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GC Troubleshooting

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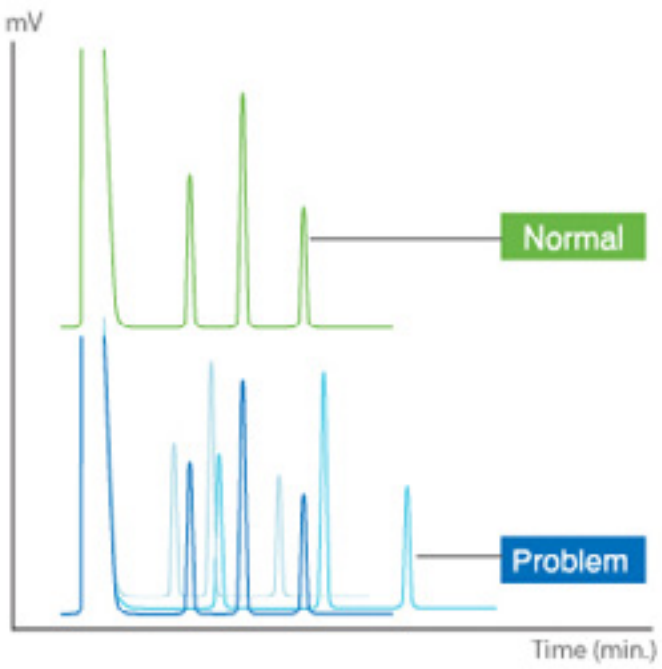
5 PICTURES WHICH REVEAL PROBLEMS WITH YOUR GC ANALYSIS – AND HOW TO FIX THEM

1) Irreproducible Retention Times

pH meters are calibrated to give the correct pH readback in aqueous solution – the buffers you verify this with are aqueous. If you measure the pH with the organic added, the pH will be different to that of measuring before organic addition. However, the most important point is to be consistent. If you do always measure pH after the organic is added, make sure you state this in the method so that everyone does it the same way. It won't be 100% accurate, but at least it will be consistent. This is probably more important than having the exact pH.



There are two types of retention time variability that can be observed. Firstly, from injection to injection, this could be from injection to injection in a run of samples or multiple injections from the same vial. This may be caused by changes in carrier gas linear velocity due to a defective electronic flow controller, leaking septum or column connection, or variable gas supply due to a leak in the gas supply tubing. Under these circumstances you would expect to see variable retention times for all analytes.

To remedy the problem ensure that the instrument settings are correct and occasionally verify that the instrument calculated flow/linear velocity matches the carrier gas flow by measuring all inlet flows using a flow meter.

Other causes of this type of variable retention time are variations in the oven temperature (either from a faulty temperature controller or from insufficient thermal equilibration time) or if the inlet pressure is not high enough to sustain flow at high gradient temperatures. In constant flow operating mode, the carrier pressure is ramped to attain a constant flow through the temperature program (as the temperature is increased carrier gas viscosity also increases, and therefore, requires a higher pressure to maintain constant flow), in constant pressure mode the inlet pressure remains constant which means

that as the carrier gas viscosity increases during the temperature program the linear velocity will decrease causing retention times to change. With mass-flow or flow sensitive detectors, this phenomenon can also manifest itself as either a gradually increasing or decreasing baseline signal depending upon the response characteristics of the detector.

Therefore, it is often better to use constant flow mode to avoid these problems. Figure 1 demonstrates the improvement in chromatography when using constant flow mode; there has been an overall gain in sensitivity (peak areas have increased) mainly due to the decrease in peak width, the rising baseline has been eliminated due to the constant flow into the mass-flow sensitive detector which allows for more reproducible integration and quantitation, and finally retention time has decreases by a factor of three.

