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. Organinc Acids?
   4. Light Gases?
   5. Nobel 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 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

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
- 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
  -<u>Do not</u> use ratio as a dilution factor

MINIMUM RECOMMENDED SPLIT RATIO

Higher flow rates

mm I.D.	Lowest ratio
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 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

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

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

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

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 μL of vapor

  4mm liners hold approx. 0.972 mL or 972 μL of vapor

Recommended injection volumes are 1-2μL or less for organic solvents, 0.5μL for water.

Liner Volume

How Do we Calculate the Vapor Volume?

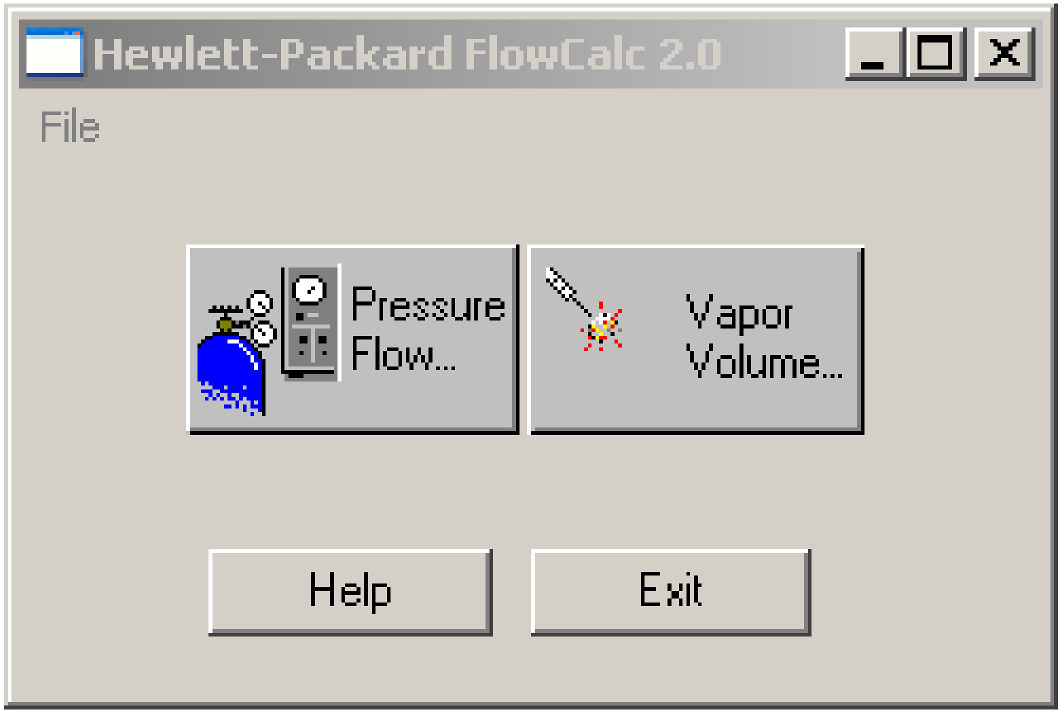
Pressure / Flow Calculator

Free download from our Website

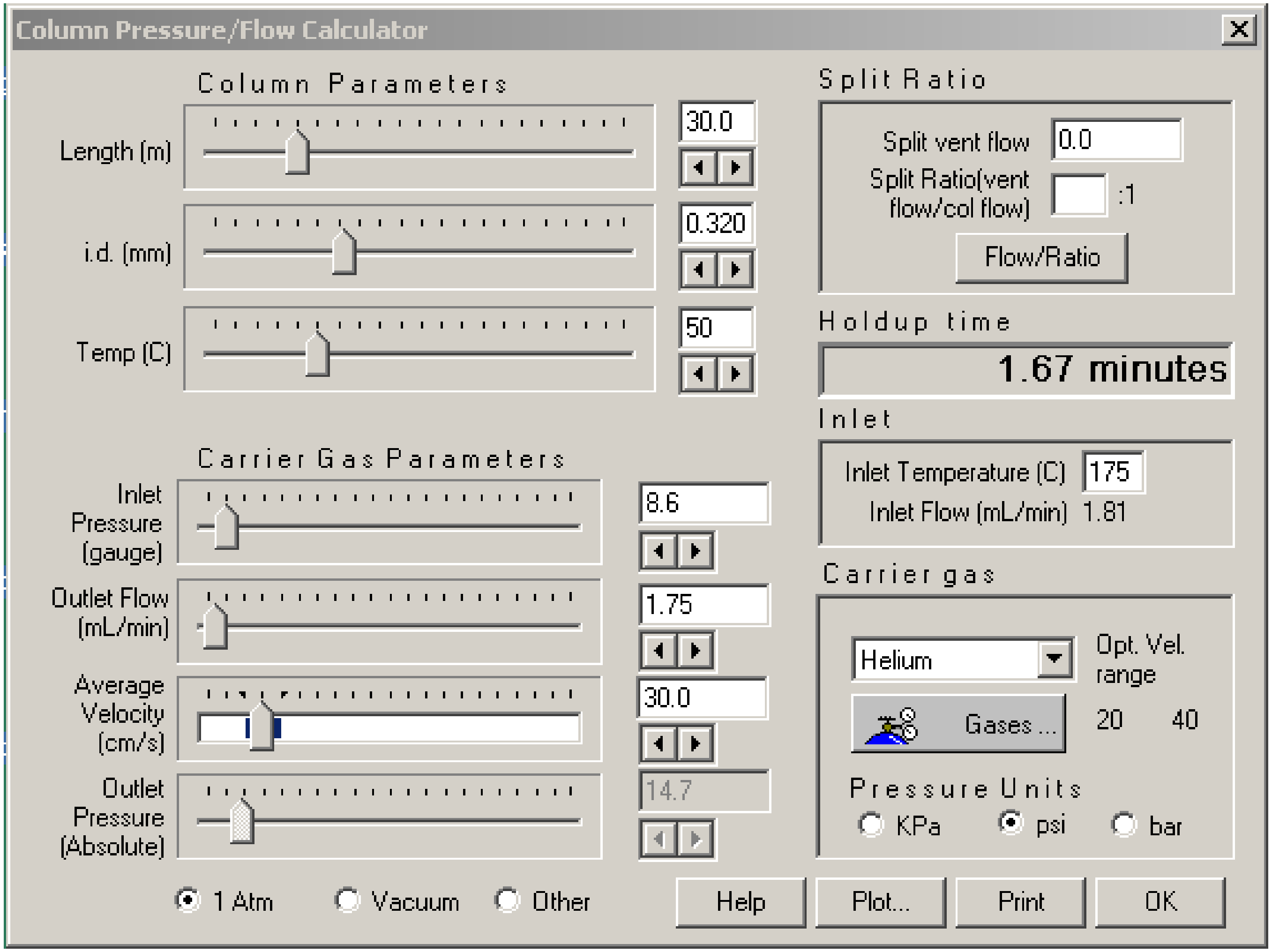
www.chem.agilent.com

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

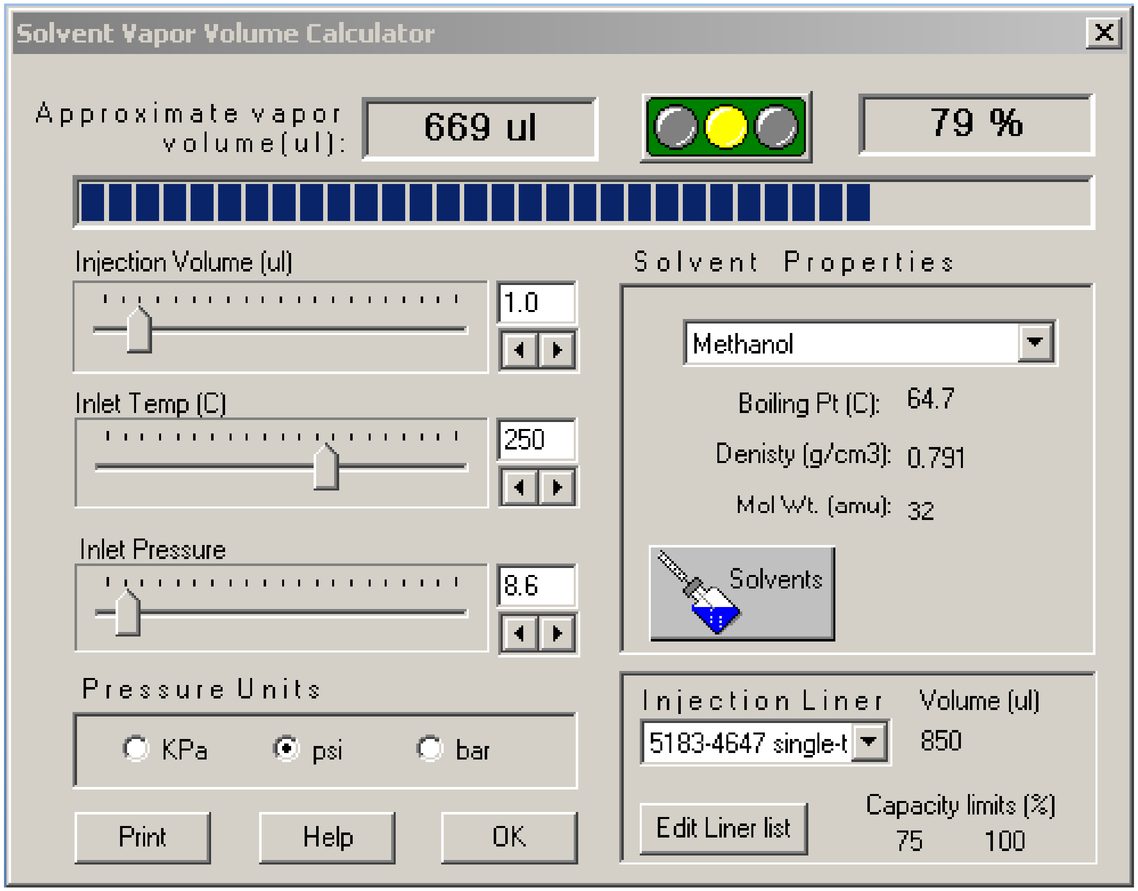
Pressure / Flow Calculator



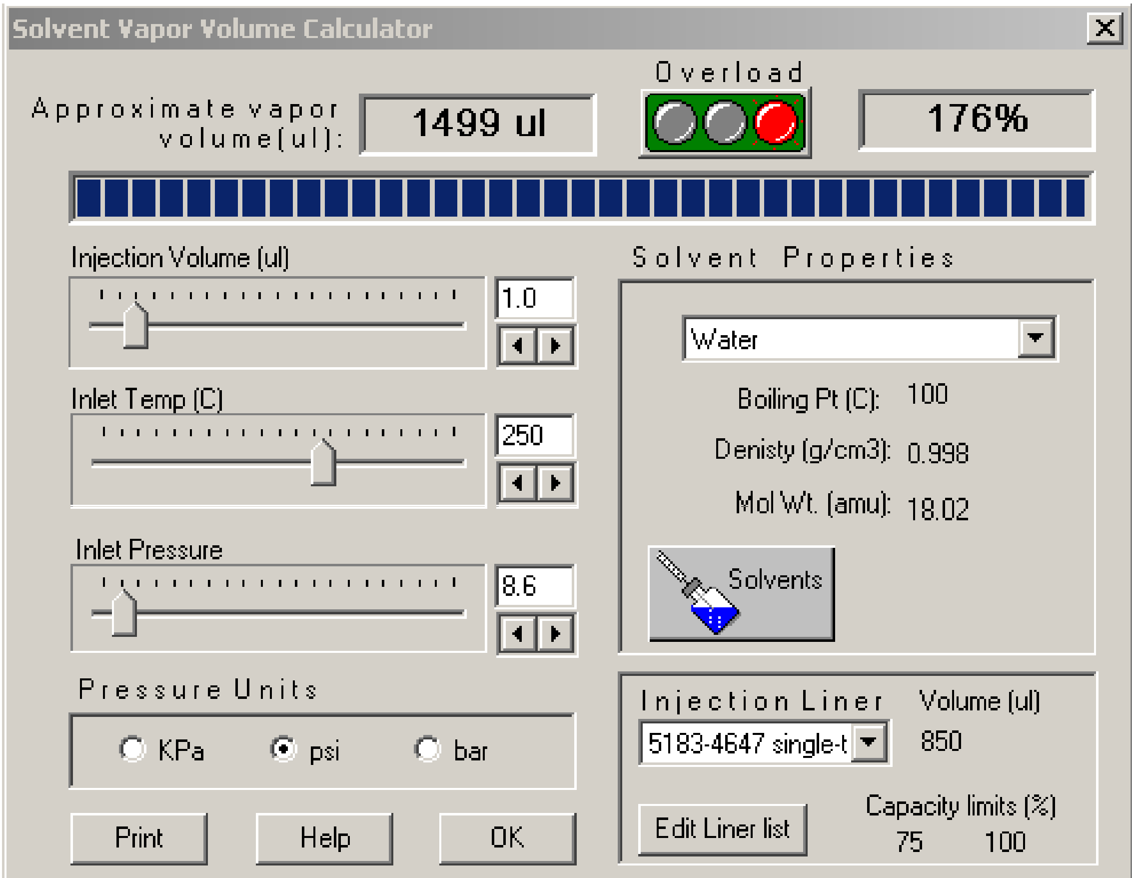
Determine what the inlet pressure will be:



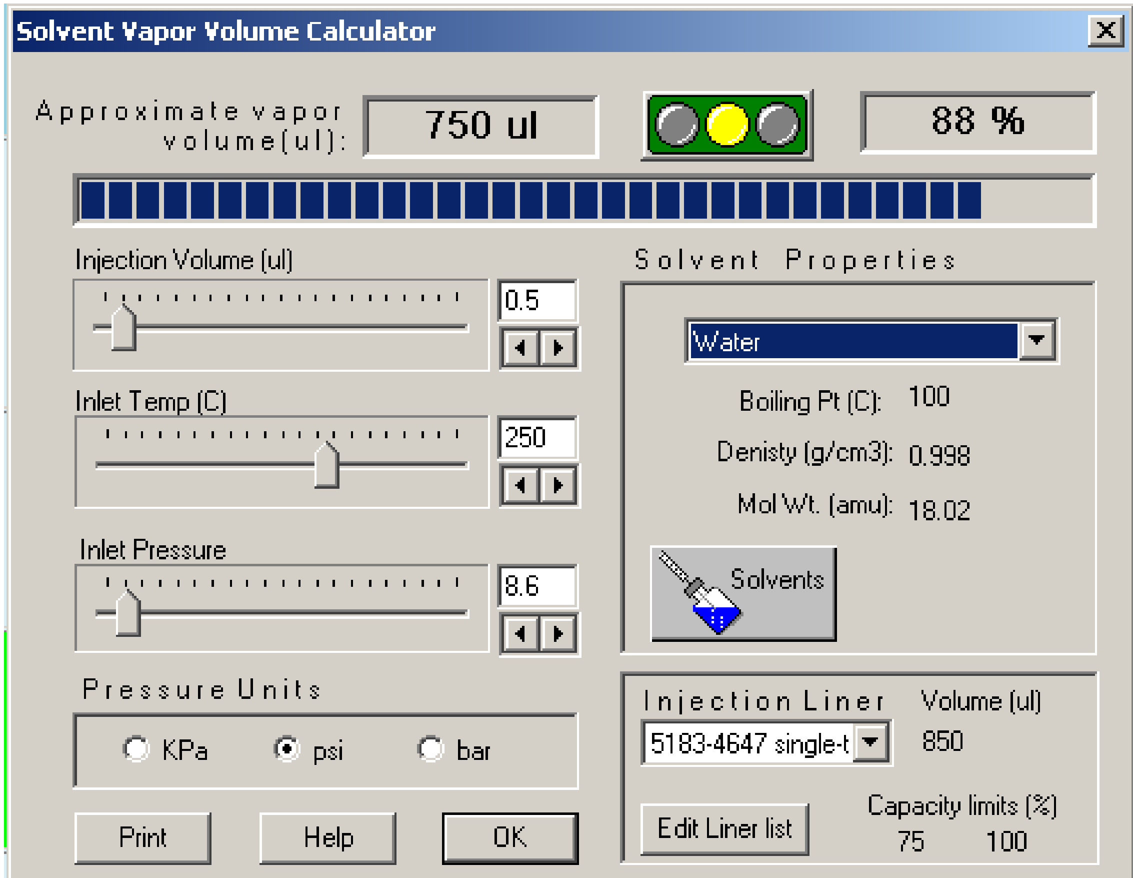
Test Inlet Conditions For Solvent Expansion



