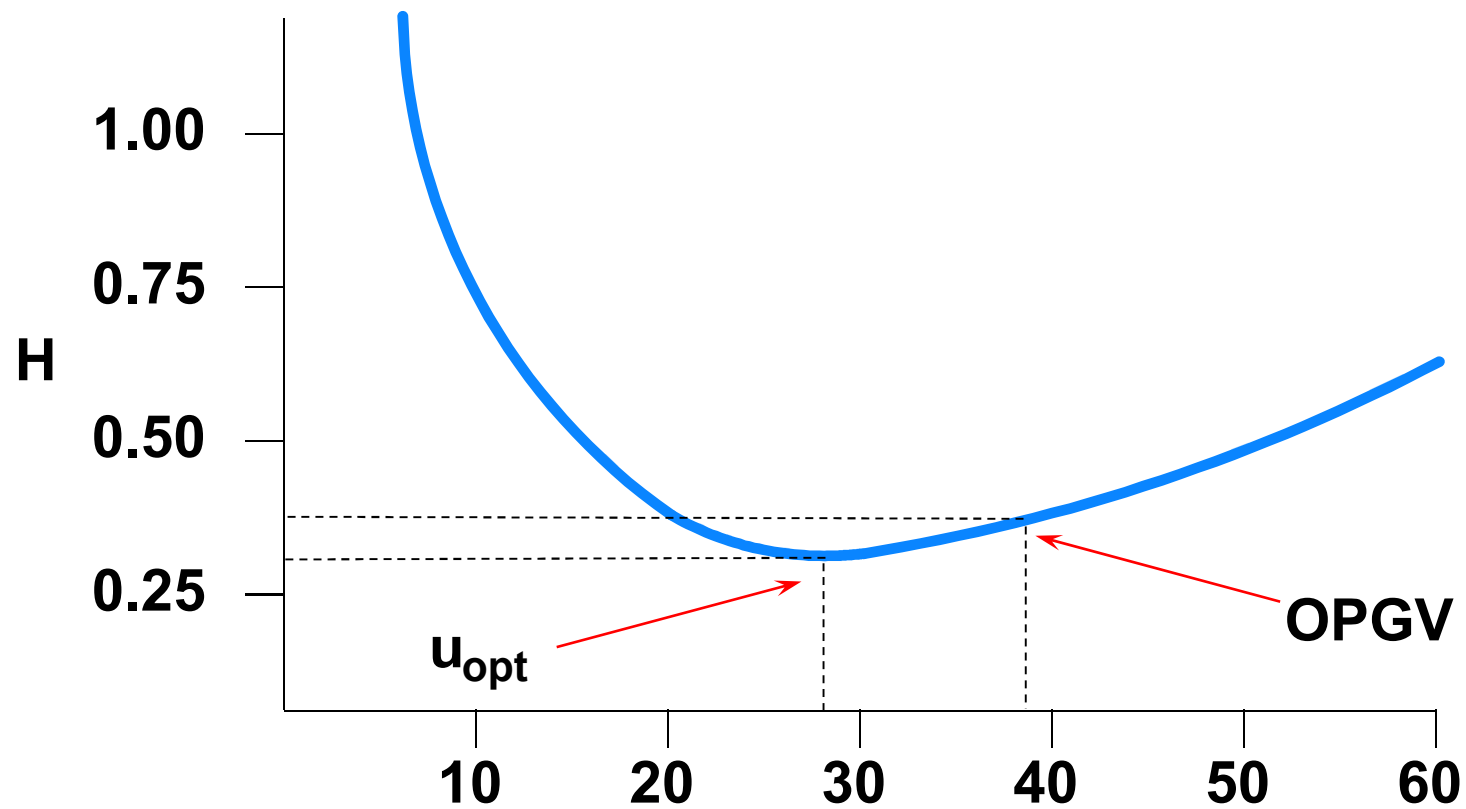
**5.8 psig**

**DB-1, 15 m x 0.32 mm ID, 0.25  $\mu$ m**  
**60°C isothermal**  
**1,3- and 1,4-Dichlorobenzene**



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# VAN DEEMTER CURVE





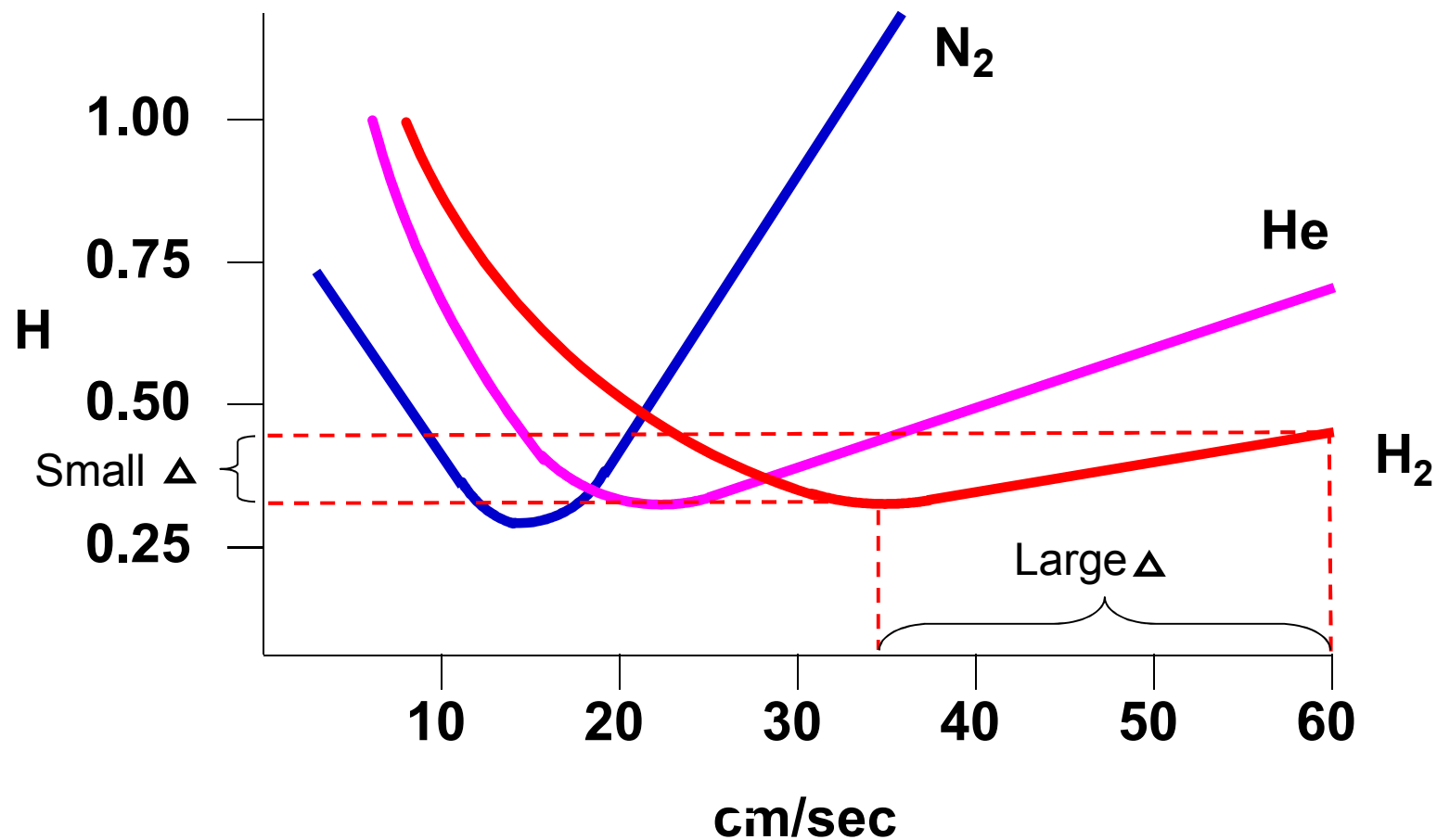
# $\bar{u}_{opt}$ and OPGV

$\bar{u}_{opt}$ : Maximum efficiency

**OPGV**: Optimal practical gas velocity  
Maximum efficiency per unit time

$$1.5 - 2x \bar{u}_{opt}$$

# VAN DEEMTER CURVES



# What Happens to the Flow as Oven Temp Increases?

The screenshot shows the 'Column Pressure/Flow Calculator' window. It is divided into several sections for parameter input and calculation results.

- Column Parameters:** Length (m) is 30.0, i.d. (mm) is 0.320, and Temp (C) is 50.
- Carrier Gas Parameters:** Inlet Pressure (gauge) is 8.6, Outlet Flow (mL/min) is 1.75, Average Velocity (cm/s) is 30.0, and Outlet Pressure (Absolute) is 14.7.
- Split Ratio:** Split vent flow is 0.0, resulting in a Split Ratio (vent flow/col flow) of 0:1.
- Inlet:** Inlet Temperature (C) is 175, and Inlet Flow (mL/min) is 1.81.
- Carrier gas:** Helium is selected with an Opt. Vel. range of 20-40. Pressure Units are set to psi.
- Holdup time:** The calculated holdup time is 1.67 minutes.

At the bottom, there are radio buttons for '1 Atm', 'Vacuum', and 'Other', along with 'Help', 'Plot...', 'Print', and 'OK' buttons.

# Carrier Gas: Constant Pressure

**Column Pressure/Flow Calculator**

**Column Parameters**

Length (m)

i.d. (mm)

Temp (C)

**Carrier Gas Parameters**

Inlet Pressure (gauge)

Outlet Flow (mL/min)

Average Velocity (cm/s)

Outlet Pressure (Absolute)

1 Atm    Vacuum    Other

**Split Ratio**

Split vent flow

Split Ratio(vent flow/col flow)  :1

**Holdup time**

**Inlet**

Inlet Temperature (C)

Inlet Flow (mL/min)

**Carrier gas**

Opt. Vel. range

**Pressure Units**

KPa    psi    bar

# Carrier Gas: Constant Flow

The screenshot shows the 'Column Pressure/Flow Calculator' window. It is divided into several sections for input and output parameters.