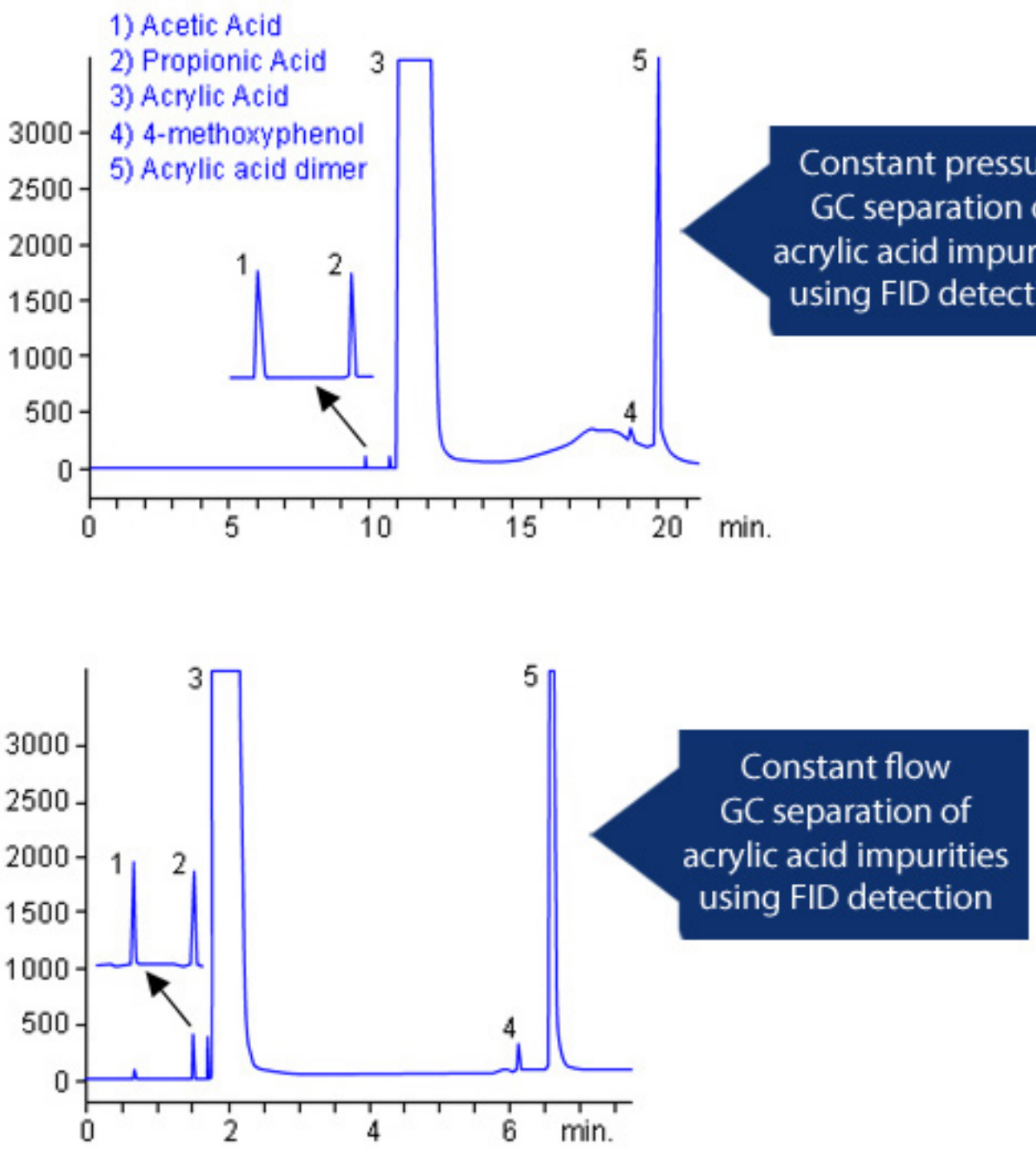


Figure 1: Constant flow vs. constant pressure analysis.