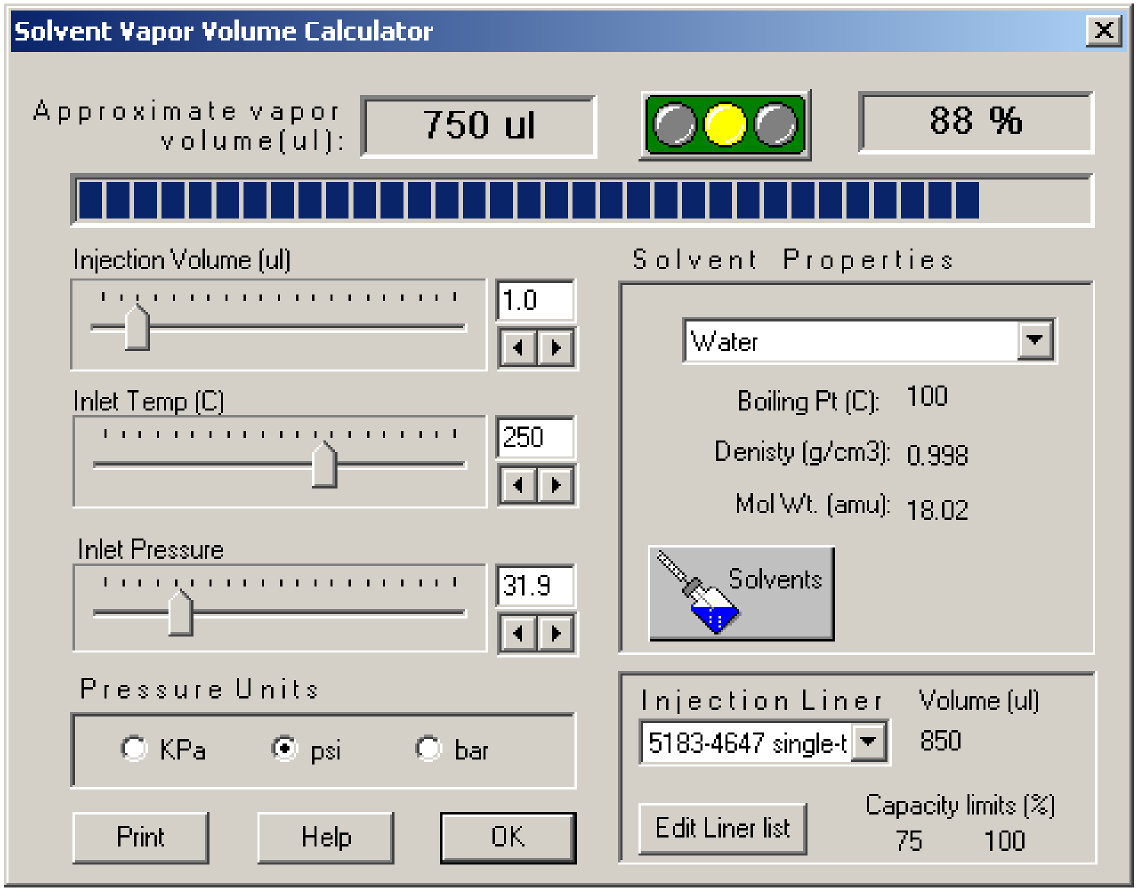
Water as Solvent



Water as Solvent
Cut Injection Volume in Half



Water as Solvent
Pulsed Injection



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:

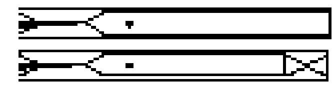
Taper (gooseneck), minimizes sample contact with gold seal.

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.)

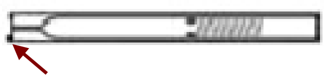
Other special characteristics include:

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

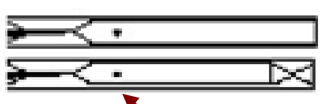
All designed to provide:

- 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.
	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 manual injection only. 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 manual injection only. 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.
 Side hole	G1544-80730 G1544-80700	Direct connect liners, single and dual taper, deactivated. Capillary column press fits into liner end, eliminating sample exposure to inlet. Ultimate protection for trace, active samples. Side hole permits use with EPC.

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

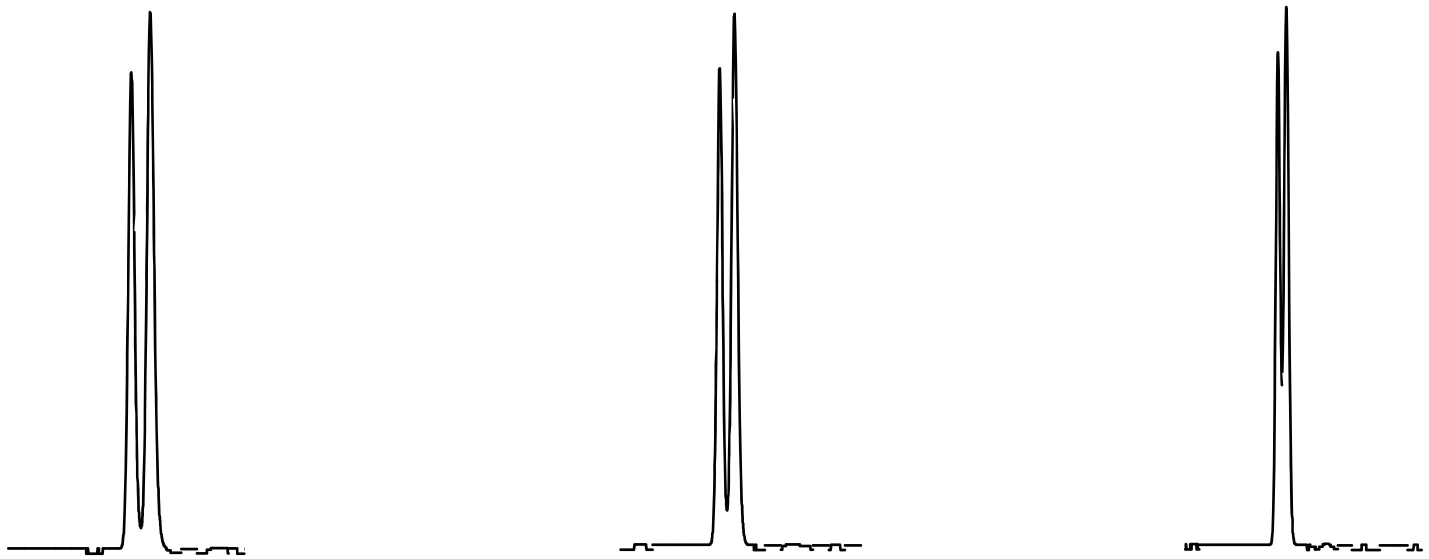
Carrier Gas Considerations

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

RESOLUTION VS. LINEAR VELOCITY

Helium

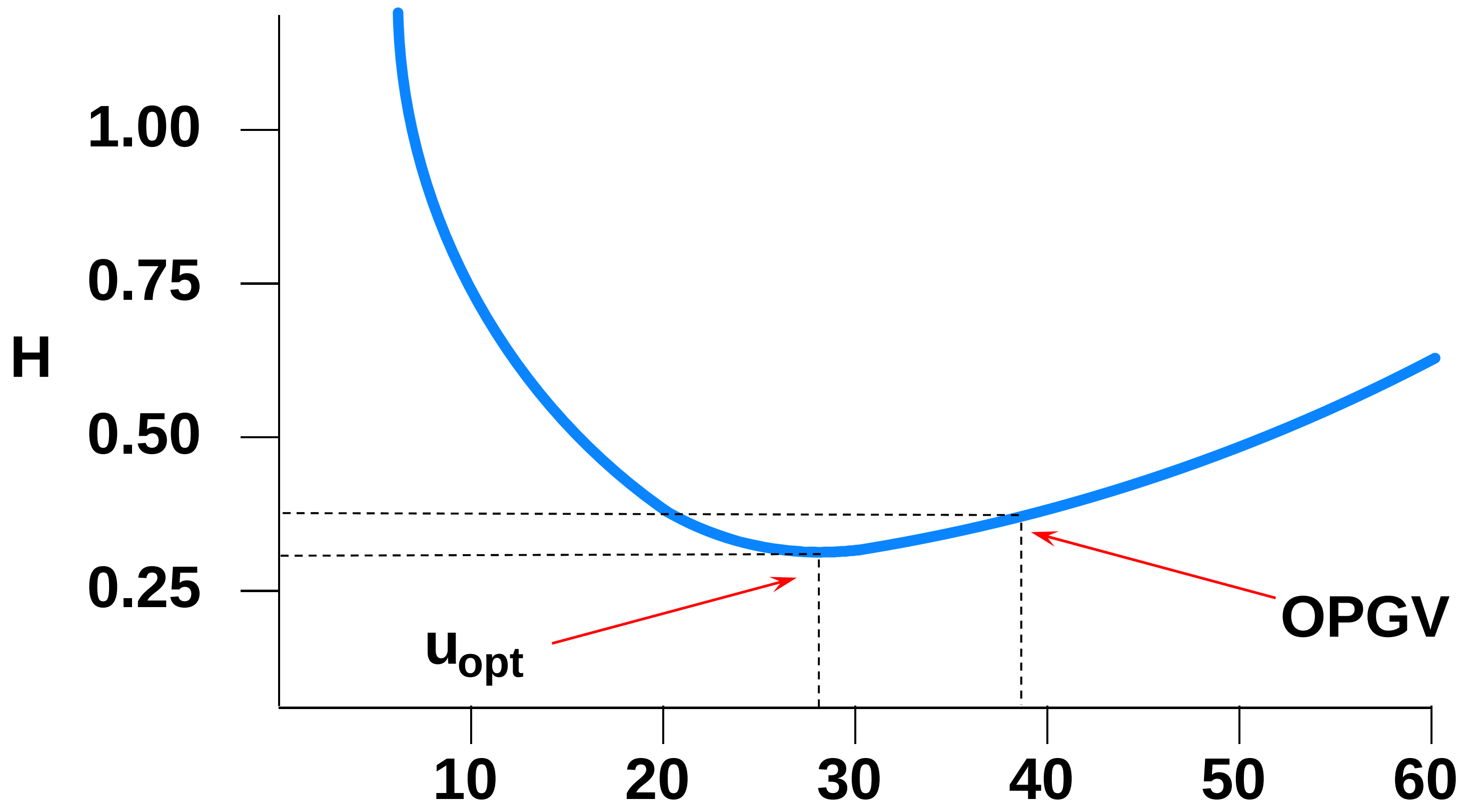
Resolution of 1.5 = baseline resolution



R = 1.46	*R = 1.31*	*R = 0.97*
30 cm/sec	*35 cm/sec*	*40 cm/sec*
4.4 psig	*5.1 psig*	*5.8 psig*

