Capillary column GC

There are sufficient differences in the performance and instrumentation involved with capillary column GC to allow separate treatment.

You must have an instrument capable of using capillary columns.

Additional decisions must be made regarding how do inject samples and optimize the separation.

Packed vs. capillary columns

	Packed	Capillary
length, M	0.5 - 5	5 - 100
ID, mm	2 - 4	0.1 - 0.7
flow, ml/min	10 - 60	0.5 - 15
head pressure, psig	10 - 40	3 - 40
total plates	4000	250,000
capacity	10 µg/peak	100 ng/peak
film thickness, µm	1 - 10	0.1 - 0.8

Capillary columns

Major differences

- Smaller ID
- Longer
- No packing
- Smaller sample capacity

This all adds up to allow components to remain on the column longer while still retaining good peak shape.

Improved sensitivity

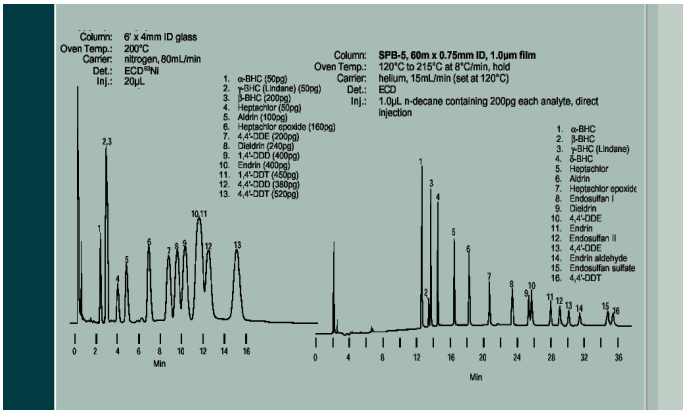
Because peaks remain narrower, the sensitivity is improved.

Packed

Capillary

Both peaks have an area of 5000 units.

Because the capillary peak is higher, you get a better S/N.



Packed vs. macrobore

Columns

Available in two basic forms

Coated - simple coating on the inside of a fused silica tube

Bonded - chemically bound via a silane bond.

Both types are coated on the outside with a polyamide to reduce breakage.

Factors influencing separation

Six major interrelated factors to consider

- Column length
- Column internal diameter
- Film thickness
- Carrier gas type
- Carrier gas velocity
- Column temperature

Carrier gas

We'll only consider hydrogen, helium and nitrogen.

Nitrogen

Generates the lowest HETP values (at the expense of speed).

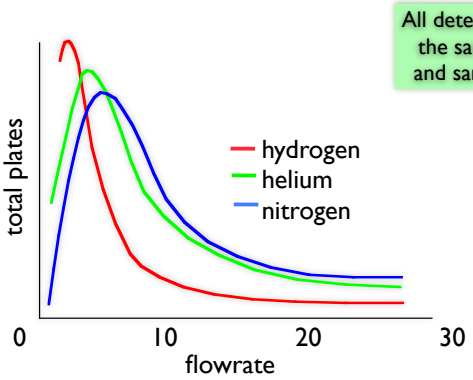
Helium

Better speed than with nitrogen with only small increase in HETP

Hydrogen

Best

Flowrate vs. carrier gas type

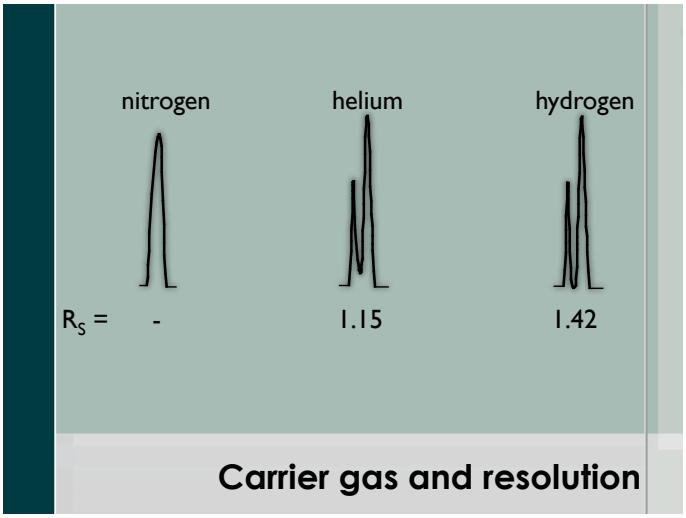


Why?