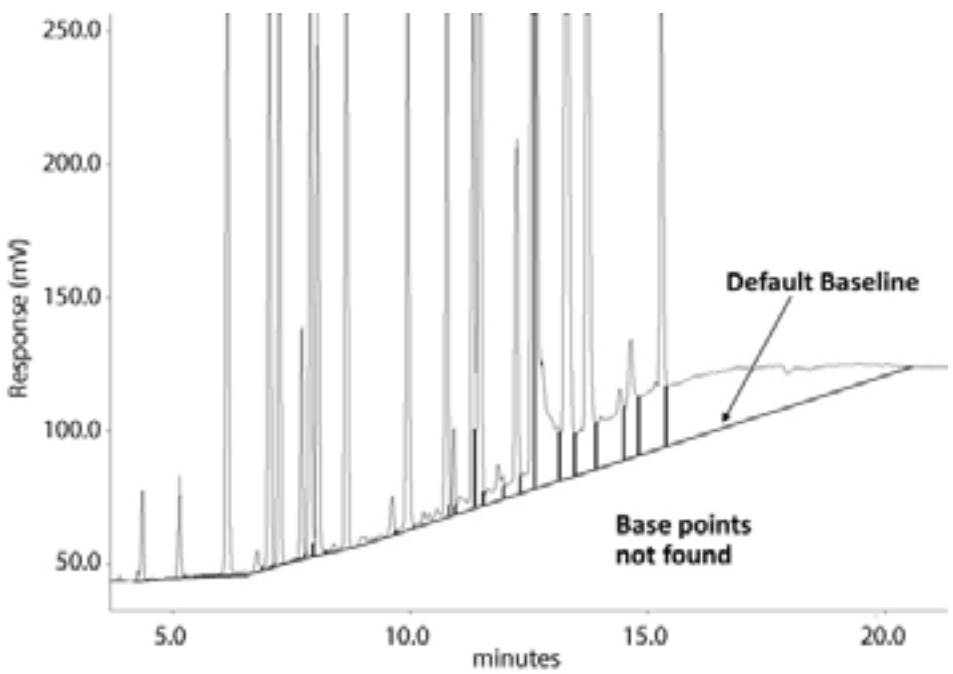
If you have long term variability in retention times, this may be attributed to changes in the column, such as, stationary phase degradation or shortened column due to trimming which when entered incorrectly into the GC data system causes the instrument to incorrectly calculate the linear velocity of the carrier gas. Remember, the EPC calculates all flows from the applied pressure, the nature of the carrier gas, and the column dimensions.

However, how important is it to know the exact length of the column? Columns are regularly cut by users, with varying unspecified lengths being removed. It would be impractical to expect users to record the exact length of the small pieces of column that are generally removed and it is totally impractical to measure the length of the existing column - the standard length being 30 m. Having to unwind and rewind 30 m would likely damage the column. In general removal of these small portions has negligible effect on the performance of the column (e.g. removal of a typical length of 3 cm from a 30 m column equates to 0.1% of total length).

The assessment of exact column length is of no benefit whatsoever for the majority of applications and is better served by applying meaningful system suitability criteria to critical parameters within the method that are affected by reducing the column length - this would be absolute retention time, resolution, and possibly some effect on peak area due to slight changes in the split ratio. The criticality of these parameters has to be assessed on a method by method basis and adequate provisions made for system suitability criteria that are relevant, meaningful, and have sensible limits applied. In general these would be tests such as retention time limits, resolution limits, and linearity.

There are certain cases where reduction in column length is more significant - either with short columns (10 m or less) or columns which are exposed to excessive contamination and need larger lengths removed more regularly. This should be captured by system suitability or by use of retention gaps. If the column is too short, retention times will be too short and vice versa.

2) Integration Issues



In this case it is difficult to reliably integrate the peaks as there is a rising baseline. The first solution to avoid this problem would be to eliminate any type of baseline drift in the first place. This can be done by reducing column bleed by properly conditioning the column, using constant flow or linear velocity mode especially when using a temperature program, or if you need to use constant pressure mode and are using a mass-flow sensitive ionizing detector you may have the ability to use variable make-up flow to compensate for the change in linear velocity during the method.

A great tip to help condition the column correctly and in the most efficient manner is, once the column has been connected to the inlet do not connect it to the detector and allow at least six column volumes of carrier gas to pass through the column in order to remove air from the column as well as sparging any dissolved oxygen from within the stationary phase (Table 1). This will allow you to condition the column for less time and at a lower temperature. Once this step has been completed, ramp the oven temperature (ensure the carrier gas is still flowing through the column) at 20 °C/min. to 20 °C above the upper temperature required by the analytical method. Once the upper temperature limit has been reached the column should be conditioned for the correct amount of time based on the dimensions and phase type (Table 2).

