

# GC Method Development

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# 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. Nobel Gases?
  6. Halogens?

# Sample Residues

## Semi-volatile residues

- Bake out

- Back flush

## Non-volatile residues

- Guard column

- Back flush

## Dirty Samples

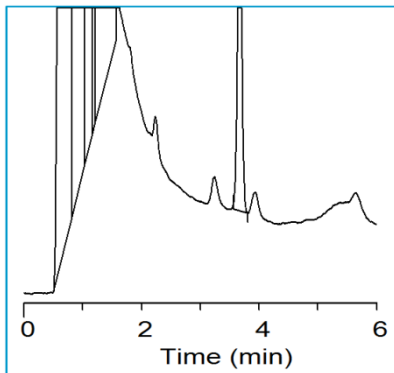
- Sample clean up?

- Back flush

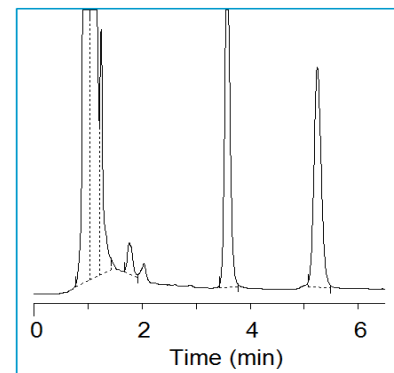
# Perform Sample Preparation

- To acquire desired sensitivity/selectivity
- To reduce contamination/carryover issues
- Use of sensitive and expensive instruments: Protect your investment!!!

Pesticides in Avocado without SP



Pesticides in Avocado with SP



# Sample Preparation techniques comparison

- **Bond Elut SPE**

- Multi-step approach for highest level of sample cleanup

- **Chem Elut (SLE)**

- Extraction by solvent exchange, substitute for LLE

- **QuEChERS (dSPE)**

- Sample cleanup by extraction of bulk interferences

- **Captiva ND (PPT)**

- Removes precipitated proteins by in-well protein precipitation

- **Captiva Filtration**

- Removes particulates

**Selectivity**

**Complexity**

**Cost**



# Enhanced Matrix Removal: EMR-Lipids

Effective Removal of lipids (fats)



*Tubes containing  
1g of EMR sorbent*

5982-1010



Protocols also require a “polish” step after EMR to remove water and dissolved solids before injection

Fits into existing workflows:

- after QUECHERS extraction
- after Liquid Extraction

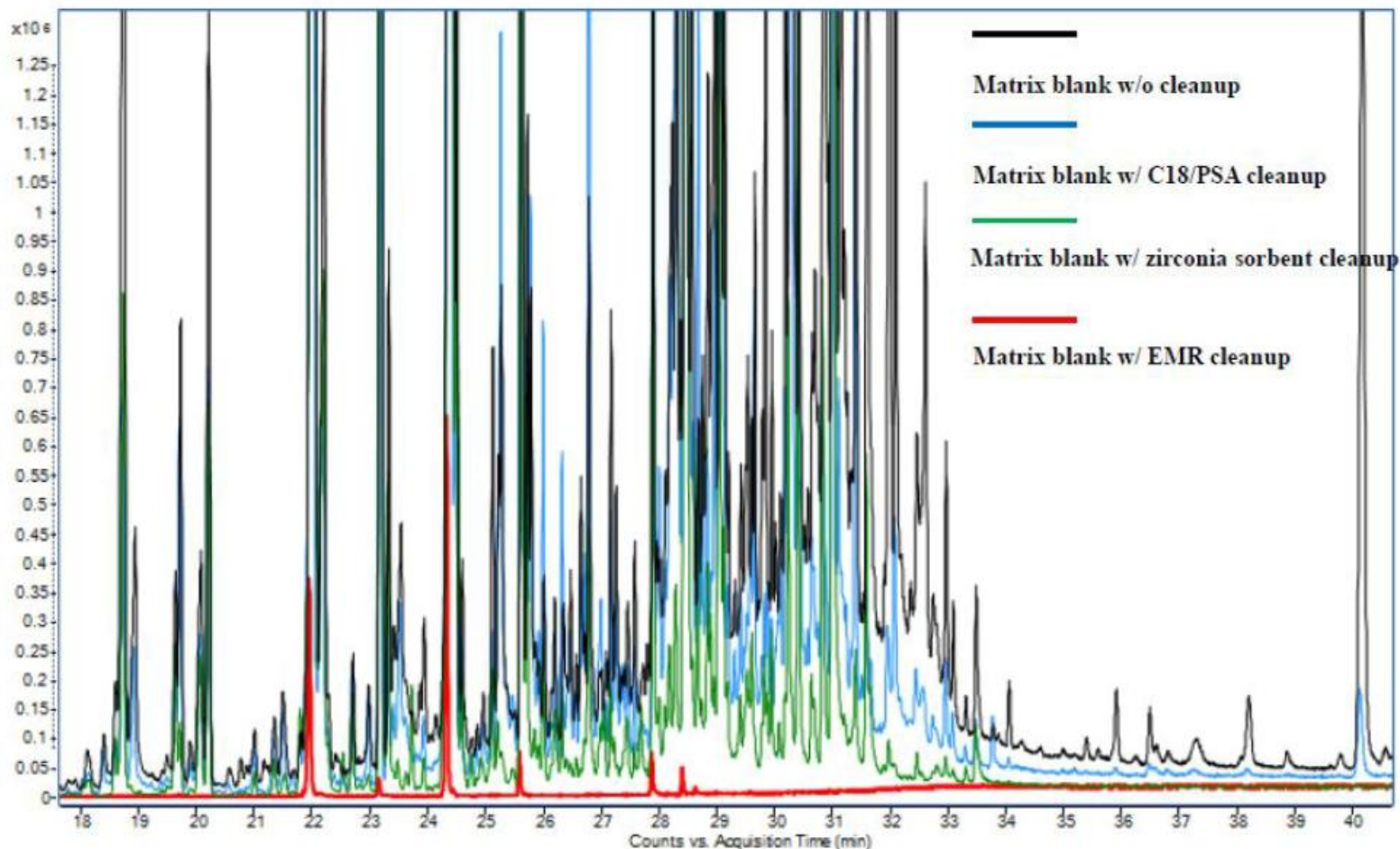
*Tubes containing  
polish salts*



5982-0101



# Comparison of GC/MS Full-scan Chromatogram for Matrix Background



**The use of EMR material cleanup provides significantly cleanup chromatographic sample background.**

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

# 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

<b>Mode</b>	<b>Sample Concentration</b>	<b>Sample to Column</b>	<b>Comments</b>
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

- 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)

Good for Large Volume Injections

*Trace Level Analysis*

Can be cooled to  $-160^{\circ}\text{C}$  with liquid Nitrogen

Can run in hot or cold, Split or Splitless mode



# PTV

## (Programmable Temperature Vaporization)

Good for Trace Level Analysis – Large Volume Injections

<b>Mode</b>	<b>Sample Concentration</b>	<b>Sample to Column</b>	<b>Comments</b>
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

<b>Mode</b>	<b>Sample Concentration</b>	<b>Sample to Column</b>	<b>Comments</b>
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

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

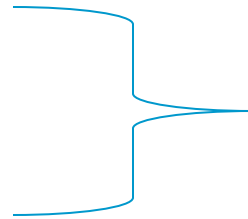
# Sample Expansion...Liners?