Hydrogen only shows a very small rise in the effect of the C term of the van Deemter equation.

This allows us to use much higher flows with little loss of resolution.

Hydrogen gives four times as many plates/second compared to helium.



Column temperature

Very strong effect on analysis.

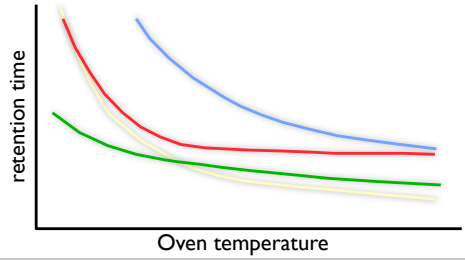
$$t_R = \frac{L}{\bar{v}}(k + 1)$$

$$k \propto \frac{1}{T}$$

Column temperature can be used to directly control retention on the column.

Increased temperature will reduce retention but all components may not be affected to the same extent.

Effect of temperature



As T increases, k is reduced for each species. The degree of this effect is not the same for each component. Resolution may improve or degrade for any pair of peaks and elution order may vary.

Column factors

Film thickness
Column diameter
Column length

Film thickness and internal diameter of the column are the most important effects.

They determine the relative amounts of each phase present in a plate

Distribution constant, K_D

$$K_D = \frac{[\ ]_{\text{liquid phase}}}{[\ ]_{\text{gas phase}}} = \frac{\text{weight}_{\text{liquid phase}}}{\text{weight}_{\text{gas phase}}} = \frac{\text{volume}_{\text{gas}}}{\text{volume}_{\text{liquid}}} = k\beta$$

k = partition ratio

β = phase ratio

Film thickness & internal diameter

Film thickness & internal diameter

$$\beta = \frac{r}{2d_f}$$

r = column internal diameter

d_f = film thickness

As β increases, you get more retention. However, the resolution will decrease and the analysis time will go up.

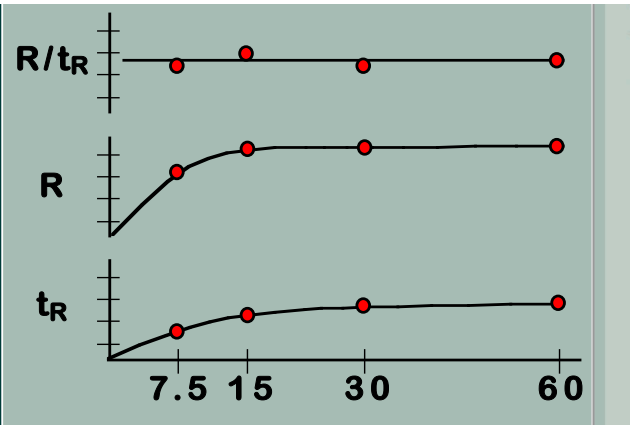
Generally, thick films are used for the analysis of more volatile samples.

Column length

One of the least significant effects.

Remember - resolution α length$^{1/2}$

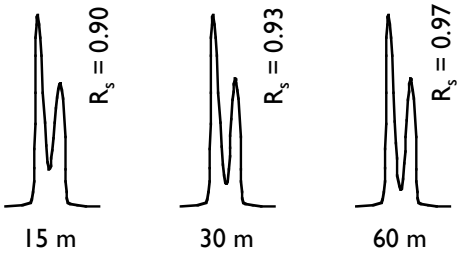
If a 10 meter column gives moderate resolution, you might need a 50 meter column to make a significant improvement.