**Column Parameters:**

- Length (m): 30.0
- i.d. (mm): 0.320
- Temp (C): 325

**Carrier Gas Parameters:**

- Inlet Pressure (gauge): 19.4
- Outlet Flow (mL/min): 1.75
- Average Velocity (cm/s): 41.6
- Outlet Pressure (Absolute): 14.7

**Split Ratio:**

- Split vent flow: 0.0
- Split Ratio (vent flow/col flow): :1

**Holdup time:** 1.20 minutes

**Inlet:**

- Inlet Temperature (C): 175
- Inlet Flow (mL/min): 1.24

**Carrier gas:**

- Gas: Helium
- Opt. Vel. range: 20 - 40

**Pressure Units:**  KPa  psi  bar

**Buttons:** Help, Plot..., Print, OK

**Radio Buttons:**  1 Atm  Vacuum  Other

# Detectors

Detector	Dynamic Range		MDL
TCD	$10^5$	Universal	400 pg Tridecane
FID	$10^7$	Responds to C-H bonds	1.8 pg Tridecane
ECD	$5 \times 10^5$	Responds to free electrons	6 fg/mL Lindane
NPD	$10^5$	Specific to N or P	0.4 pgN/s 0.06 pg P /s
FPD	$10^3$ S, $10^4$ P	Specific to S or P	60 fg P/s 3.6 pg S/s
SCD	$10^4$	Specific & Selective to S	0.5 pg S/s
NCD	$10^4$	Specific & Selective to N	3 pg N/s
MSD		Universal	S/N 400:1 1 pg/uL OFN