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

VAN DEEMTER CURVE



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

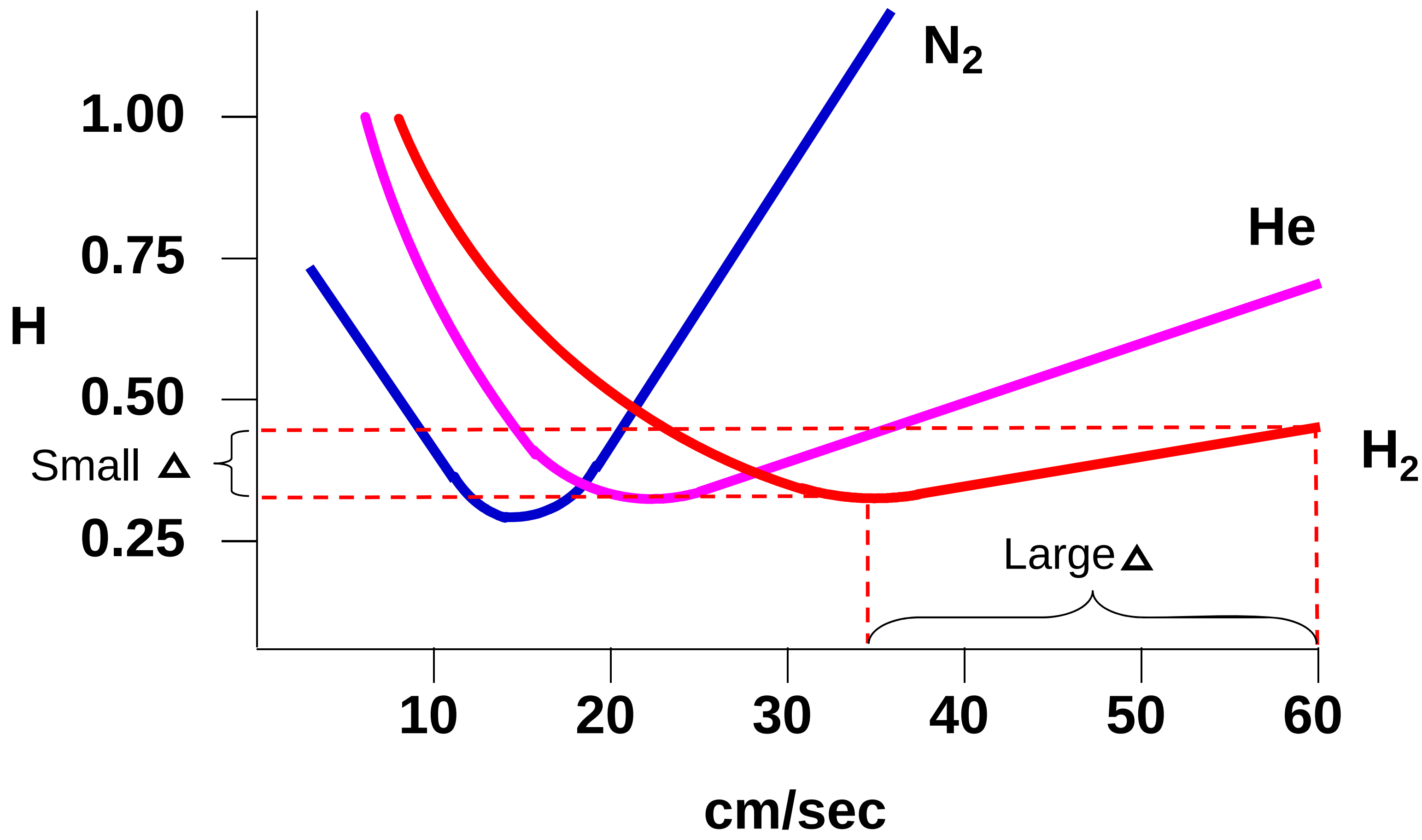
$\bar{u}_{opt}$: Maximum efficiency

OPGV: Optimal practical gas velocity

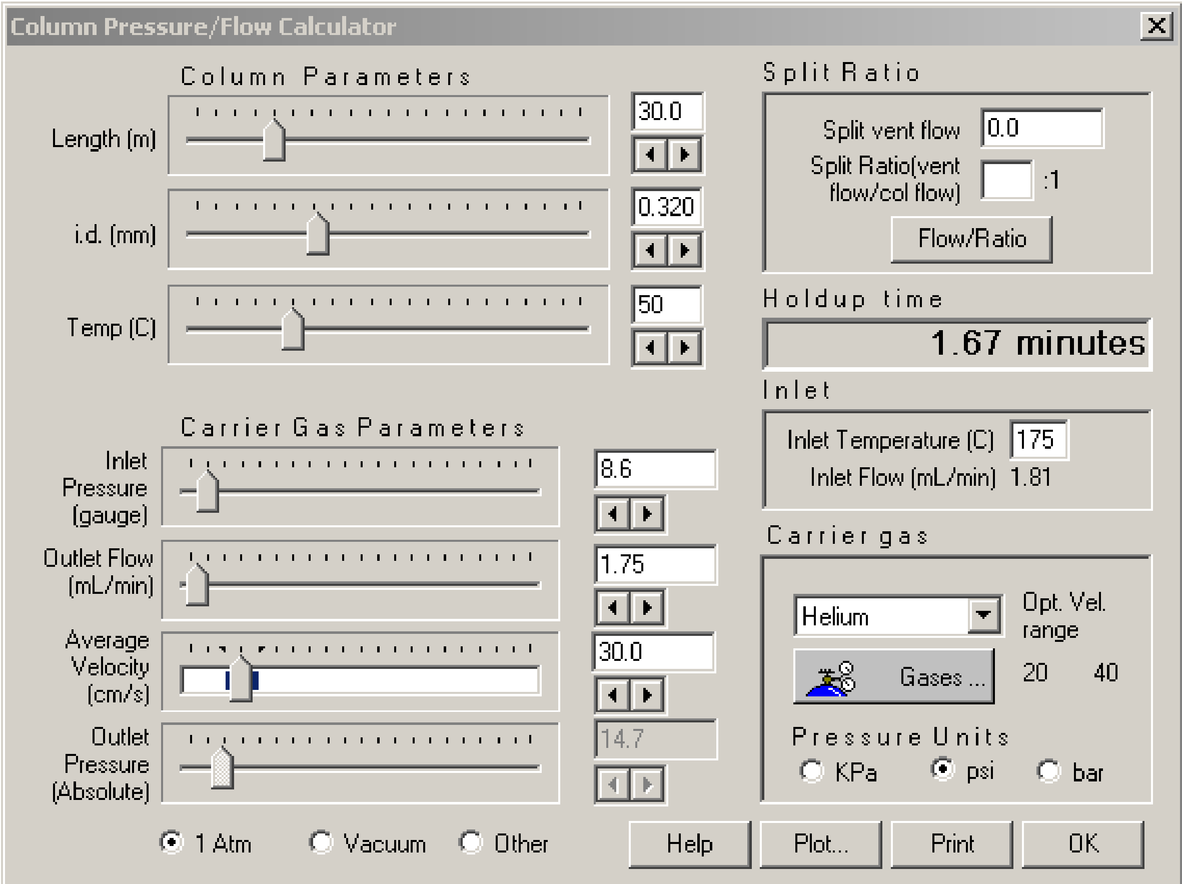
Maximum efficiency per unit time

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

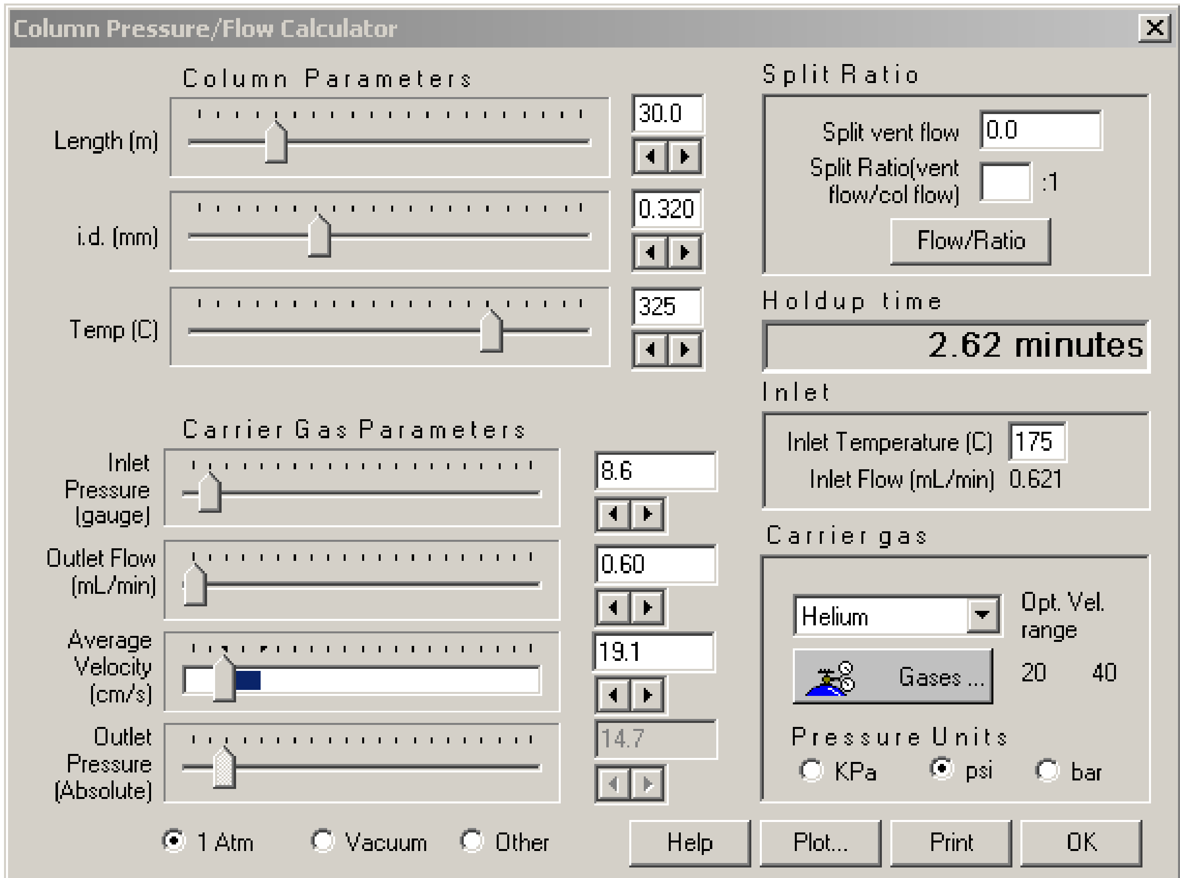
VAN DEEMTER CURVES



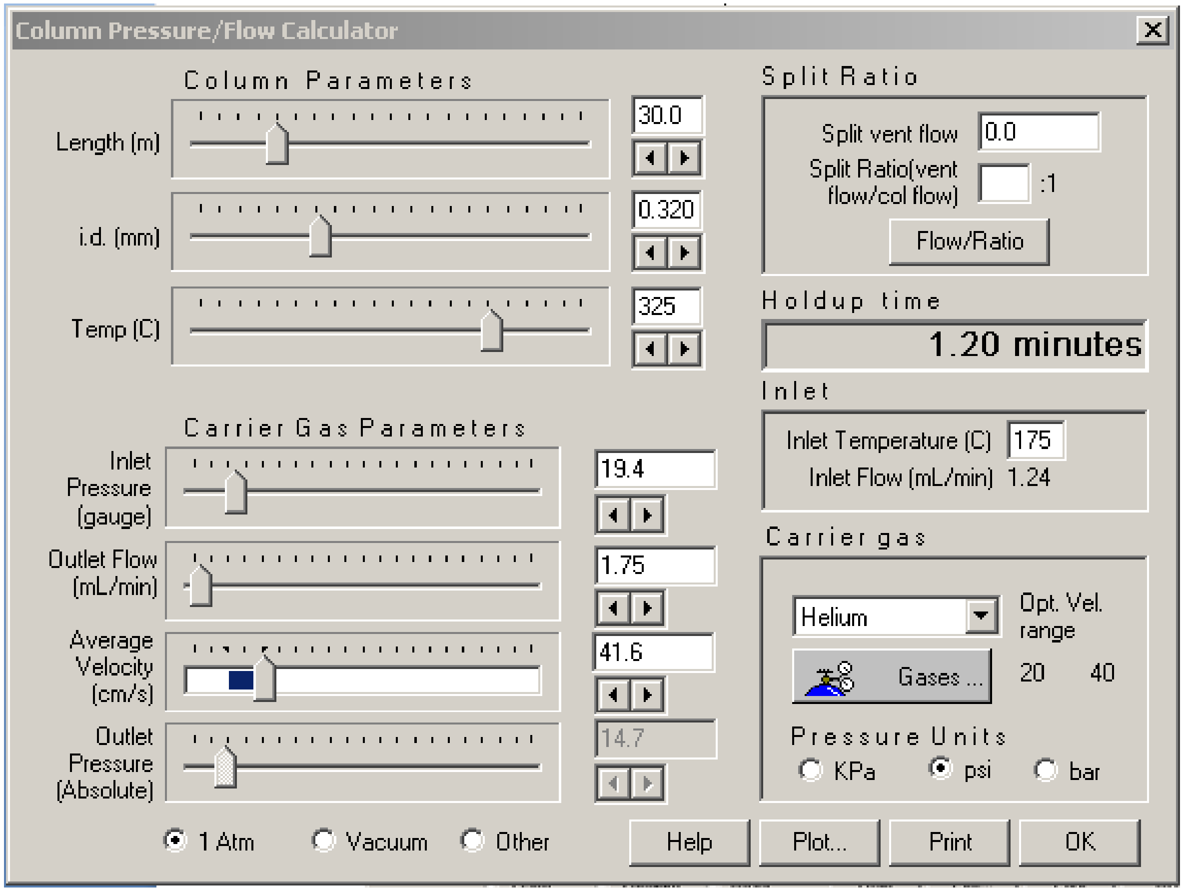
What Happens to the Flow as Oven Temp Increases?



Carrier Gas: Constant Pressure



Carrier Gas: Constant Flow



Detectors

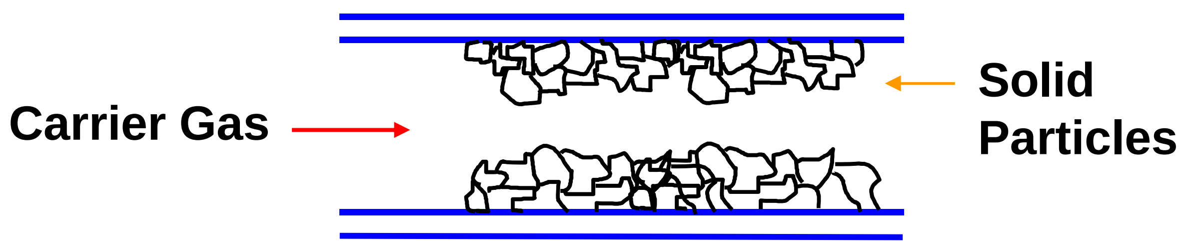
Detector	Dynamic Range		MDL
TCD	10^5	Universal	400 pg Tridecane
FID	10^7	Responds to C-H bonds	1.8 pg Tridecane
ECD	$5x10^5$	Responds to free electrons	6 fg/mL Lindane
NPD	10^5	Specific to N or P	0.4 pgN/s 0.06 pg P /s
FPD	10^3S, 10^4P	Specific to S or P	60 fg P/s 3.6 pg S/s
SCD	10^4	Specific & Selective 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

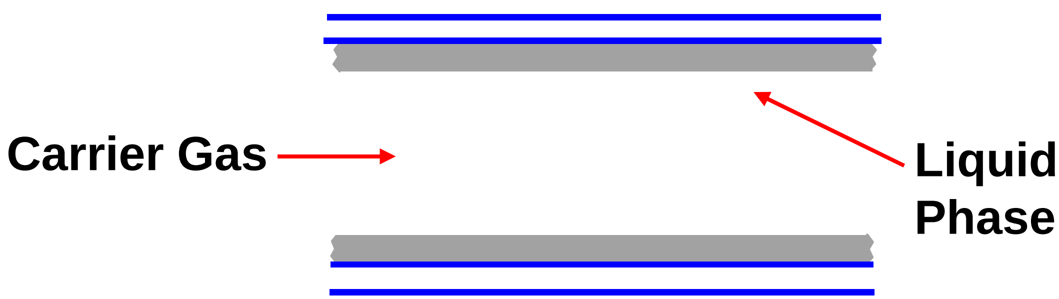
Understanding the Stationary Phase

CAPILLARY COLUMN TYPES

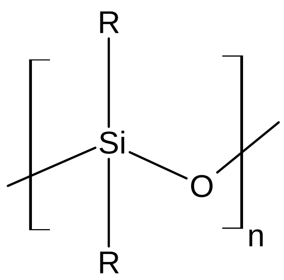