Column length

Column length vs. resolution

0.25 mm id columns
0.1 µm film thickness
Isothermal run



15 m 30 m 60 m

Instrumental considerations

To account for capillary work, the injection and detection systems must be modified.

- Flows and sample amounts are smaller
- Peaks are much narrower

Detectors - must be redesigned to reduce 'dead' volumes.

Injectors - modified to account for low column capacity and alternate injection methods.

Instrumental considerations

- With detectors, you either have one capable of working with capillary columns or not. Newer instruments can handle most packed and capillary columns.
- Injection ports usually are either for capillary or packed column work. Capillary column injection ports can be used with packed columns if need be. The opposite is not true.

Injection methods

Capillary columns have a much smaller sample capacity compared to packed columns

(1/100th to 1/1000th)

Methods are available to either reduce total sample size (split injection) or reduce the amount of solvent that enters a column (Grob injection).

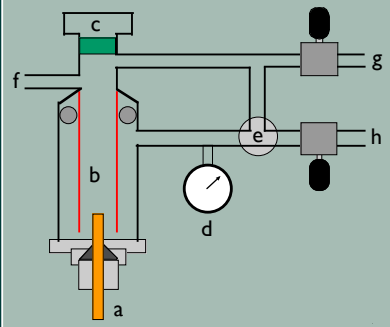
First, lets look at an injection system.

Packed Capillary

Additional flow control is required for capillary column injection.

High pressure control is also required.

Packed vs. Capillary injection

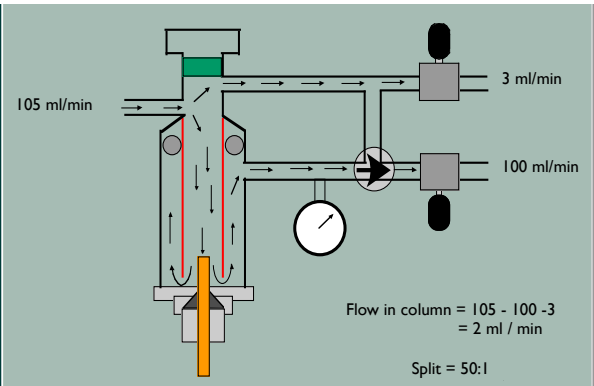


a) column
b) liner
c) septum
d) column head pressure gauge
e) purge control valve
f) total flow in
g) septum flow
h) split flow

Injection system

Split injection

- It would be difficult to introduce volumes much less than 1 μl directly.
- One way to accomplish the same thing is to split the sample after injection - reducing the total load entering the column.
- After volatilization and mixing with carrier gas, most of the flow can be directed out the split vent.
- Only a fraction of the sample actually enters the column.



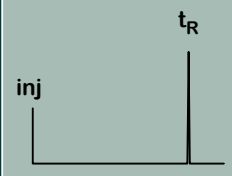
Split mode

Calculation of the split ratio

Split vent flow can be measured directly at the split vent with a bubble meter.

Determining the column flow is a bit more difficult.

$$\text{Split ratio} = \frac{\text{split vent flow} + \text{column flow}}{\text{column flow}}$$



Set GC to run isothermal at the desired starting temperature.

Inject a detectable, non-retained species (air, methane, ...) and measure retention time.

Determine column volume from

$$V_M = \pi \times \text{column radius}^2 \times \text{length}$$

Determining column flow

Determining column flow

Example.

For a 30 m x 0.2 mm id column, the t_R for a non-retained species was 1.45 minutes.