Column Internal Diameter (mm)	Minimum Flow Rate (mL/min.)	Minimum Purge Time (min.)
0.53	5	10
0.32	1.5	20
0.25	1	25
0.18	0.8	30
0.1	0.5	40

Table 1: GC column purge times.

Phase Type	Length (m)	Film Thickness (mm)	Conditioning Time (min.)
Non-polar	< 30	<0.5 0.5-1.0 >1.0	15 30 60
	30-60	<0.5 0.5-1.0 >1.0	30 45 60
	>60	<0.5 0.5-1.0 >1.0	60 90 120
Mid-polarity	< 30	<0.5 0.5-1.0 >1.0	20 40 60
	30-60	<0.5 0.5-1.0 >1.0	40 60 80
	>60	<0.5 0.5-1.0 >1.0	80 120 160
Polar	< 30	<0.5 0.5-1.0 >1.0	30 45 60
	30-60	<0.5 0.5-1.0 >1.0	60 90 120
	>60	<0.5 0.5-1.0 >1.0	80 120 160

Table 2: Recommended conditioning times for various capillary GC column types.

It is always good policy to record the bleed profile for a column when new (Figure 2), so that the level of bleed can be referenced at a later date in order to assess the degradation of the stationary phase over time, and perhaps a performance limit established for column replacement.

Simply run the method without making an injection or inject a small amount of sample solvent. If you are using mass spectral detectors, the presence of ions at m/z 207, 281, and 355 indicate column bleed.

These will almost always be there – it is whether they are causing problems either spectrally or in terms of reproducibility of integration that really matters.

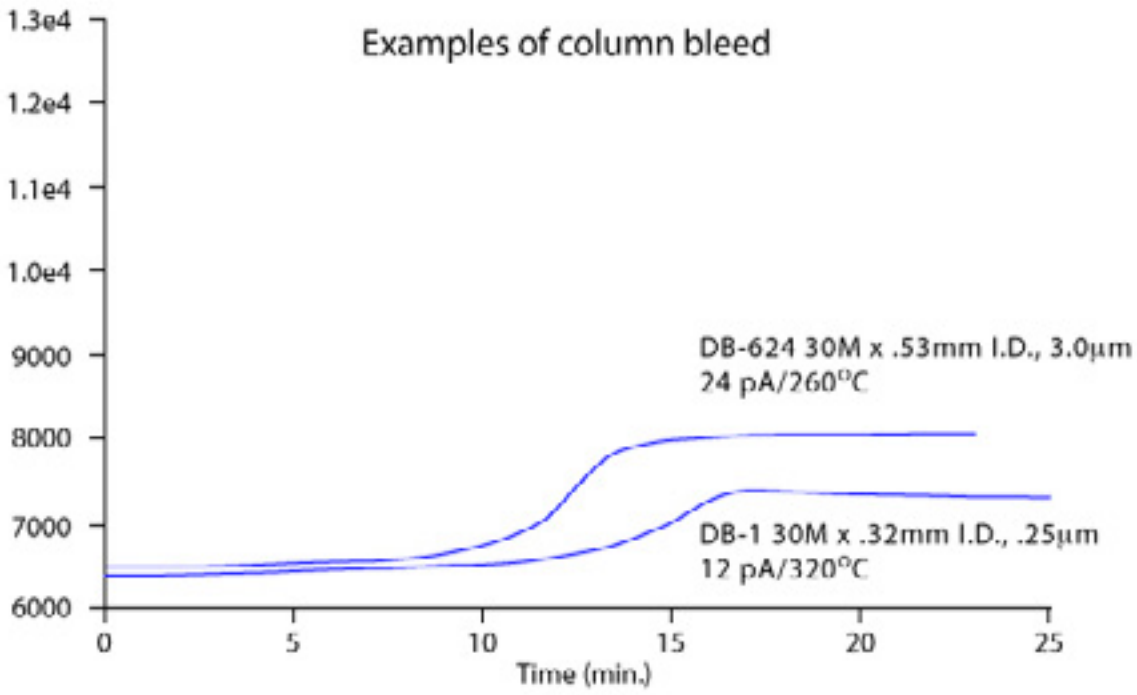


Figure 2: Typical capillary GC stationary phase bleed profile for two different polysiloxane based stationary phases.