Split / Splitless Inlet

Multimode Inlet

Packed inlet

PTV



Use the same liners

# 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?

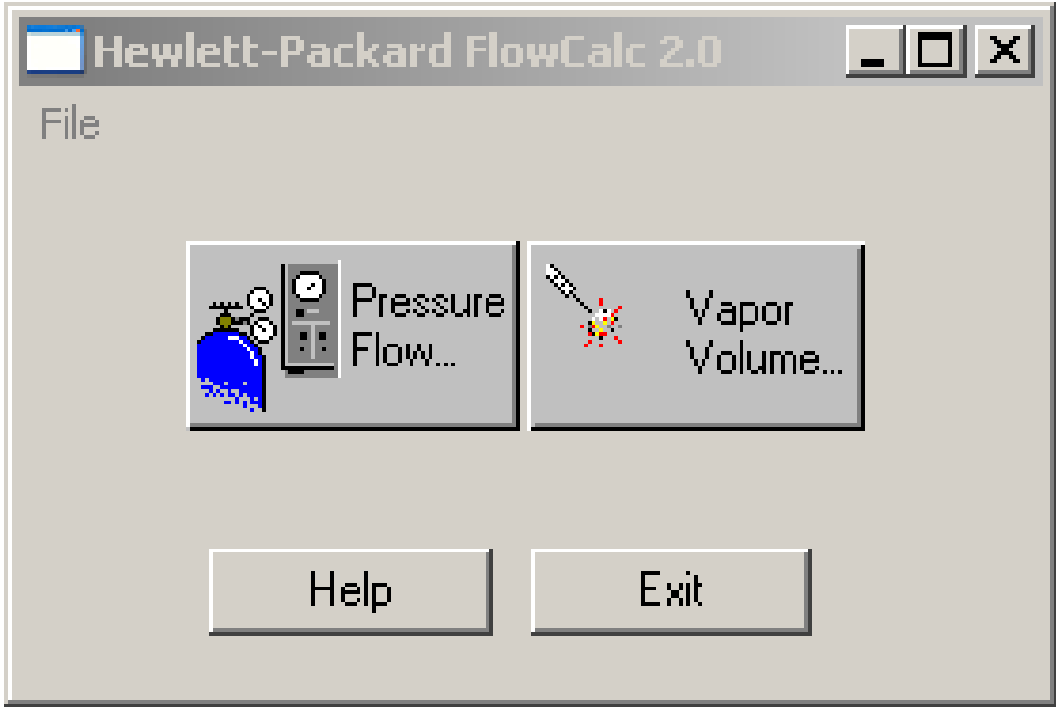
**Pressure / Flow Calculator**

Free download from our Website

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

<https://www.agilent.com/en-us/support/gas-chromatography/gccalculators>

# 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

# Determine what the inlet pressure will be:

**Pressure Flow Calculator**

Length (m)	30.00	Split Vent Flow (mL/min)	0.000
Inner Diameter ( $\mu\text{m}$ )	320	Split Ratio (vent flow/col flow)	0.000 : 1
Film Thickness ( $\mu\text{m}$ )	0.25	Holdup Time	1.66 min
Temperature ( $^{\circ}\text{C}$ )	50	Inlet Temp ( $^{\circ}\text{C}$ )	250
Inlet Pressure (gauge)	8.599	Inlet Liner Flow (mL/min)	2.125
Outlet Flow (mL/min)	1.759	Liner Volume ( $\mu\text{L}$ )	880
Average Velocity (cm/s)	30.146	Suggested Splitless Purge Time:	0.4 min
Outlet Pressure (absolute)	14.696	Carrier Gas	Helium

Pressure Units

KPa  psi  bar


1 Atm  
 Vacuum  
 Other


Optimum velocity range (cm/s)

20 40

# 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

**Solvent Vapor Volume Calculator** [X]

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



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

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

**Inlet Pressure**  
Slider: 8.6

**Pressure Units**  
 KPa     psi     bar

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

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

**Capacity limits (%)**  
75    100

Print    Help    OK    Edit Liner list

# Water as Solvent

## Cut Injection Volume in Half

The screenshot shows the 'Solvent Vapor Volume Calculator' window. At the top, it displays 'Approximate vapor volume(ul): 750 ul' and '88 %'. Below this is a progress bar. The main interface is divided into several sections: 'Injection Volume (ul)' with a slider set to 0.5; '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 fields 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 field set to 850. There are also buttons for 'Print', 'Help', 'OK', and 'Edit Liner list', and a 'Capacity limits (%)' section with values 75 and 100.

**Solvent Vapor Volume Calculator**

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

Injection Volume (ul): **0.5**

Inlet Temp (C): **250**

Inlet Pressure: **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

Solvents

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

Capacity limits (%): 75 100

Buttons: Print, Help, OK, Edit Liner list

# Water as Solvent

## Pulsed Injection

The screenshot shows the 'Solvent Vapor Volume Calculator' window. At the top, it displays 'Approximate vapor volume(ul): 750 ul' and '88 %'. 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 31.9; '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 (100), Density (0.998), and Mol Wt. (18.02); and 'Injection Liner' with a dropdown set to '5183-4647 single-t' and a 'Volume (ul)' of 850. A table at the bottom right shows 'Capacity limits (%)' with values 75 and 100. Buttons for 'Print', 'Help', 'OK', and 'Edit Liner list' are also visible.