V_M = 3.141 x (0.01 cm)^2 x 3000 cm

= 0.9423 ml

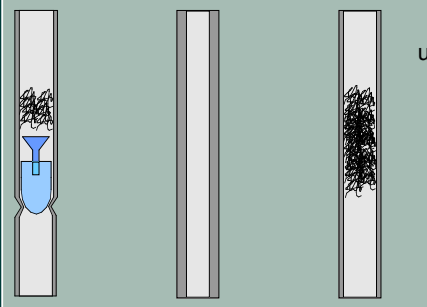
flowrate = 0.9423 ml / 1.45 min

= 0.65 ml / min

Split injection

Key to method is assuring total vaporization and mixing with the carrier gas. This is accomplished in the split liner.

- Provides for efficient heat transfer
- Promotes mixing of sample and carrier
- Surface is used to trap non-volatile components.



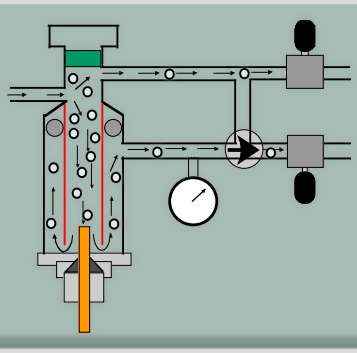
The liner you use is dependent on the method of injection

It must be replaced at regular intervals

Most crud ends up in the liner

Liners

Liner overload



If too much sample is introduced, it will overload the liner.

The can result in some sample escaping through the purge vent giving erratic results.

You must keep the sample volume < volume liner.

Liner overload

General rule of thumb

"Keep expanded volume below 0.5 ml"

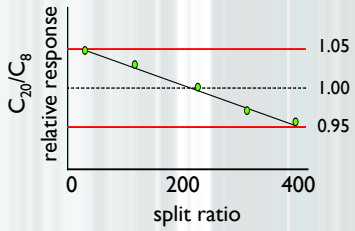
Expansion of some common solvents

250 °C and 13 psig

water	1277:1	ethyl acetate	235:1
MeOH	567:1	pentane	197:1
$MeCl_2$	360:1	hexane	176:1
$MeCl_3$	286:1	isooctane	139:1

Split selectivity

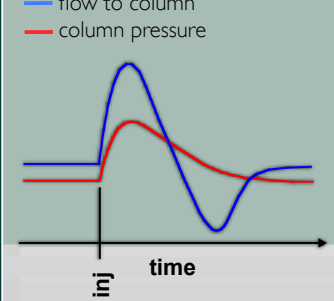
When the split ratio is changed, you don't always get a 1:1 variation for each species.



Higher BP materials take more time to volatilize, don't expand as much.

Higher splits push sample through injection port more rapidly.

Pressure and flow changes during an injection



During an injection, the expansion of the sample results in a brief pressure pulse.

This causes a change in the flow entering the column.

Using small sample sizes, low expanding solvents and consistent injection will minimize this effect.

Splitless injection

Split injection is best when you have relatively high levels of the eluents of interest.

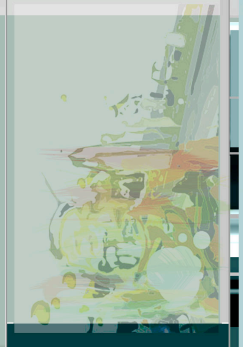
For trace analysis, splitless injection can be used.

Two approaches:

Splitless - total sample enters column

Splitless/Split - Grob injection

Attempt to selectively remove solvent



Splitless injection

A brute force approach.

Simply turn off the split and introduce the entire sample.

This is very rough on the column and gives poor results.

The large amount of solvent will saturate the column and the gas phase.

Splitless/Split

Developed by Grob so it's sometimes called a Grob injection.

Two step process

Initial injection under splitless conditions

Change to a split mode after a fixed period of time - purge time.

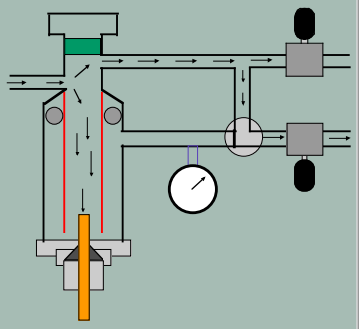
Goal is to introduce the majority of the sample components but not the solvent.