Porous Layer Open Tube (PLOT)



Wall Coated Open Tube (WCOT)

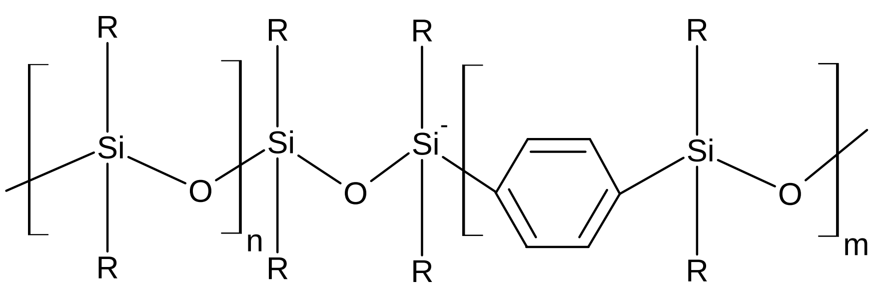


STATIONARY PHASE POLYMERS

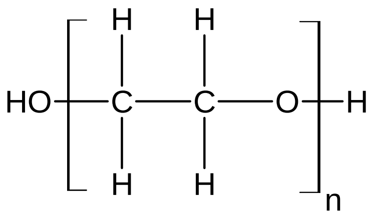


R= methyl, cyanopropyl, cyanopropylphenyl, trifluoropropyl

Siloxane



Arylene



Polyethylene glycol backbone

Selectivity Interactions

- Dispersion
- Dipole
- Hydrogen bonding

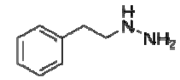
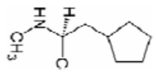
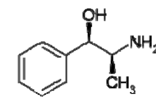
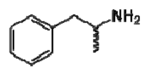
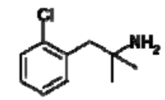
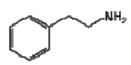
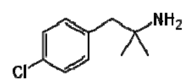
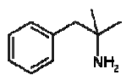
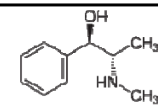
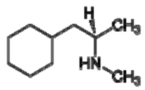
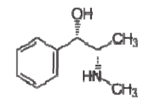
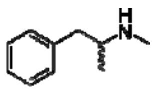
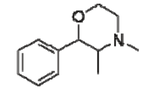
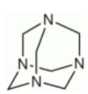
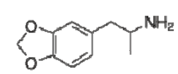
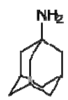
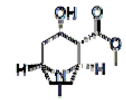
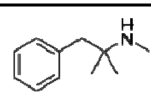
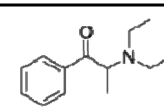
Selectivity