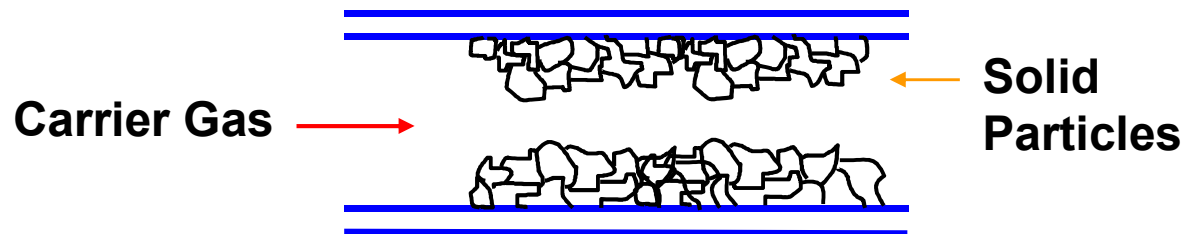
# Selecting the RIGHT Column

Understanding the Stationary Phase

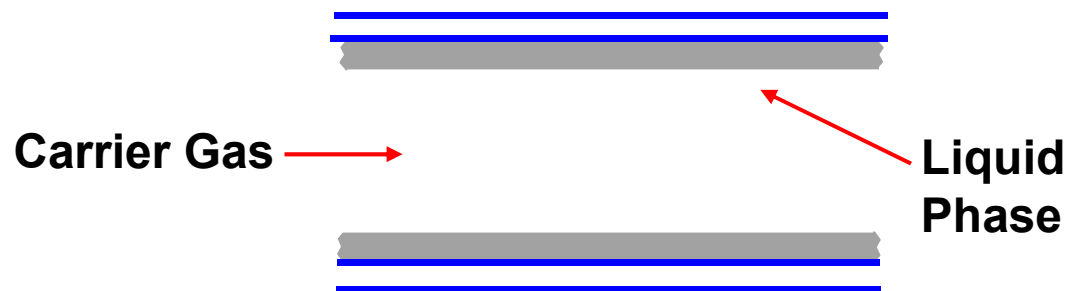


# CAPILLARY COLUMN TYPES

## Porous Layer Open Tube (PLOT)

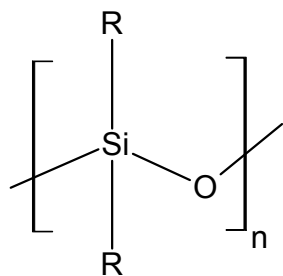


## Wall Coated Open Tube (WCOT)



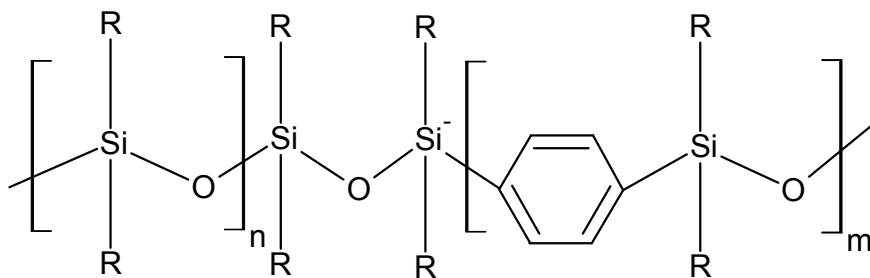


# STATIONARY PHASE POLYMERS

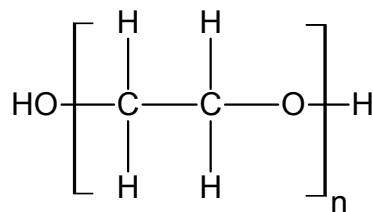


**R= methyl, cyanopropyl, cyanopropylphenyl,  
trifluoropropyl**

**Siloxane**



**Arylene**



**Polyethylene glycol backbone**

# Selectivity Interactions

- Dispersion
- Dipole
- Hydrogen bonding


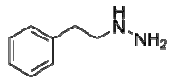
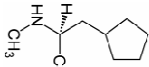
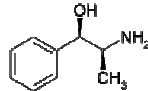
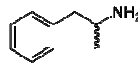
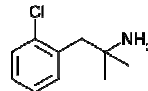
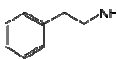
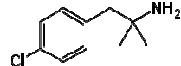
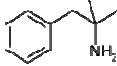
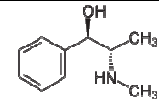
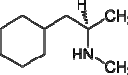
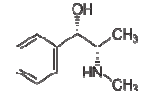
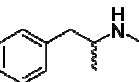
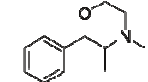

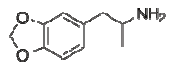

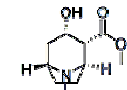
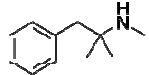
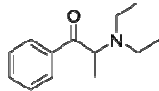
# Selectivity

## Interaction Strengths

Phase	Dispersion	Dipole	H Bonding
Methyl	Strong	None	None
Phenyl	Strong	None	Weak
Cyanopropyl	Strong	Strong	Moderate
Trifluoropropyl	Strong	Moderate	Weak
PEG	Strong	Strong	Moderate

**Now Let's Apply What We learned**

# Sample List (drugs)

1. Cadaverine		11. Phenelzine	
2. Cyclopentamine		12. Phenylpropanolamine	
3. Amphetamine		13. Clortermine	
4. Phenethylamine		14. Chlorphentermine	
5. Phentermine		15. Ephedrine	
6. Propylhexedrine		16. Pseudoephedrine	
7. Methamphetamine		17. Phendimetrazine	
8. Methenamine		18. MDA	
9. Amantidine		19. Ecgonine methyl ester	
10. Mephentermine		20. diethylpropion	

## Starting Method Parameters

Column: DB-5 30m X 0.32mm X 0.25um

S/SI Inlet: Split 50:1 Temp 250°

FID: Temp 350°

Carrier: He

Constant flow 30cm/sec

Oven: 50°C Hold for 5 min

10°C/min to 325°C Hold for 5 min

# Am I Going to Have Backflash?


The screenshot shows the 'Column Pressure/Flow Calculator' window with the following settings:


- Column Parameters:**
  - Length (m): 30.0
  - i.d. (mm): 0.320
  - Temp (C): 50
- Carrier Gas Parameters:**
  - Inlet Pressure (gauge): 8.6
  - Outlet Flow (mL/min): 1.75
  - Average Velocity (cm/s): 30.0
  - Outlet Pressure (Absolute): 14.7
- Split Ratio:**
  - Split vent flow: 0.0
  - Split Ratio (vent flow/col flow): :1
- Inlet:**
  - Inlet Temperature (C): 175
  - Inlet Flow (mL/min): 1.81
- Carrier gas:**
  - Gas: Helium
  - Opt. Vel. range: 20 - 40
  - Pressure Units:  KPa,  psi,  bar
- Buttons:** Help, Plot..., Print, OK
- Radio Buttons:**  1 Atm,  Vacuum,  Other

The calculated holdup time is 1.67 minutes.

# Injection Volume / Solvent Expansion

**Solvent Vapor Volume Calculator** [X]

Approximate vapor volume(ul): **669 ul**  **79 %**




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

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

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

**Pressure Units**  
 KPa  psi  bar

**Solvent Properties**  
Solvent: **Methanol** [v]  
Boiling Pt (C): 64.7  
Density (g/cm3): 0.791  
Mol Wt. (amu): 32  
 Solvents