Initially the purge is off.

All flow entering the injection port will go into the column.

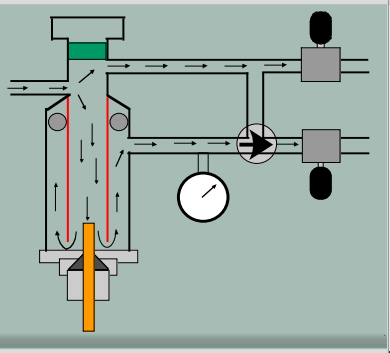
The normal 'split' flow bypasses the liner.

This is done to help maintain the column head pressure



Step one - purge off

Step two - purge on



After a fixed period of time, the purge is turned back on - split mode.

Any remaining material is essentially flushed from the injection port.

How it works

- The solvent must be more volatile than any component of interest.
- The initial column temperature must be 5-10°C below the boiling point of the solvent.
- The injection port must be hot enough to volatilize all components of interest.
- Under these constraints, only a small portion of the solvent will enter the column but will act to collect and focus the solutes.

Focusing

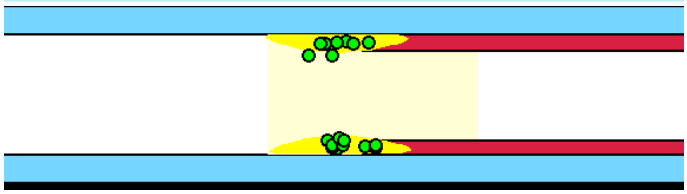
Not only can you get rid of much of the solvent, you can reduce the band width of you injected peak - focusing

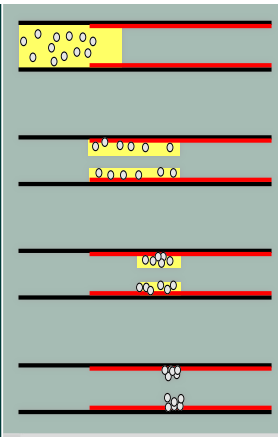
Three types

- Solvent focusing - solutes collect in condensed solvent.
- Stationary phase focusing - solutes collect in stationary phase.
- Temperature focusing - solutes simply condense at start of column at low T.

Focusing

For complex samples you often get a combination of each type - based on solvent used, temperature and volatility of sample components.





Solvent and sample enter the column.

Solvent saturates the start of the column. Gives a place for the solutes to dissolve.

The purge is turned on and no more solvent enters. As the temperature increases, the solvent evaporates.

The solutes get concentrated into a narrow band.

Solvent focusing

Initial column temperatures

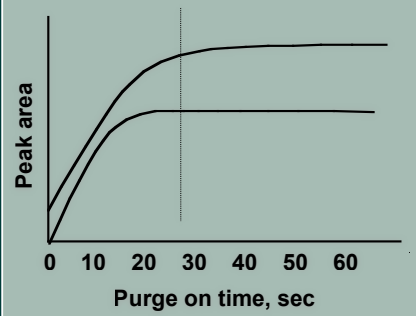
Solvent	BP	°C Initial Temp.
diethyl ether	35	30
dichloromethane	36	30
carbon disulfide	46	40
hexane	69	60
octane	125	120

Purge time

You must allow sufficient time to for eluents to enter the column.

If the time is too long, to much solvent enters and the focusing is poorer.

Best time for your sample can be found through a few simple injections with different purge on times.