**Solvent Vapor Volume Calculator**

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

Injection Volume (ul): 1.0

Inlet Temp (C): 250

Inlet Pressure: 31.9

Pressure Units:  KPa  psi  bar

Solvent Properties:

- Solvent: 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

Capacity limits (%): 75 100

Buttons: 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:

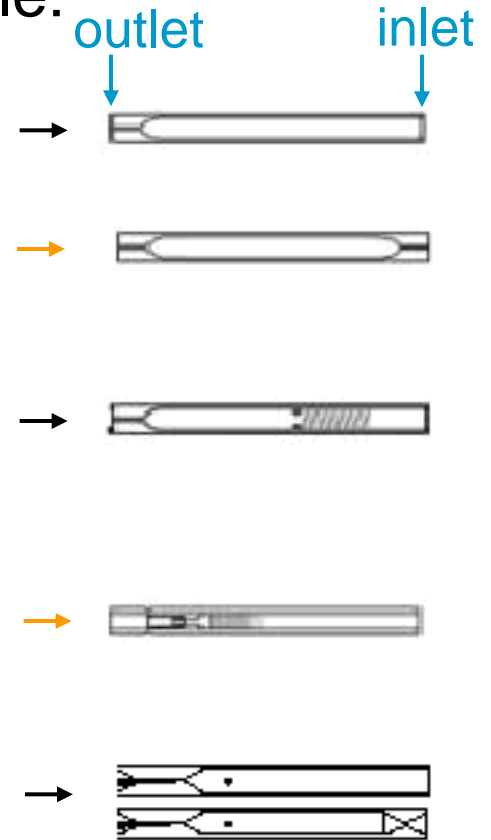
Taper (gooseneck), minimizes sample contact with gold seal.

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
	5190-2294	Simplest split liner, glass wool, UI deactivation, large volume, 990µL volume. Use for general purpose. Also used for Splitless mode.
 Glass nub	5190-2295	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 Liners

Liner	Part No.	Comments
	5190-2292	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.
	5190-2293	Single taper, deactivated, with glass wool, 900µL volume. Glass wool aides volatilization and protects column. For trace (dirty) samples.
	5190-3983	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.



# 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

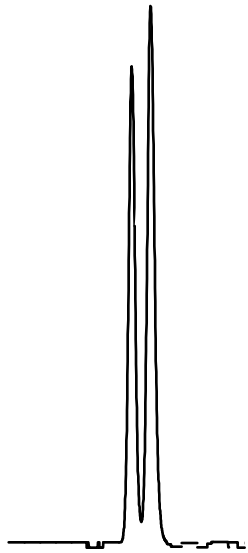
# Carrier Gas Considerations

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

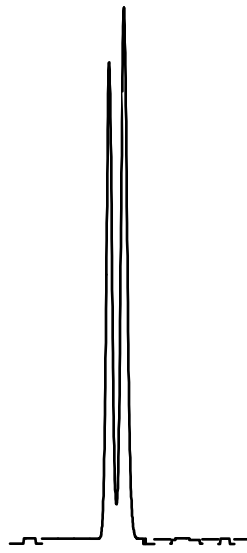
# RESOLUTION VS. LINEAR VELOCITY

Helium

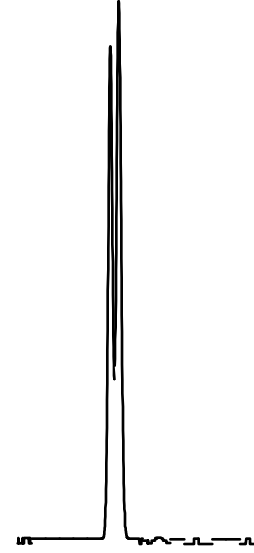
Resolution of 1.5 = baseline resolution



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



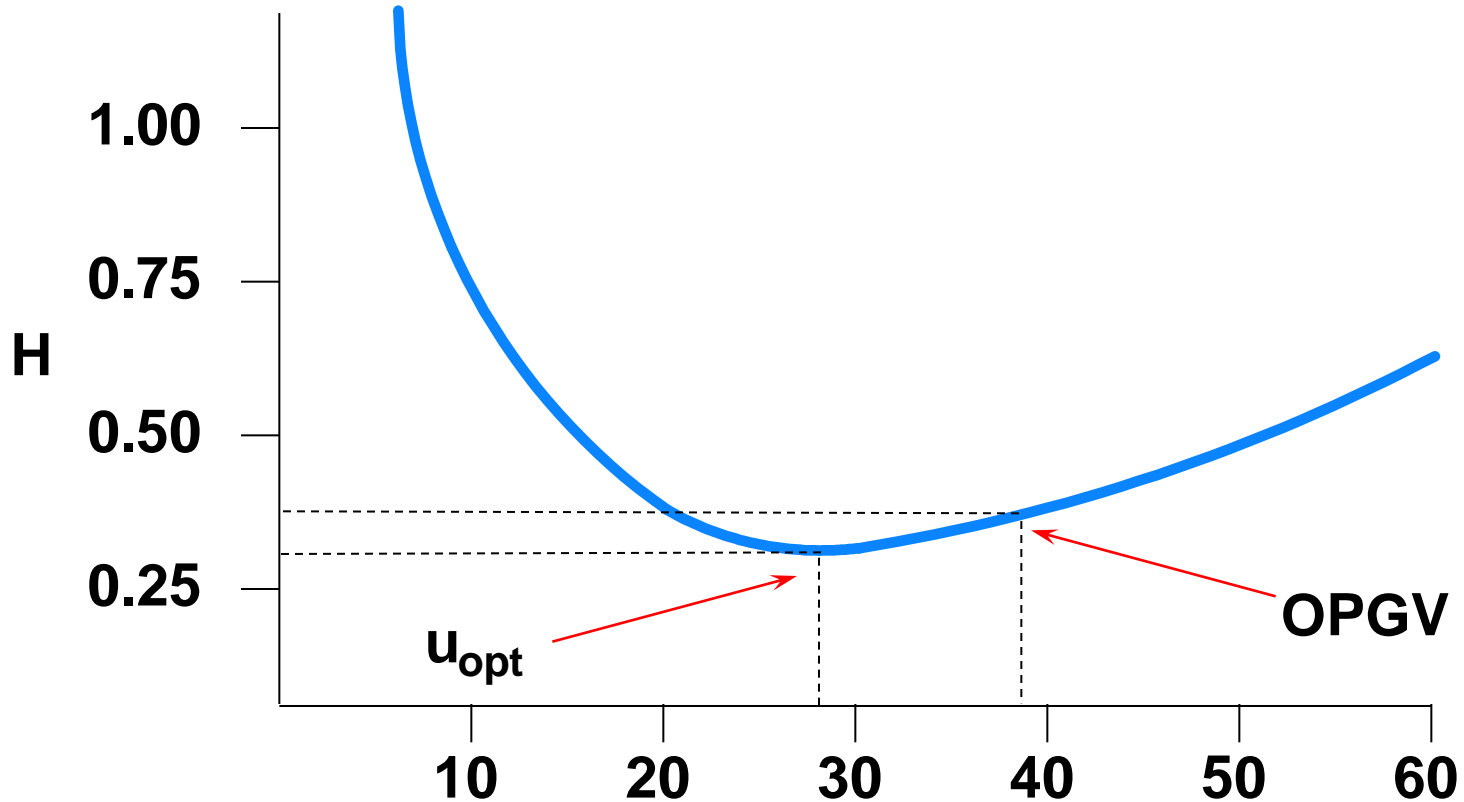
**$R = 1.31$**   
**35 cm/sec**  
**5.1 psig**