Another approach to avoiding column bleed is to consider the stationary phase and column type you are using. High polarity columns inherently bleed more; therefore, using the lowest polarity column possible can help to avoid some unwanted bleed. You can also consider using a GC-MS designated column (even if you are running a GC method) as they have been designed to be low bleed. They will provide better sensitivity due to the improved signal to noise ratio and will give better mass spectral purity from the absence of background bleed ions (Figure 3).

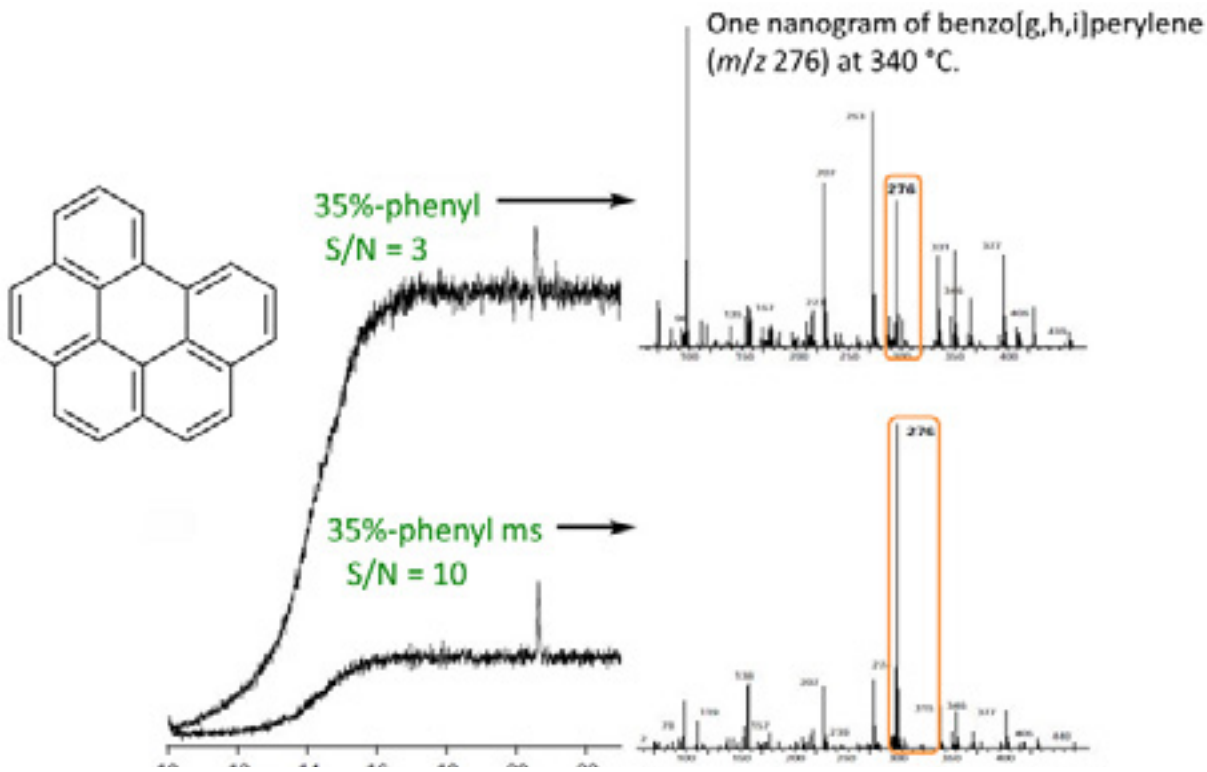


Figure 3: Comparison of 35% phenyl standard GC and GC-MS designated column.