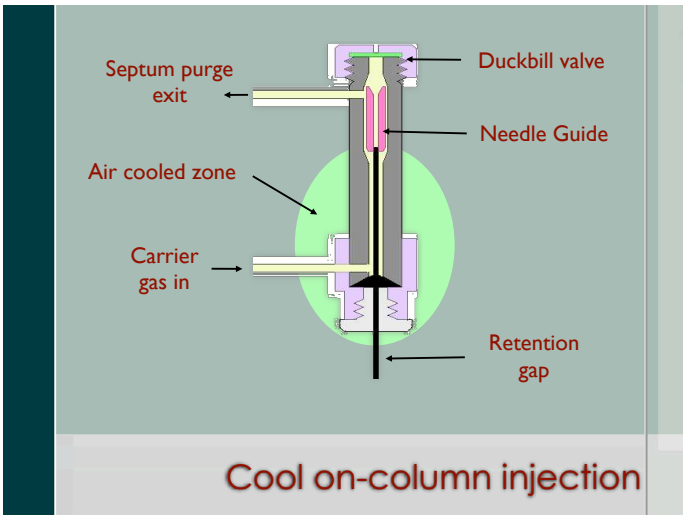
Optimum time is when all components of interest have reach a maximum response.

Typically, this is in the 30 - 60 second range.

Purge time

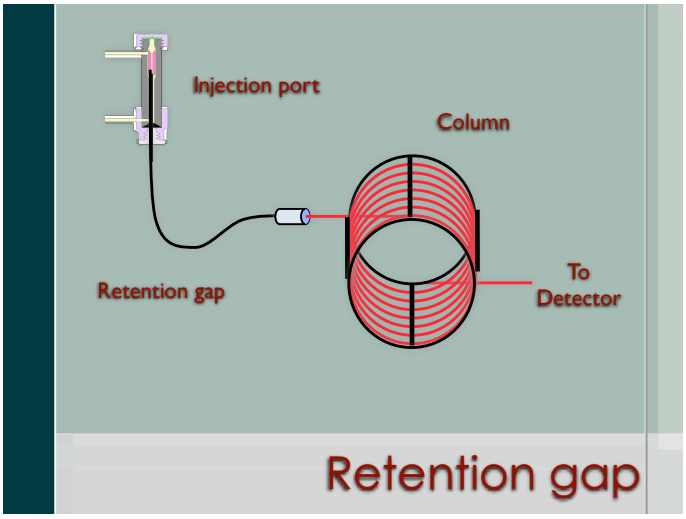
Cool on-column injection

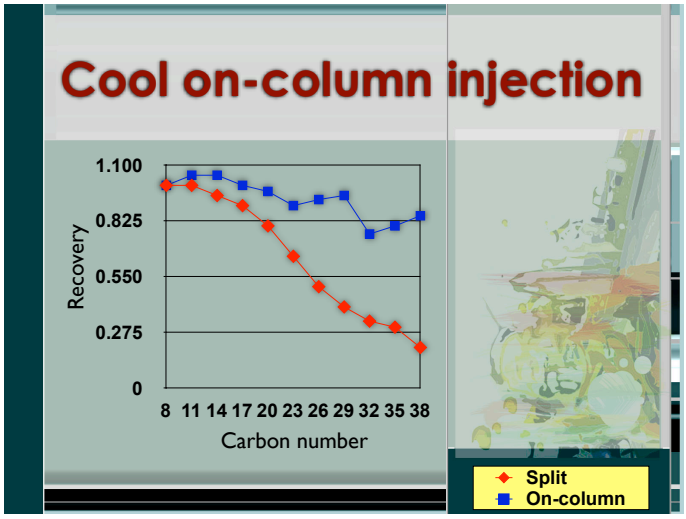
- The process of initial sample volatilization can degrade some samples.
- On-column injection is a method to directly place sample on the column under low temperature conditions.
- A special injector is required as the 'needle' is a section of capillary column that actually fits inside the analytical column.
- The injector is typically kept cool using fan driven air.