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/Sl Inlet: Split 50:1 Temp 250°

FID: Temp 350°

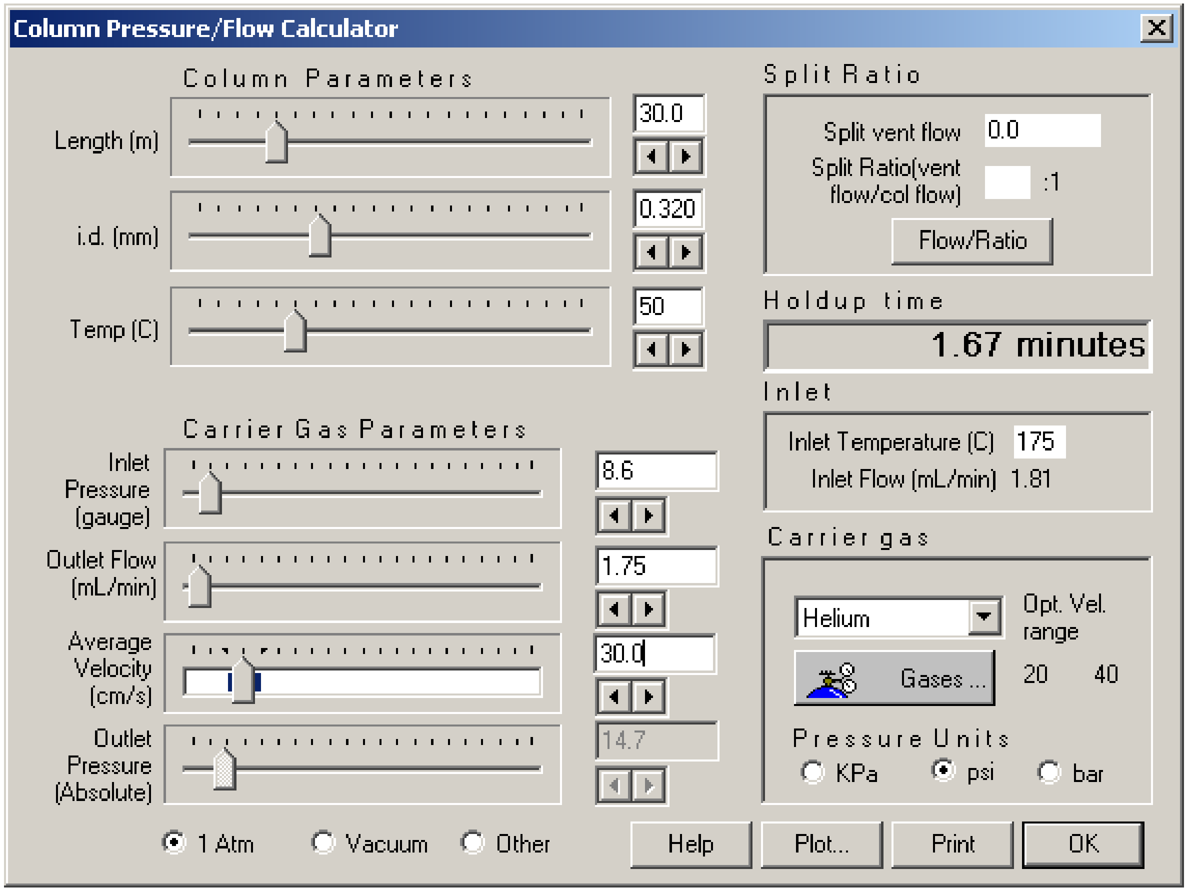
Carrier: He

Constant flow 30cm/sec

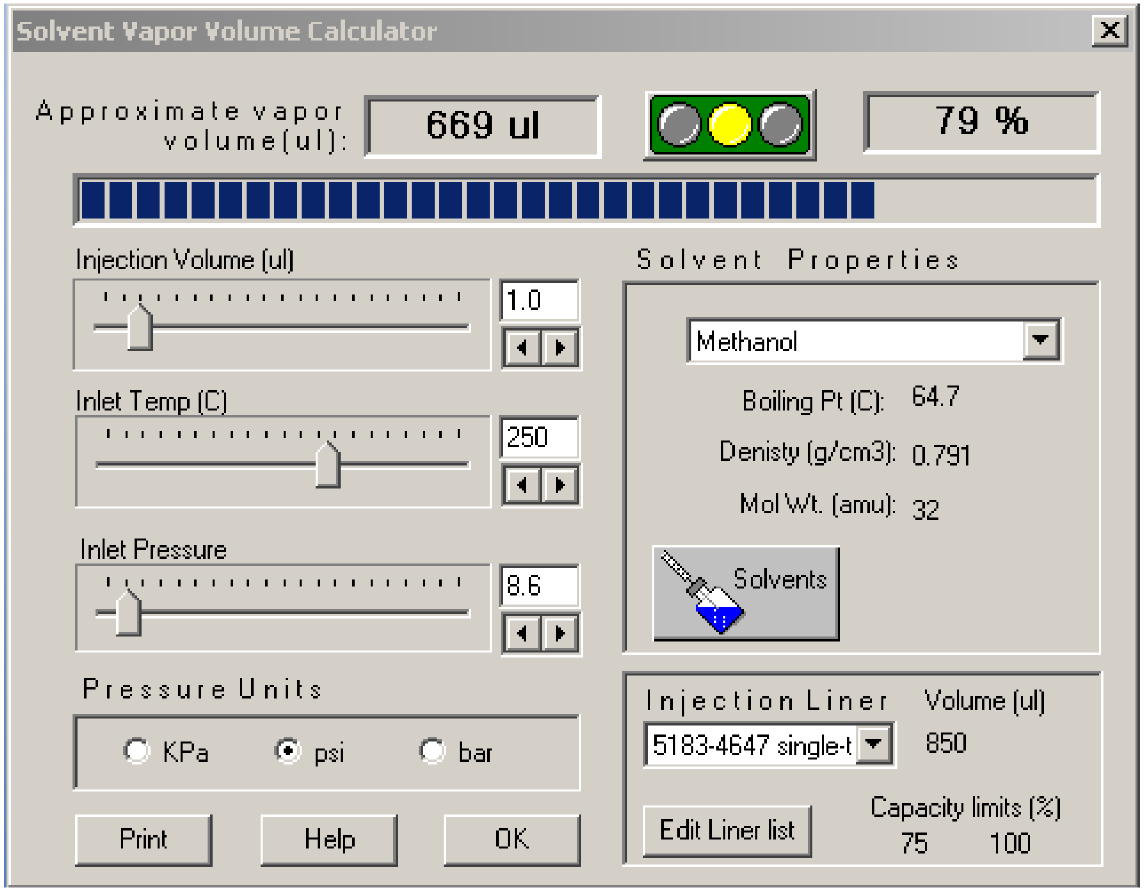
Oven: 50°C Hold for 5 min

10°C/min to 325°C Hold for 5 min

Am I Going to Have Backflash?