In the real world rising baselines are often inevitable, therefore, spending some time familiarizing yourself with the advanced integration settings in your data system will allow you to properly integrate challenging chromatograms - these settings include threshold, slope sensitivity, baseline reset points, and different integration methods, such as, valley-to-valley. Remember to always use the same integration method every time to provide consistent results (Figure 4 and 5).

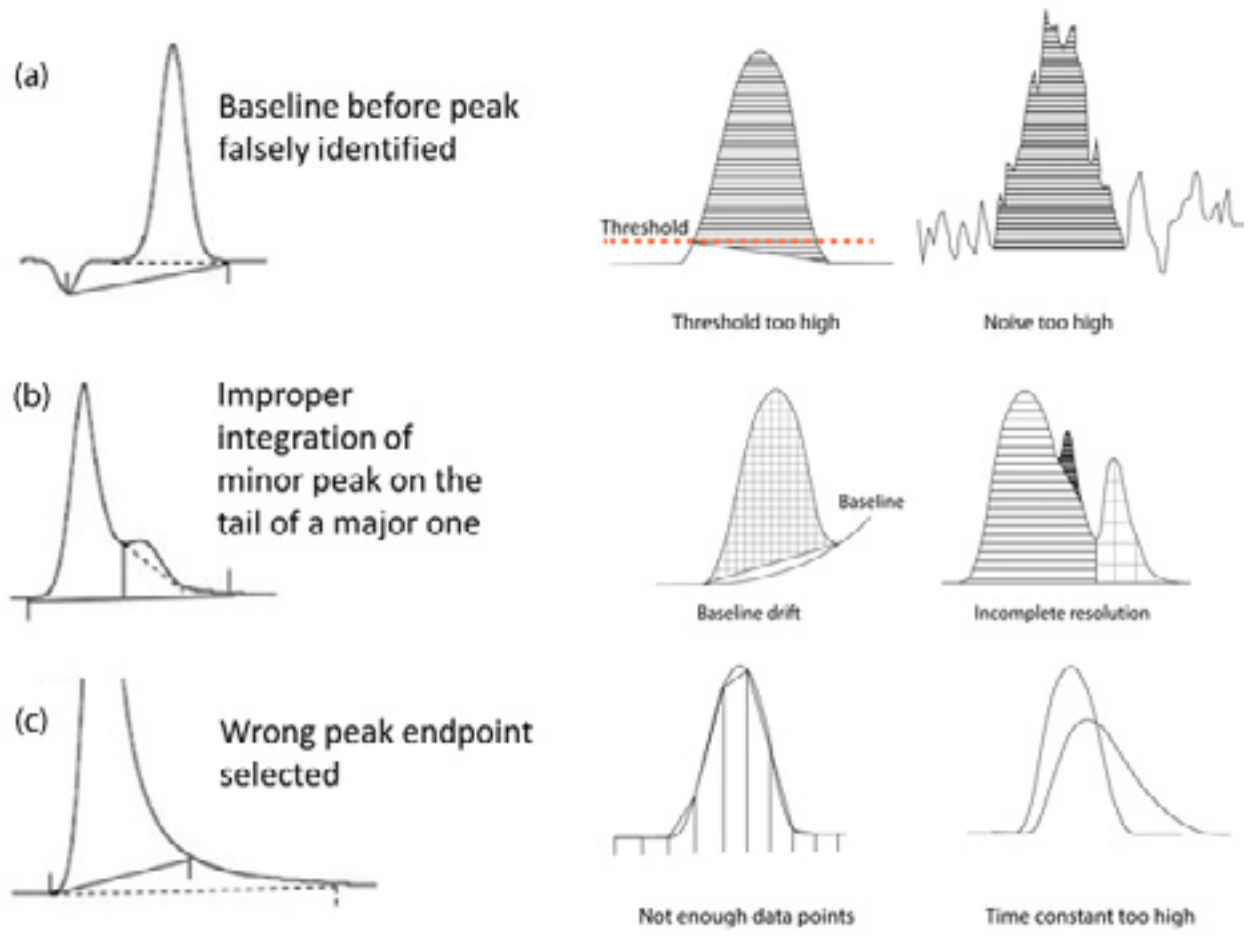


Figure 4: Common peak integration errors.