**Injection Liner** Volume (ul)  
5183-4647 single-t [v] 850  
Capacity limits (%): 75 100

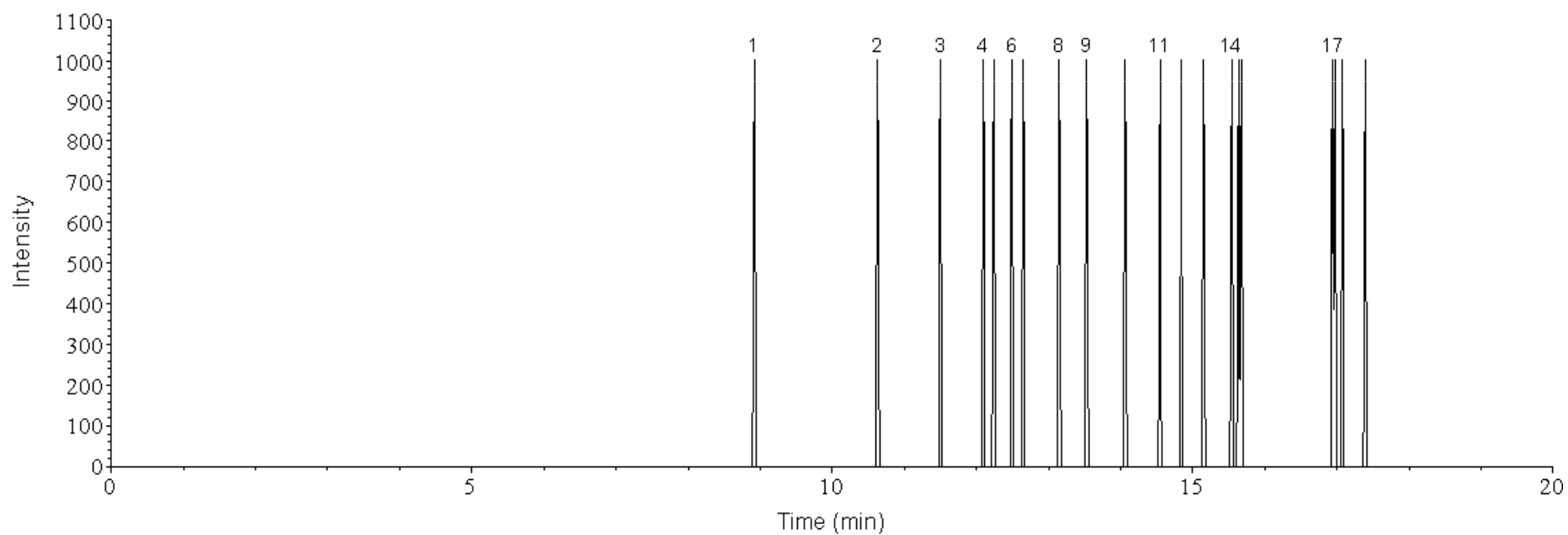


# Developing Temperature Program

## Initial Run

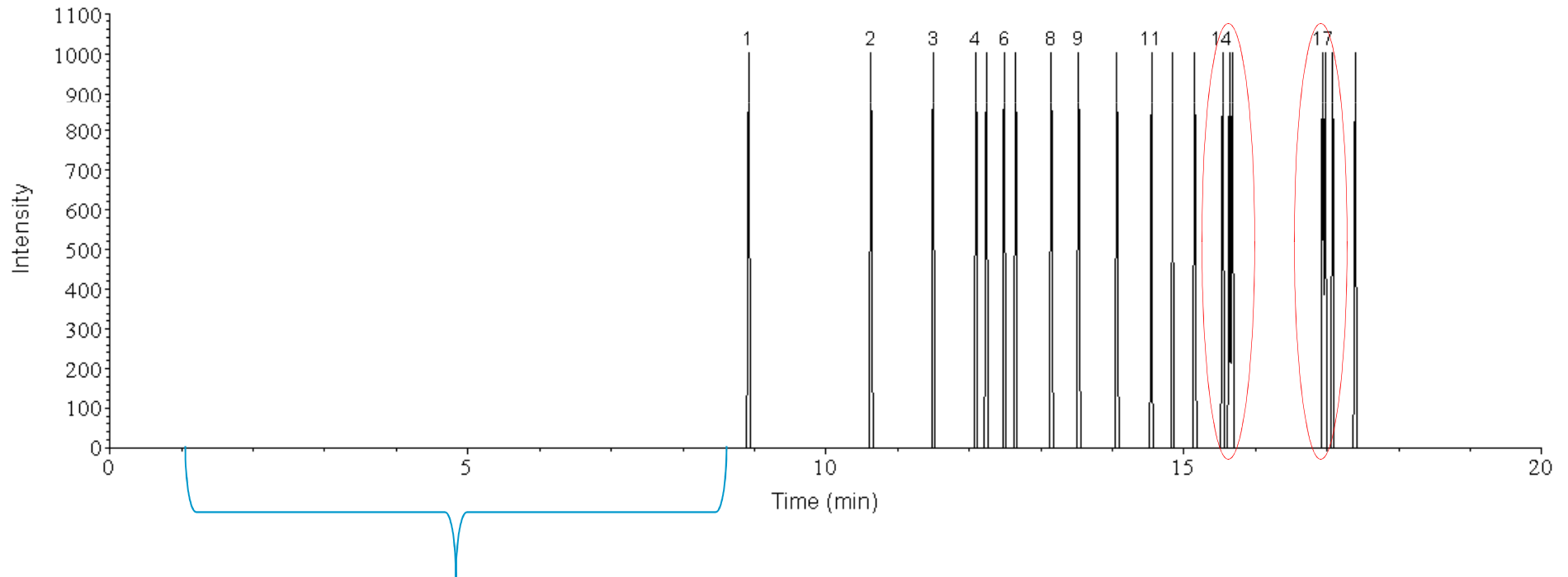
Initial Temp 50°C Hold for 5 min

Ramp 10°C/min to 325°C Hold for 5 min



# Developing Temperature Program

## Initial Run - Define Areas for Improvement



## Next Step...

When does the first peak come out?