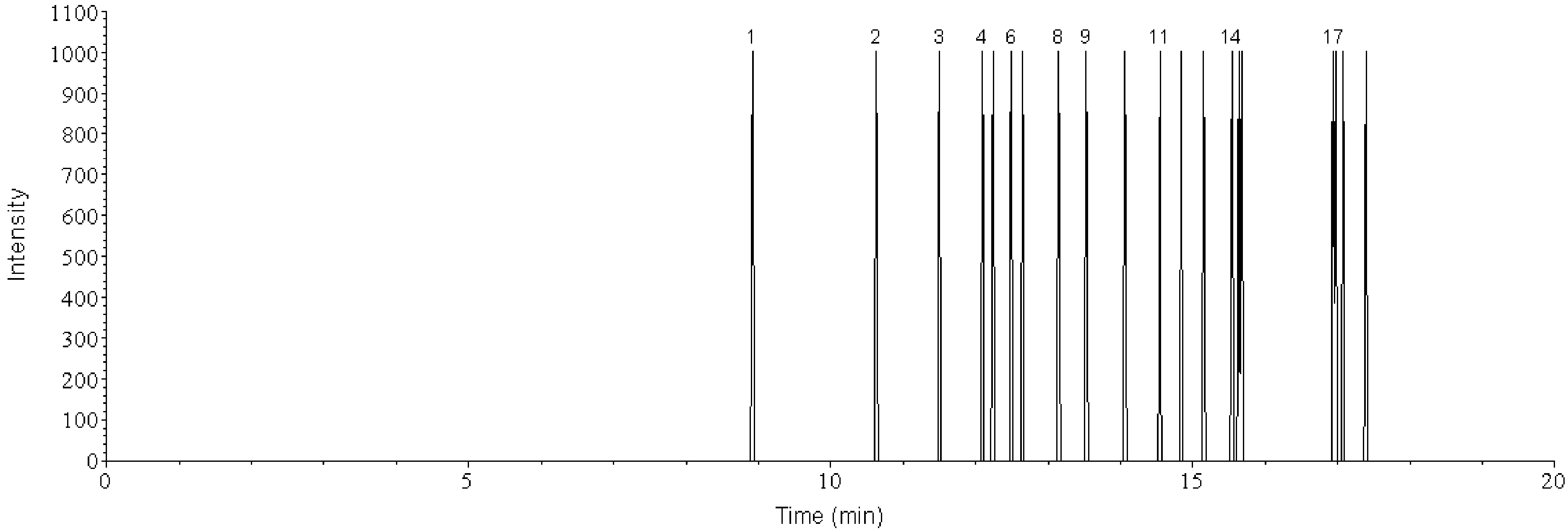


Injection Volume / Solvent Expansion



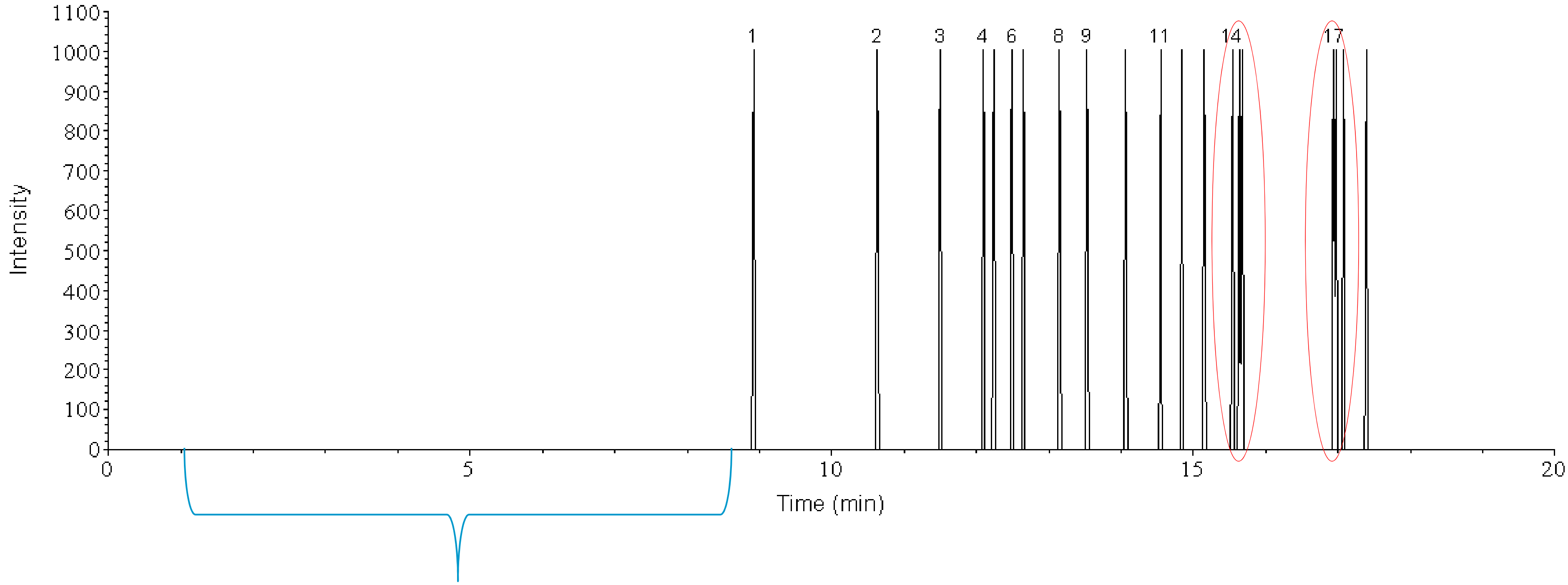
Developing Temperature Program
Initial Run

Initial Temp 50ºC Hold for 5 min
Ramp 10ºC/min to 325ºC 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:

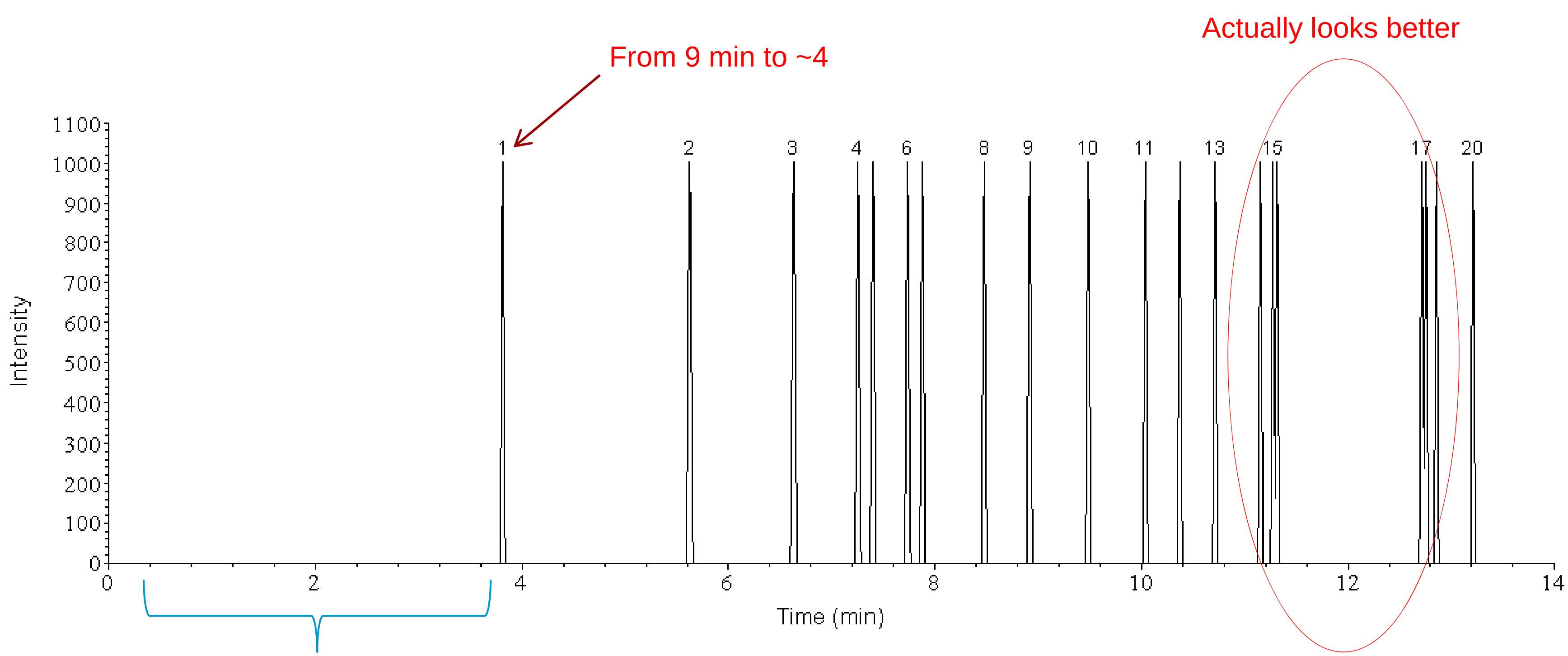
$50^{o}C$ for 5 minutes

$10^{o}C$ to $325^{o}C$

1st Peak comes out at $90^{o}C$

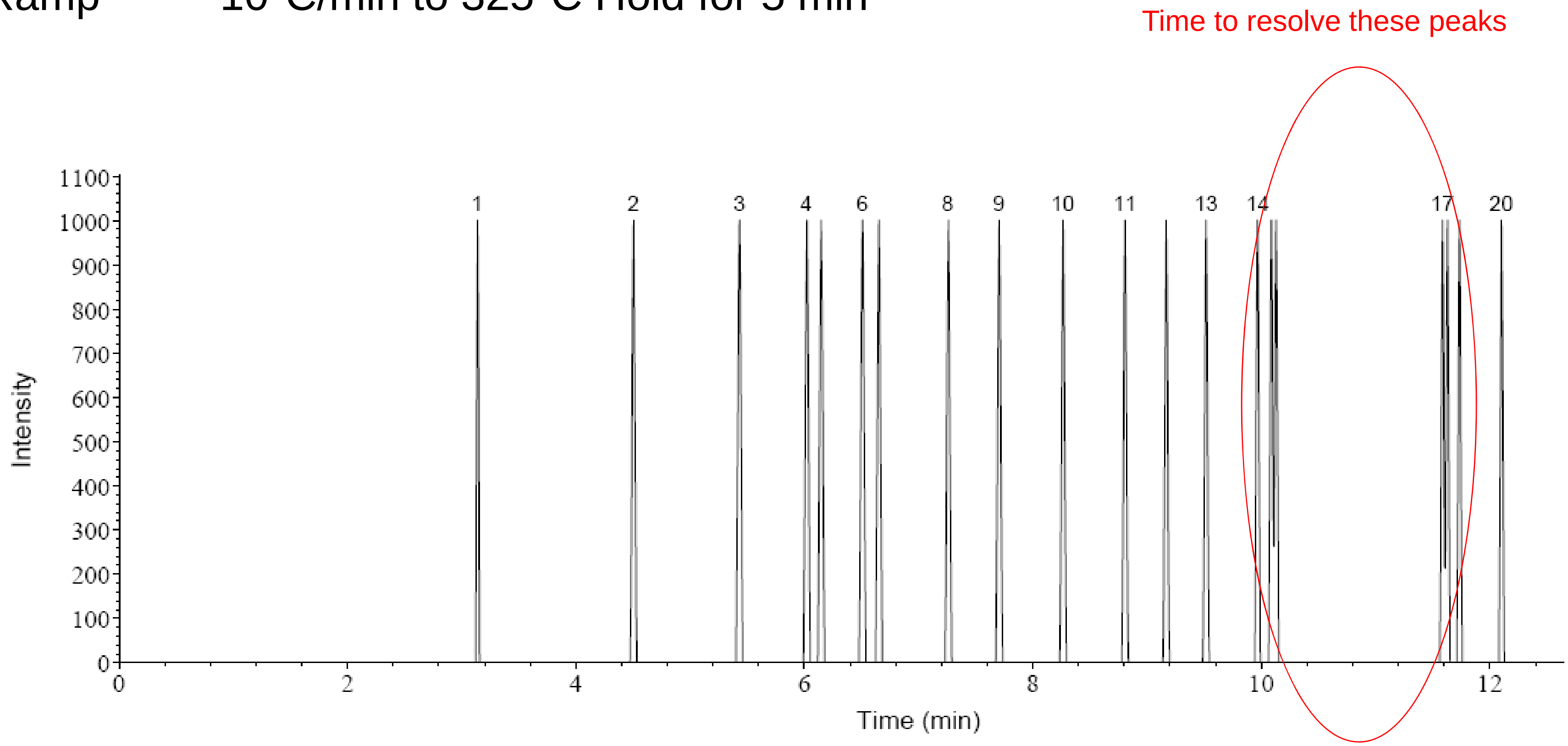
Developing Temperature Program
2nd Try

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^{o}C Hold for 5 min
Ramp 10^{o}C/min to 325^{o}C Hold for 5 min



Resolve Co-elutions

Add a hold 20-30^{o} below the elution temperature

Co-elutions occur at 10 minutes

100^{o}C hold for 5 minutes
10^{o}C/min to 325^{o}C

Co-elutions occur at 150^{o}C

Set hold at 130^{o}C

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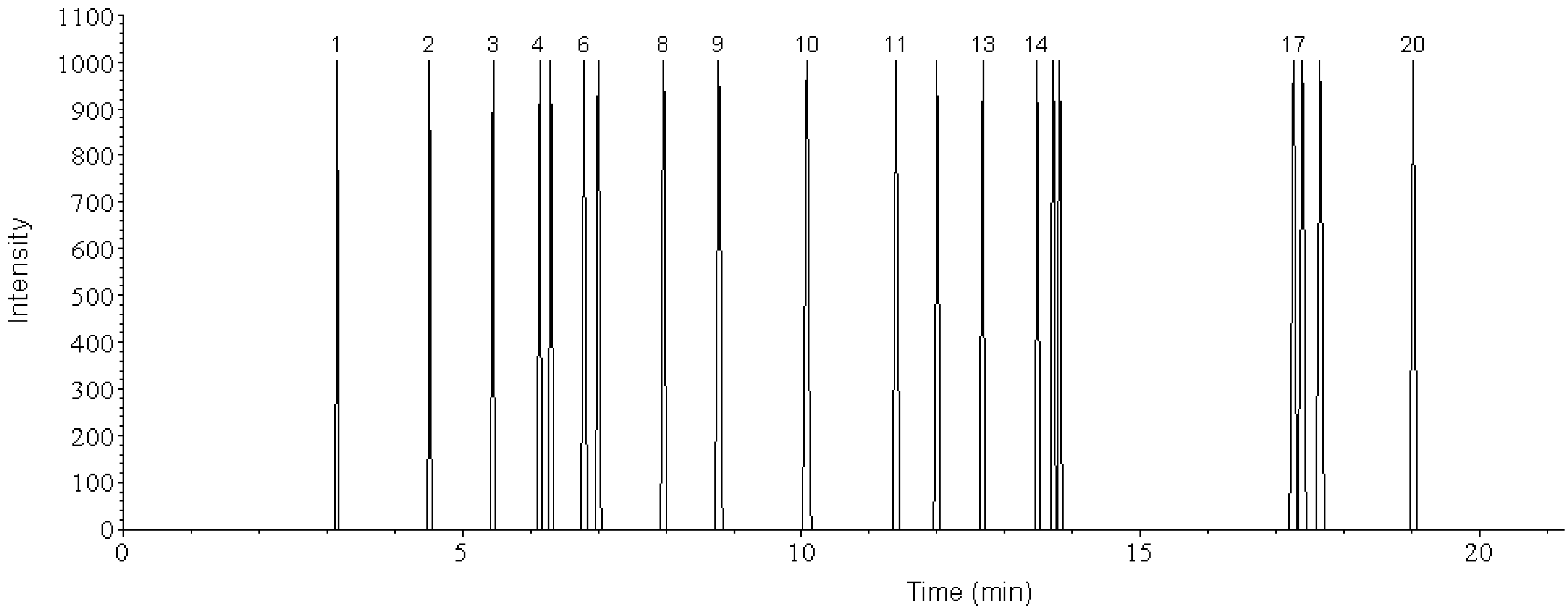
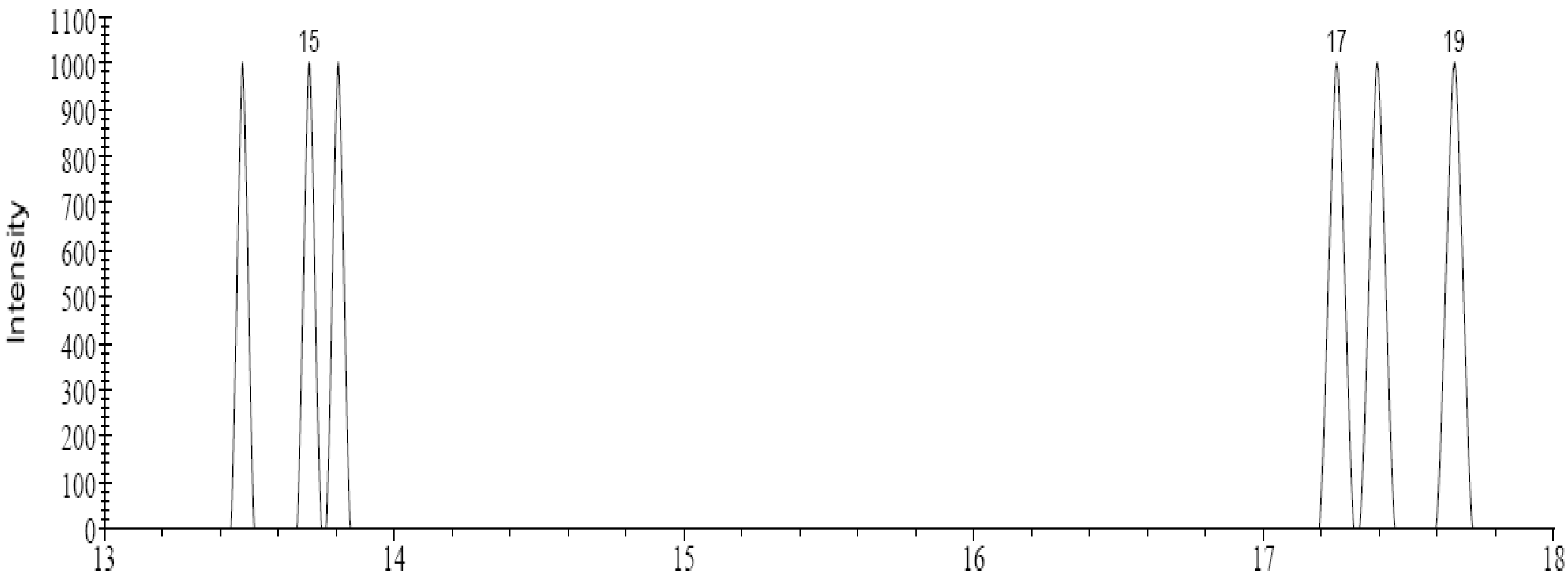
Oven: 100^{o}C Hold for 10 minutes
10^{o}C/min to 130^{o}C hold for 5 min
10^{o}C/min to 325^{o}C



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

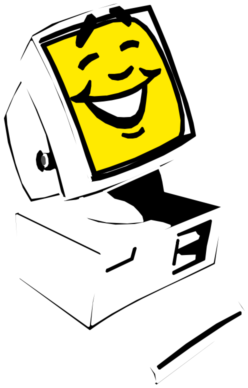
Agilent J&W Scientific Technical Support

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

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

866-422-5571 (fax)

GC-Column-support@agilent.com



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