**$R = 0.97$**   
**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**

# VAN DEEMTER CURVE



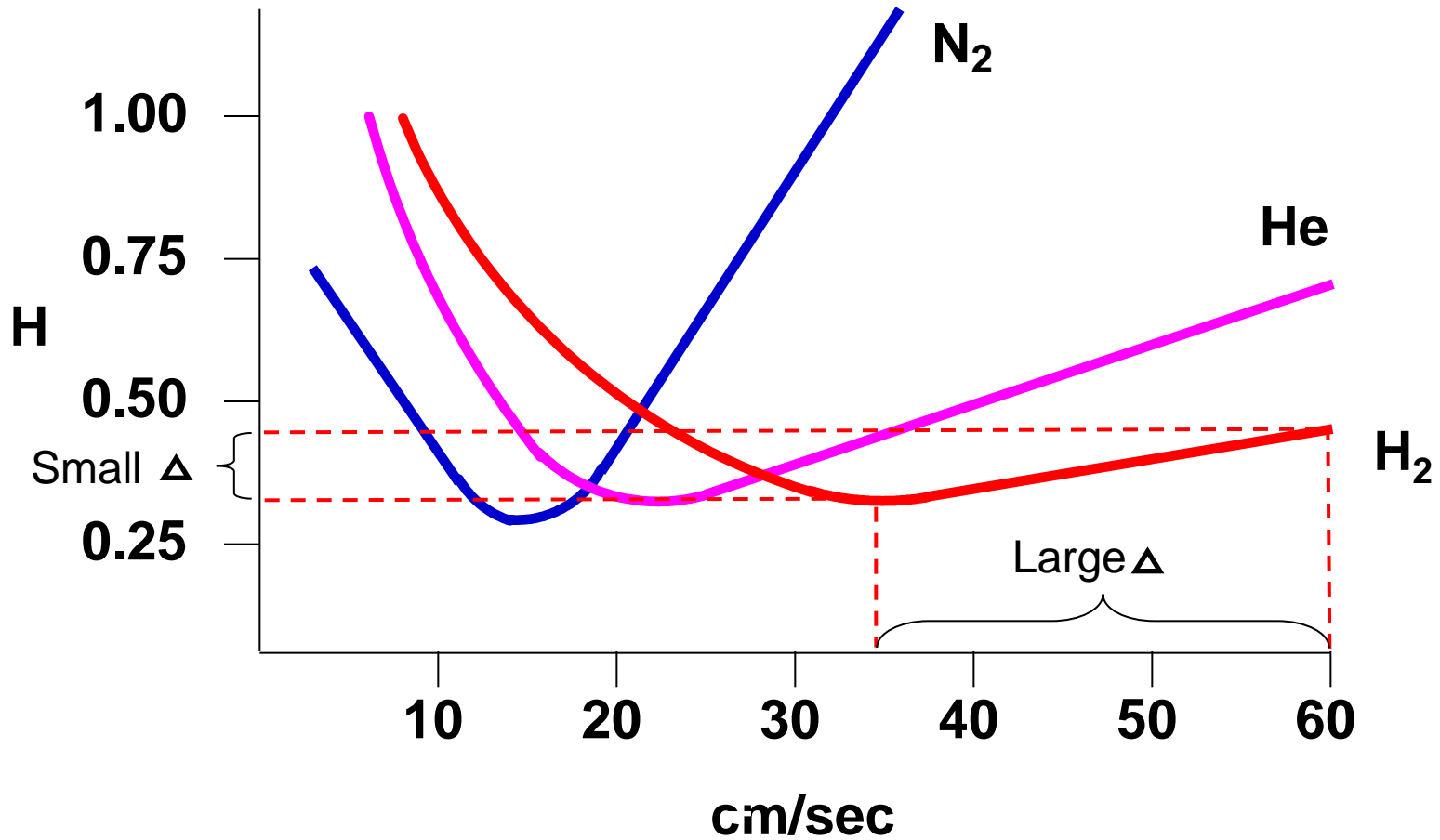
# $\bar{u}_{opt}$ and OPGV

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

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

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

# VAN DEEMTER CURVES



# What Happens to the Flow as Oven Temp Increases?

**Column Pressure/Flow Calculator**

**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

1 Atm  Vacuum  Other

**Split Ratio**

Split vent flow 0.0

Split Ratio(vent flow/col flow) :1

Flow/Ratio

**Holdup time**

1.67 minutes

**Inlet**

Inlet Temperature (C) 175

Inlet Flow (mL/min) 1.81

**Carrier gas**

Helium Opt. Vel. range 20 40

Gases ...

Pressure Units  KPa  psi  bar

Help Plot... Print OK



# 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**

**2.62 minutes**

**Inlet**

Inlet Temperature (C)

Inlet Flow (mL/min) 0.621

**Carrier gas**

Helium  Opt. Vel. range 20 40

**Pressure Units**

KPa  psi  bar

# Carrier Gas: Constant Flow

**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

Flow/Ratio

**Holdup time**

**1.20 minutes**

**Inlet**

Inlet Temperature (C)

Inlet Flow (mL/min) 1.24

**Carrier gas**

Helium Opt. Vel. range 20 40

Gases...