~9 minutes

What temperature does it come out at?

Temp program:

50°C for 5 minutes

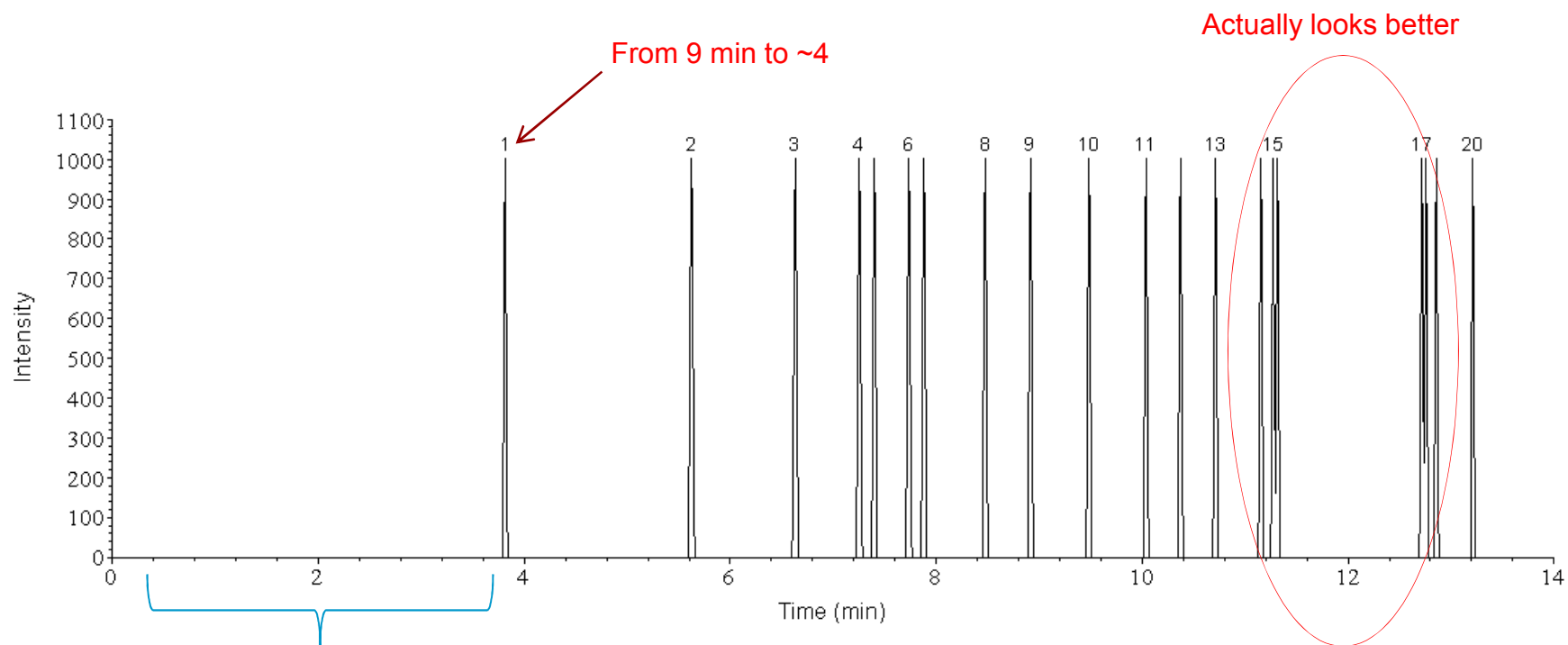
10°C to 325°C

1<sup>st</sup> Peak comes out at 90°C

# Developing Temperature Program

## 2<sup>nd</sup> Try

Initial Temp 90°C Hold for 5 min  
Ramp 10°C/min to 325°C Hold for 5 min

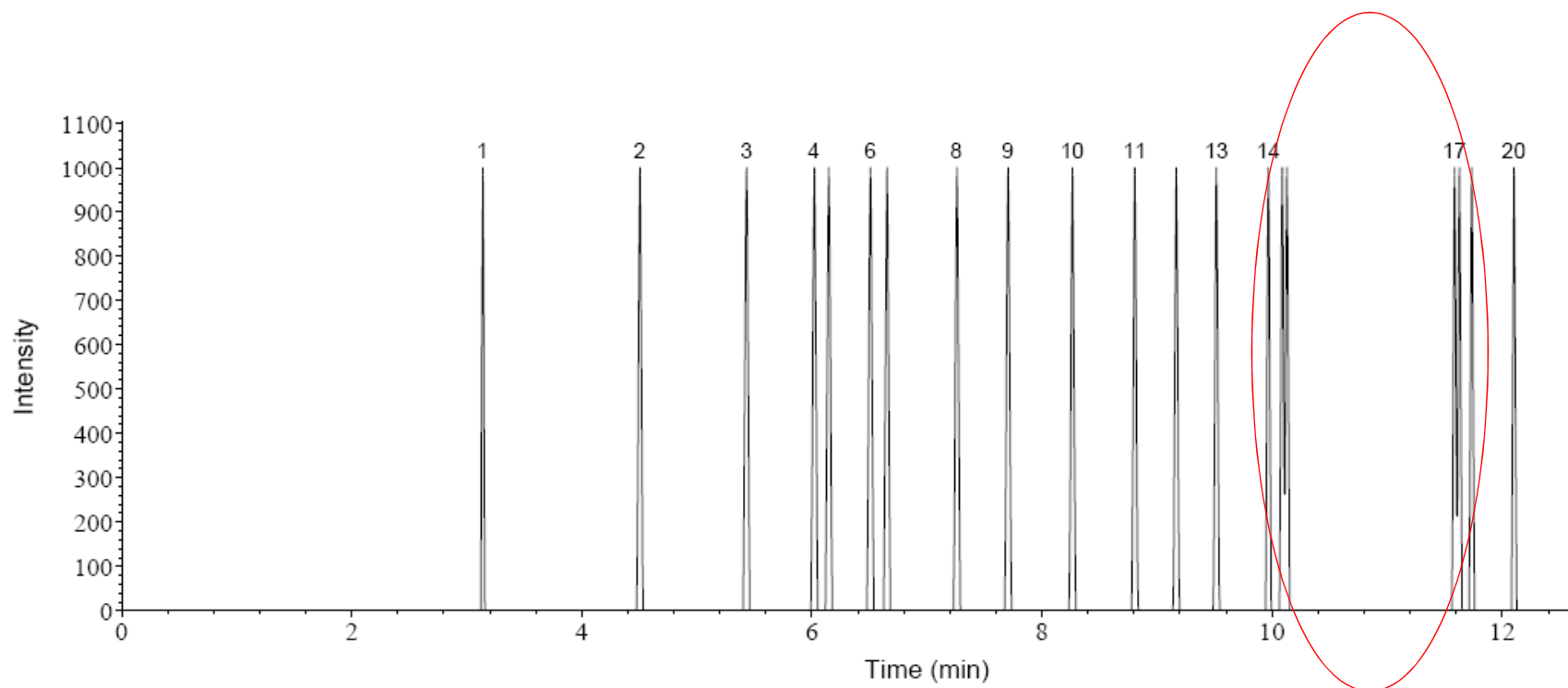