Cool on-column injection

- Solvent effect or cold trapping needs to occur for the best results - similar to Grob injections.
- A retention gap is required for most on-column injectors - empty section of capillary tube.
- Failure to use a retention gap often results in very broad and irregular peaks.
- The retention gap also acts as an area for non-volatile components to deposit and not contaminate the column.

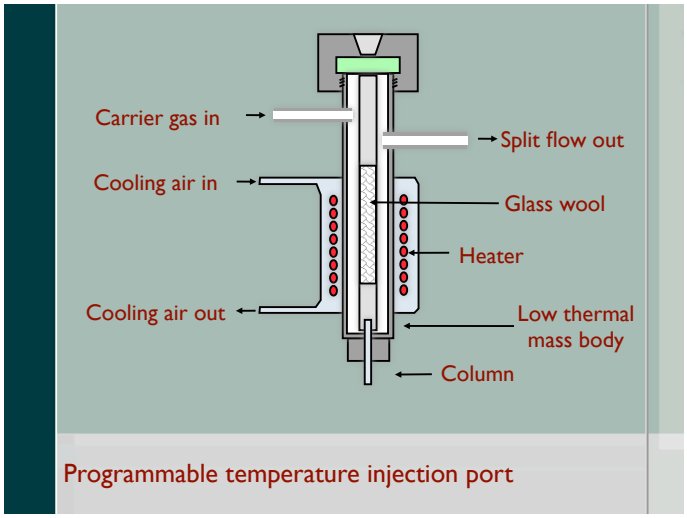




Programmable temperature injection port

Relatively new equipment.

- A modified split/splitless port where the temperature can be rapidly changed under controlled conditions.
- Both heating and cooling control is provided.
- The body has a lower thermal mass, making it easier to change the temperature.
- Samples are injected and stay in the port until the temperature is raised.



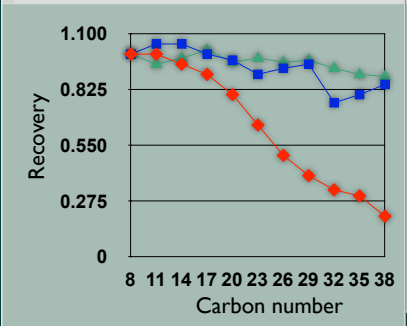
Programmable temperature injection port

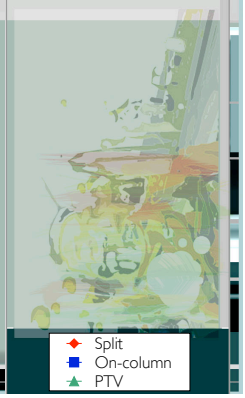
With a PTV, it is possible to:

- Inject larger samples and selectively remove the solvent prior to vaporizing the sample.
- Make multiple injections prior to vaporization.
- Selectively introduce groups of sample components if there is a large enough difference in their BP.

Unlike a normal injection, vaporization is slower. This results in a more controlled transfer to the capillary column.

Discrimination





Column selection

- There is no where near the range of capillary columns compared to packed.
- Some special phases are available like chiral columns.
- Major choice is do you want a non-polar, moderately polar or polar column.
- You might also consider film thickness and internal diameter wanted.

Column Selection

Phase polarity examples.

Non-polar
(0-5% pheny)- methylpolysiloxane

Intermediate
(20-50% pheny)- methylpolysiloxane
(5-15% cyanopropyl-phenyl)-methylpolysiloxane

Polar
Carbowax 20M
(50%-trifluoropropyl)-methylpolysiloxane
Polyethylene glycol

Column Selection

- Select the least polar phase that will perform your separation
- Non-polar phases separate mostly by order of increasing BP.
- Columns that differ more in their H bonding capacities are best separated on polar phases like polyethylene glycol or Carbowax.
- Above are only starting points.

Column selection

Stationary phase thickness.

Increasing the thickness will allow for a greater sample capacity.

It will result in wider peaks and lower resolutions. They also tend to degrade more rapidly.