**Pressure Units**

KPa  psi  bar

Help Plot... Print OK

# Detectors

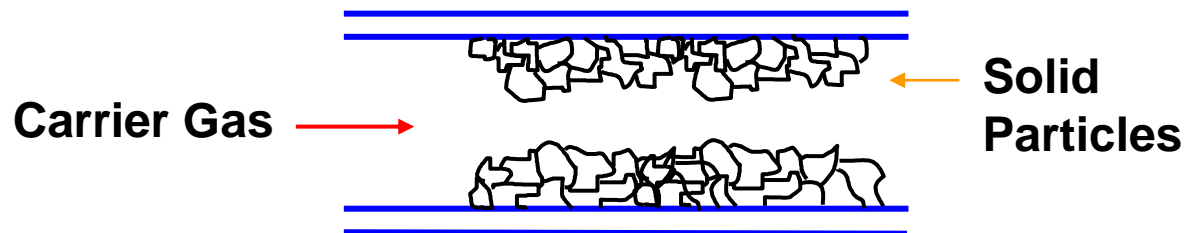
Detector	Dynamic Range		MDL
TCD	$10^5$	Universal	400 pg Tridecane
FID	$10^7$	Responds to C-H bonds	1.8 pg Tridecane
ECD	$5 \times 10^5$	Responds to free electrons	6 fg/mL Lindane
NPD	$10^5$	Specific to N or P	0.4 pgN/s 0.06 pg P /s
FPD	$10^3$ S, $10^4$ P	Specific to S or P	60 fg P/s 3.6 pg S/s
SCD	$10^4$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

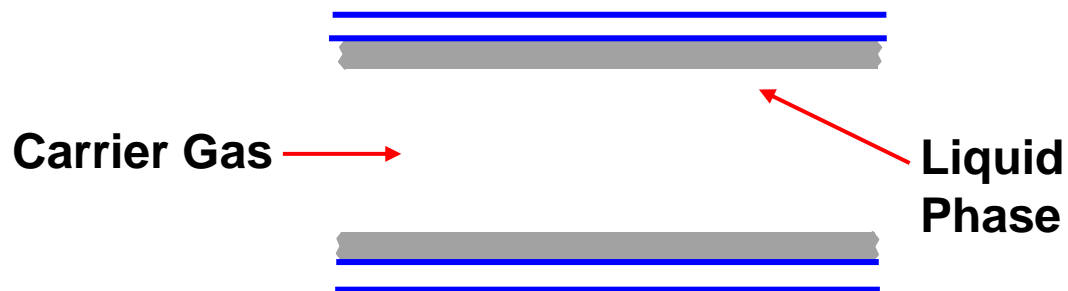
## Understanding the Stationary Phase

# CAPILLARY COLUMN TYPES

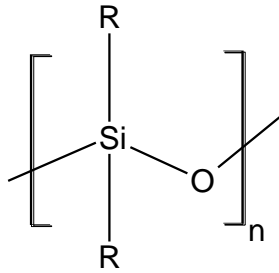
## Porous Layer Open Tube (PLOT)



## Wall Coated Open Tube (WCOT)

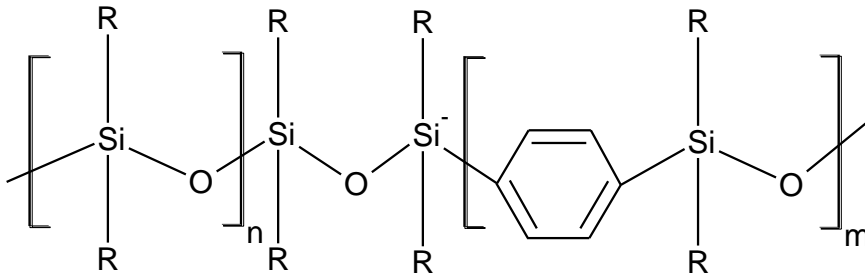


# STATIONARY PHASE POLYMERS

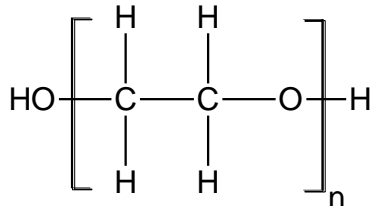


**R= methyl, cyanopropyl, cyanopropylphenyl,  
trifluoropropyl**

**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
Cvanopropyl	Strong	Strong	Moderate
Trifluoropropyl	Strong	Moderate	Weak
PEG	Strong	Strong	Moderate



# Selecting the Correct Column

Match analyte polarity to column polarity  
'Like dissolves like'

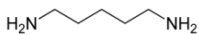
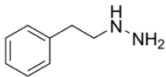
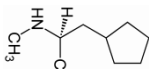
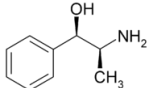
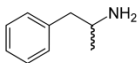
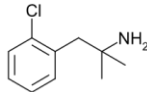
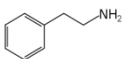
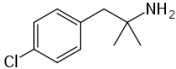
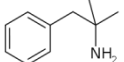
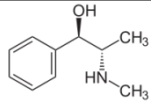
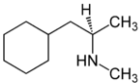
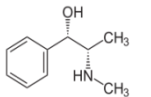
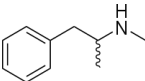
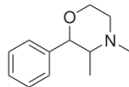

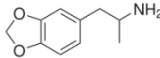

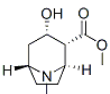
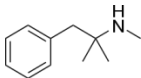
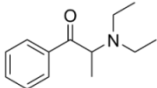
Look for unique interactions that analytes may have with a phase

Use preexisting information

Use the Agilent GC Application Support Team:  
[gc-column-support@agilent.com](mailto:gc-column-support@agilent.com)

Now Let's Apply What We Have Learned

# Sample List (drugs)

1. Cadaverine		11. Phenelzine	
2. Cyclopentamine		12. Phenylpropanolamine	
3. Amphetamine		13. Clortermine	
4. Phenethylamine		14. Chlorphentermine	
5. Pentermine		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 30 cm/sec