# Developing Temperature Program

## 3rd Try

Initial Temp 100°C Hold for 5 min  
Ramp 10°C/min to 325°C Hold for 5 min

Time to resolve these peaks



# Resolve Co-elutions

Add a hold 20-30° below the elution temperature

Co-elutions occur at 10 minutes

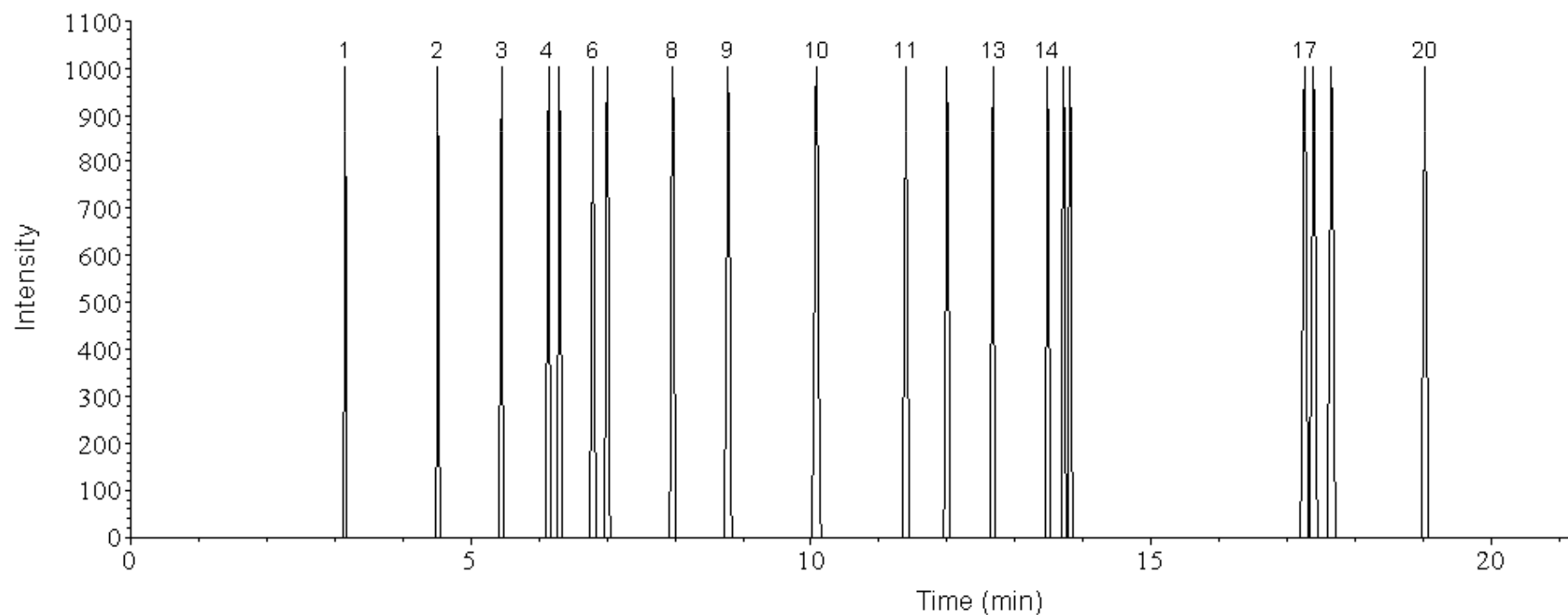
100°C hold for 5 minutes  
10°C/min to 325°C