Thin film - 0.10 - 0.25 μm
Thick film - 1 - 5 μm

Column Selection

Internal diameter

As diameter increases, the pressure requirements are reduced. The sample capacity increases and resolution decreases.

Diameter mm	plates / m	capacity ng/peak
0.2 - 0.25	4000-5000	5 - 100
0.32	3000	400 - 500
0.53	1600	1000 - 15,000
2 (packed)	2000	20 000

Column selection

Summary

- For simple samples, you most likely don't need to use a capillary column
- For complex samples, pick the column that best reflects the overall polarity of your sample.
- Typically best to use small ID columns with thin films for general work.

Column care and feeding

Capillary columns can cost 10 times that of packed columns.

Without a major investment in equipment and training, you can't simply make one.

You had better know how to take care of them.

Column care and feeding

The columns appear to be quite sturdy.

The polyamide coating helps the fused silica handle quite a bit of stress.

If the coating is scratched, the column will simply snap.

This is the way that columns are cut.

Column care and feeding

Usable lifetime is limited.

The coating becomes contaminated and will 'wear away' - even for bonded phases.

A few tricks can help extend column life if done properly.

Column care and feeding

Column should be conditioned before use.

For new columns - this removes residual traces of any solvent used when produced.

For older columns - this helps removed traces of air that might have entered during storage.

Column care and feeding

Conditioning

Attach column to injection port, leave detector end unattached.

Pressurize the column and verify that there is flow.

Slowly ramp the GC oven to about 50 - 100 °C and hold overnight.

Column care and feeding

Column storage

- Seal the column ends to protect the liquid phase. You want to keep air, water and other trace contaminates out.
- Return the column to its protective box to prevent the possibility of scratching it.

Column care and feeding

Other things to consider.

Avoid using large samples / splitless injections.

If you see some loss in column performance, try breaking off the first meter of the column - removes some contaminates.

Solvent washing can be tried as a method of last resort.

Column care and feeding

Most columns are tested prior to shipping. You will find a copy of the results included in the box.

The same types of tests can be used 'in-house' to track column performance.

Let's look at some of the standard test information.

Column evaluation

Base / Acid ratio

Two components are used

1-decylamine and 4-chlorophenol

$$\text{Base/Acid ratio} = \frac{\text{1-decylamine height}}{\text{4-chlorophenol height}}$$

This test shows if there is any acid or base selectivity to the column - due to active Si-OH sites.

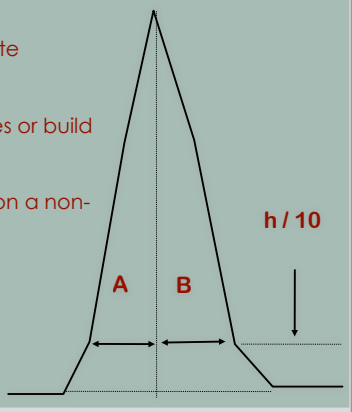
Tailing factor

Another test to evaluate column performance.

Will indicate active sites or build up of contamination

Use polar compound on a non-polar column, ...

Ratio should be ~1



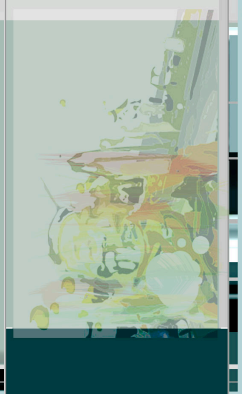
Column evaluation

Column evaluation

Trennzahl Separation Number

Measures the number of peaks of similar geometry that can be placed between two test peaks - a modified version of peak capacity.

It measures column efficiency and can be used even under temperature program conditions.



Trennzahl Separation Number

$$TZ = \frac{t_{R_b} - t_{R_a}}{\frac{W_b + W_a}{2}} - 1$$

$$t_{R_b} > t_{R_a}$$

Keeping track of this number at regular intervals will show how fast your column is degrading.

Column evaluation