Oven: 50°C Hold for 5 min

10°C/min to 325°C Hold for 5 min

# Am I Going to Have Backflash?

**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**


Opt. Vel. range


**Pressure Units**

KPa    psi    bar

# 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

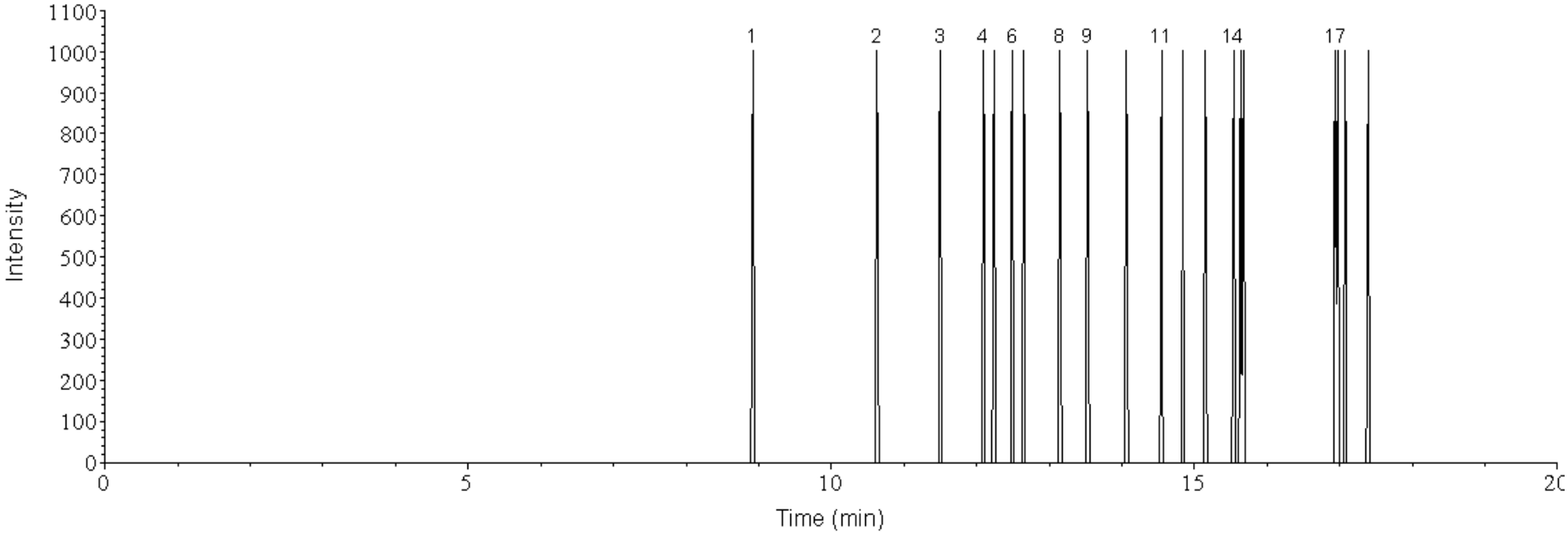
**Solvent Properties**  
Methanol [v]  
Boiling Pt (C): 64.7  
Denisty (g/cm3): 0.791  
Mol Wt. (amu): 32  
 Solvents