Co-elutions occur at 150°C

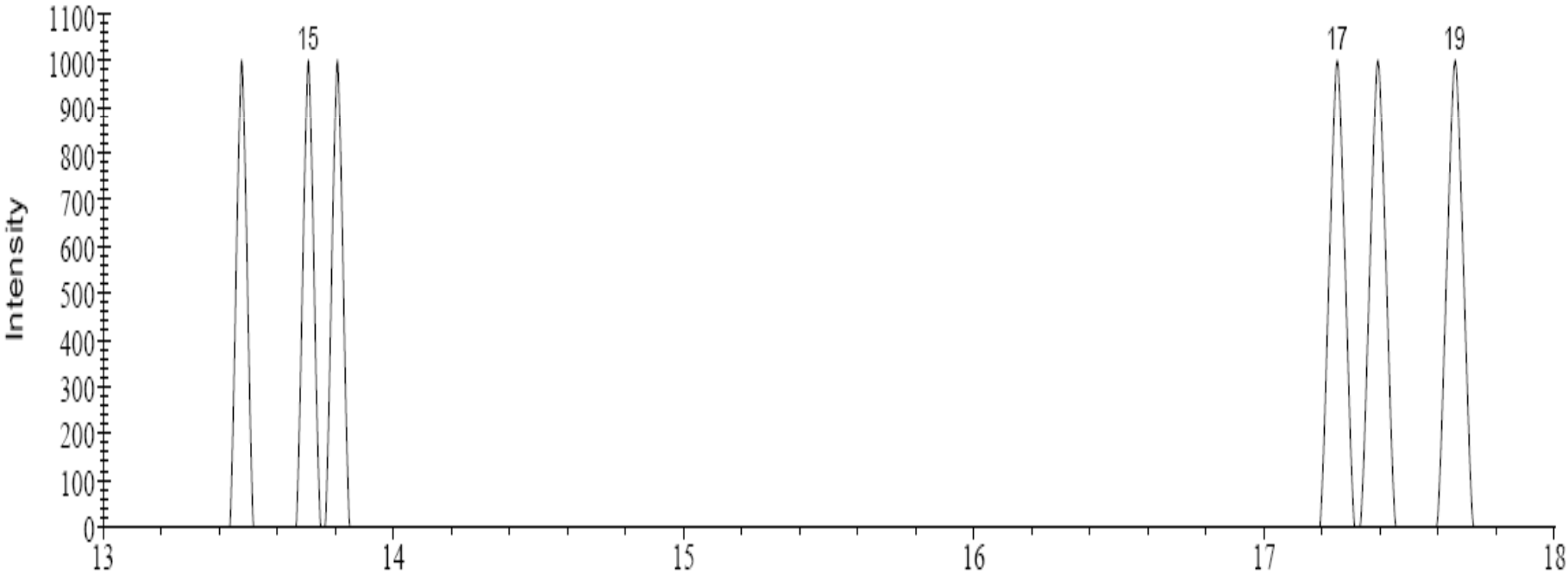
Set hold at 130°C

# Developing a Temperature Program

Oven: 100°C Hold for 10 minutes  
10°C/min to 130°C hold for 5 min  
10°C/min to 325°C



# Developing a Temperature Program





## Conclusions:

Think about the sample first

**\*\*Is it chromatographable by GC?**

sample composition

sample clean up

level of detection

Use information sources first when choosing a column

Mild oven program to begin with

*Utilize Technical Support*

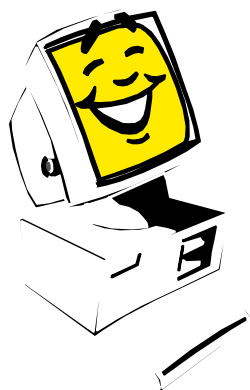
# Agilent J&W Scientific Technical Support

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

*\* Select option 3, then 3, then 1.*

**866-422-5571 (fax)**

**GC-Column-support@agilent.com**



**www.chem.agilent.com**



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