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

# Developing Temperature Program

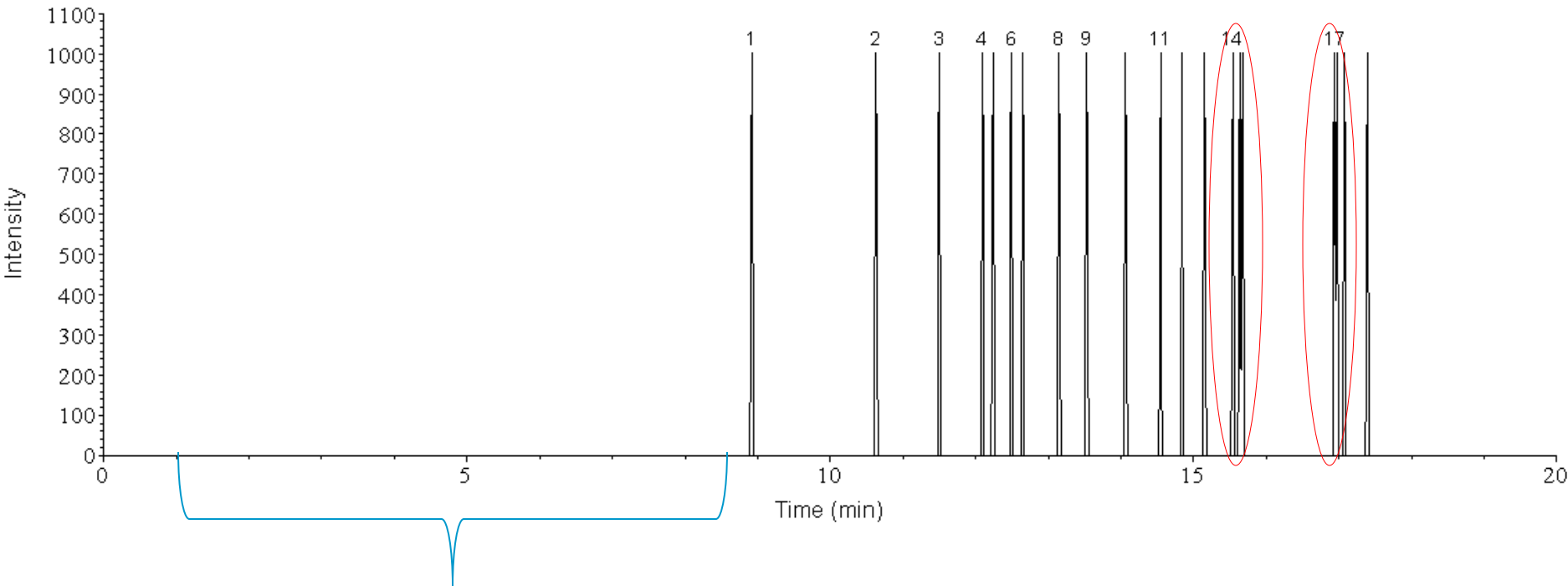
## Initial Run

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

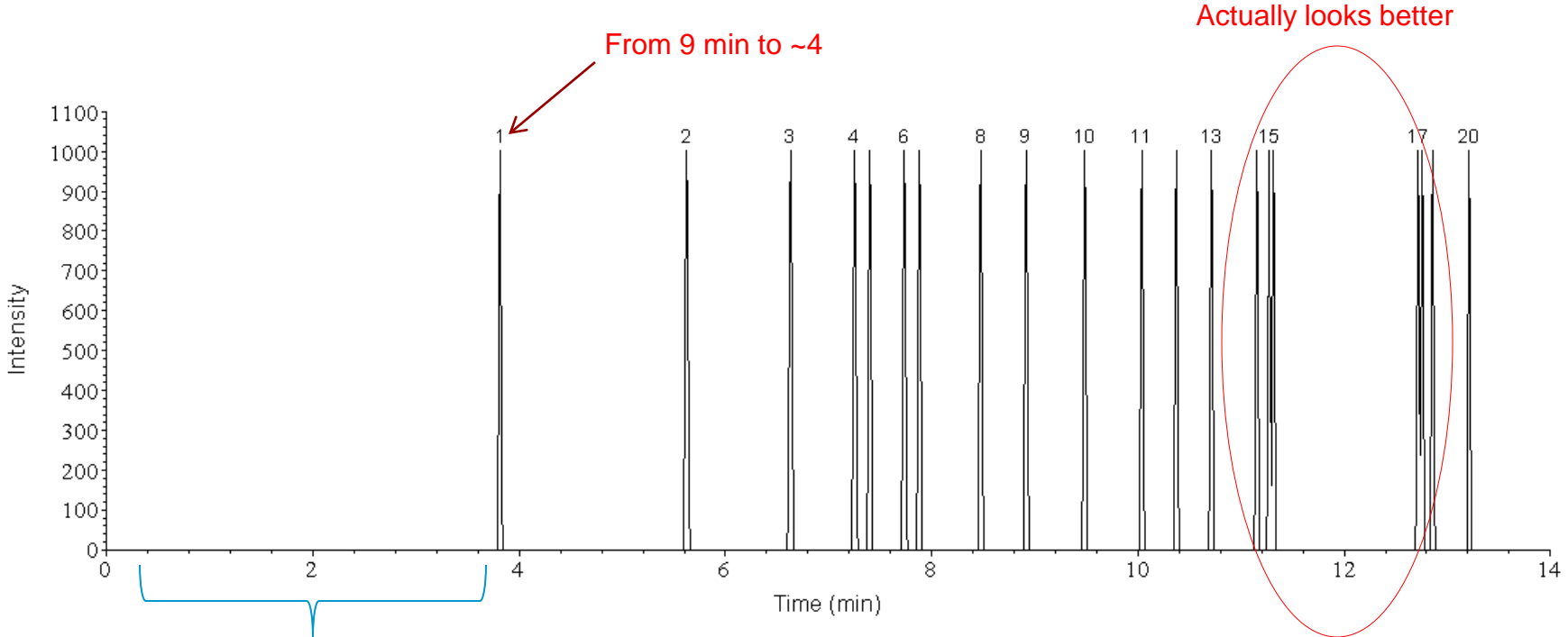
10°C to 325°C

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

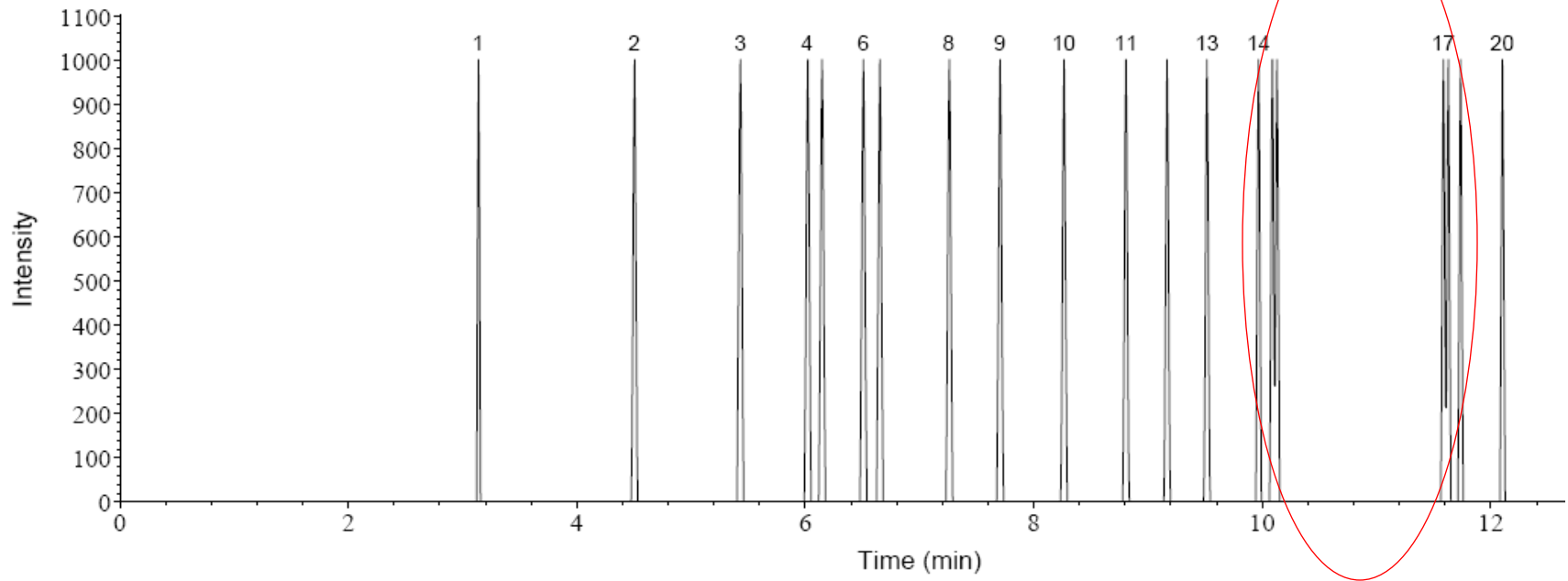


# Developing Temperature Program

## 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

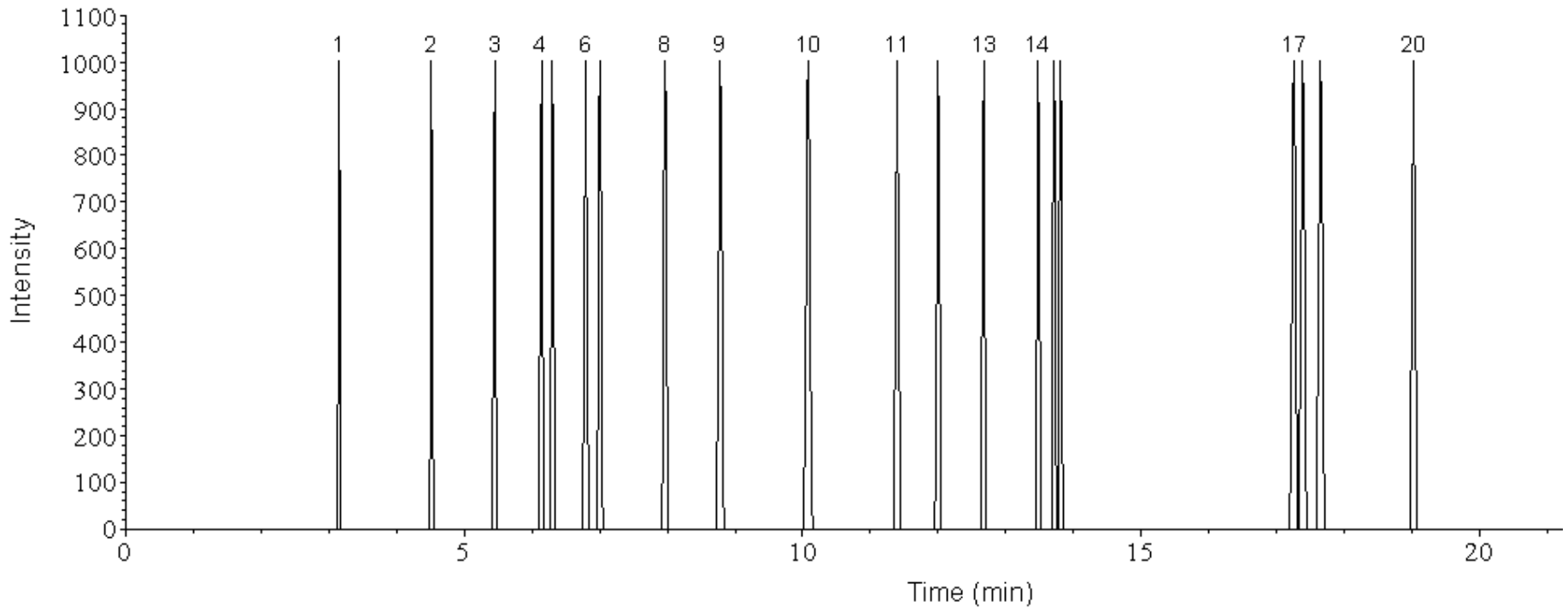
100°C hold for 5 minutes  
10°C/min to 325°C

Co-elutions occur at 150°C

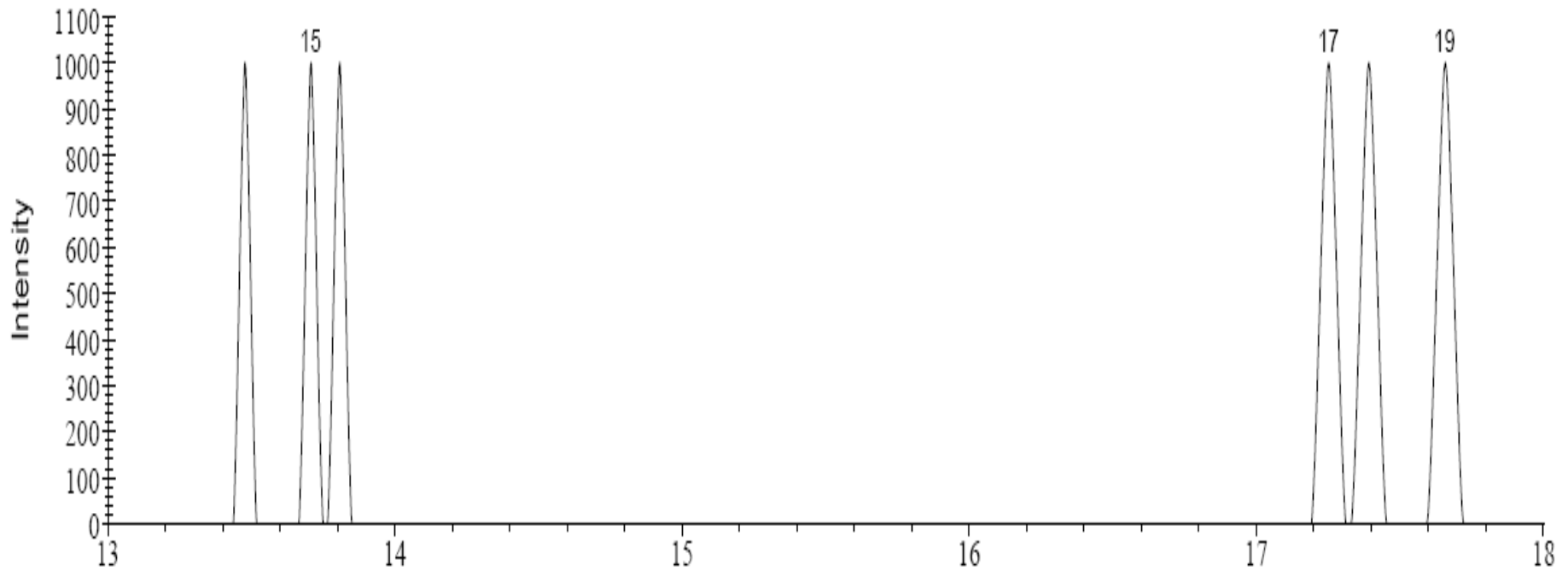
**Set hold at 130°C**

# Developing a Temperature Program

Oven: 100°C Hold for 5 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*

# Conclusions: Starting Parameters

--Assuming S/SI – FID system

Inlet Temp: 250°C

Split 50:1

Carrier Gas: Helium ~ 30 cm/sec, Hydrogen ~45 cm/sec

Oven Temp: 40°C hold for 5 minutes

10°C/min Ramp to Isothermal Limit of column

hold for 5-10 minutes

Detector Temp: 20°C above the highest oven temp



# Additional Resources and Application Support

## Sample preparation eSeminar Series

<https://www.agilent.com/en-us/training-events/eseminars/sample-preparation>

## Reference Materials and Guides:

Agilent Enhanced Matrix Removal – Lipid Brochure (Publication Number: 5991-6052EN)

[https://www.agilent.com/cs/library/brochures/EMR%20Brochure%20CPOD%20Final\\_LoResSglPgs.pdf](https://www.agilent.com/cs/library/brochures/EMR%20Brochure%20CPOD%20Final_LoResSglPgs.pdf)

<https://www.agilent.com/en-us/products/sample-preparation/sample-preparation-methods/sample-preparation-methods/enhanced-matrix-removal-lipid>

Agilent Sample Preparation Landing Page

<https://www.agilent.com/en-us/products/sample-preparation/sample-preparation-methods>

Agilent Sample Preparation Catalog (Publication Number: 5991-1057EN)

<http://www.agilent.com/cs/library/catalogs/public/5991-1057EN%20Sample%20Prep%20Catalog.pdf>



# Agilent J&W Scientific Technical Support

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

GC column/application support\* *Select option 3..3..1.*

Sample Prep Supplies/Support *Select option 3..3..3*

[gc-column-support@agilent.com](mailto:gc-column-support@agilent.com)

[spp-support@agilent.com](mailto:spp-support@agilent.com)

866-422-5571 (fax)

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