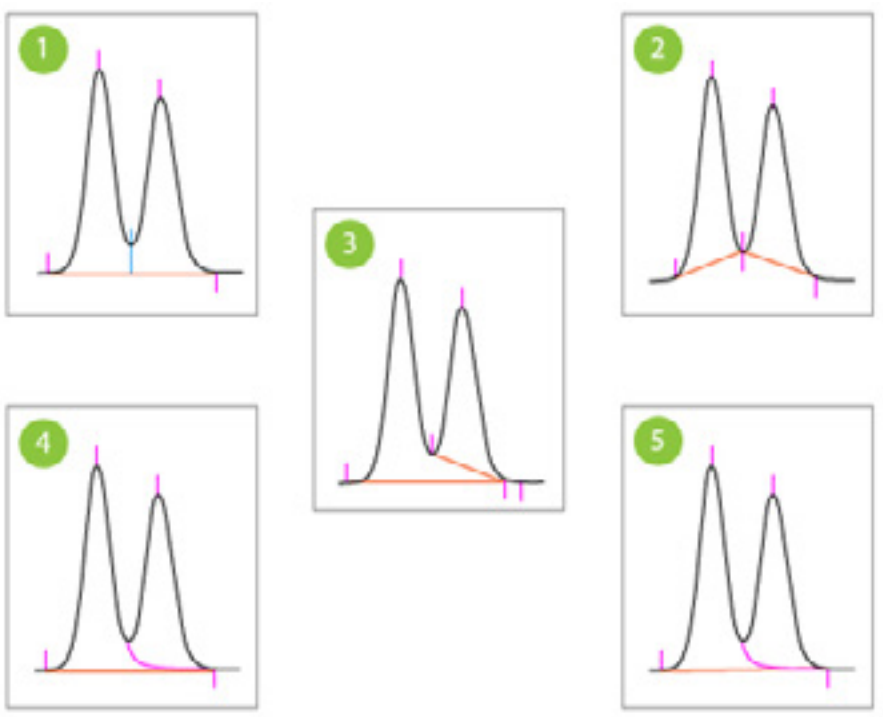
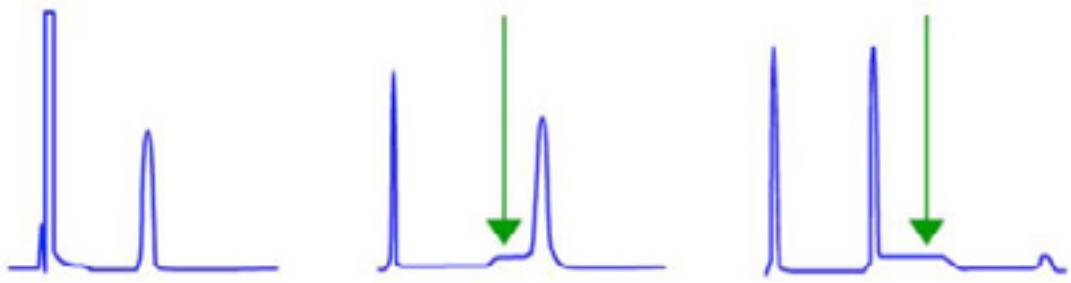


Figure 5: Peak integration methods. 1) Drop perpendicular, 2) valley to valley, 3) tangential skim, 4) exponential skim, 5) Gaussian skim

3) Step-Shaped Peaks



The step-shape can either be before or after the peak, and may also be accompanied by tailing or a shoulder, and is due to analyte thermal degradation in the inlet. To remedy this problem reduce the inlet temperature in 20 °C steps until a normal peak shape is achieved. However, take care not to lower the inlet to a temperature that is too low to achieve proper volatilization of all of your analytes, if this happens you may begin to see irreproducible peak areas.
To choose an appropriate inlet temperature the following steps can be followed:

- A good starting point for method development and for new analyte applications is 250 °C
- A scouting temperature program can be used to estimate the elution temperature of the highest boiling component (Figure 6). Set the inlet temperature at least 50 °C above this temperature to ensure sufficient sample volatilization

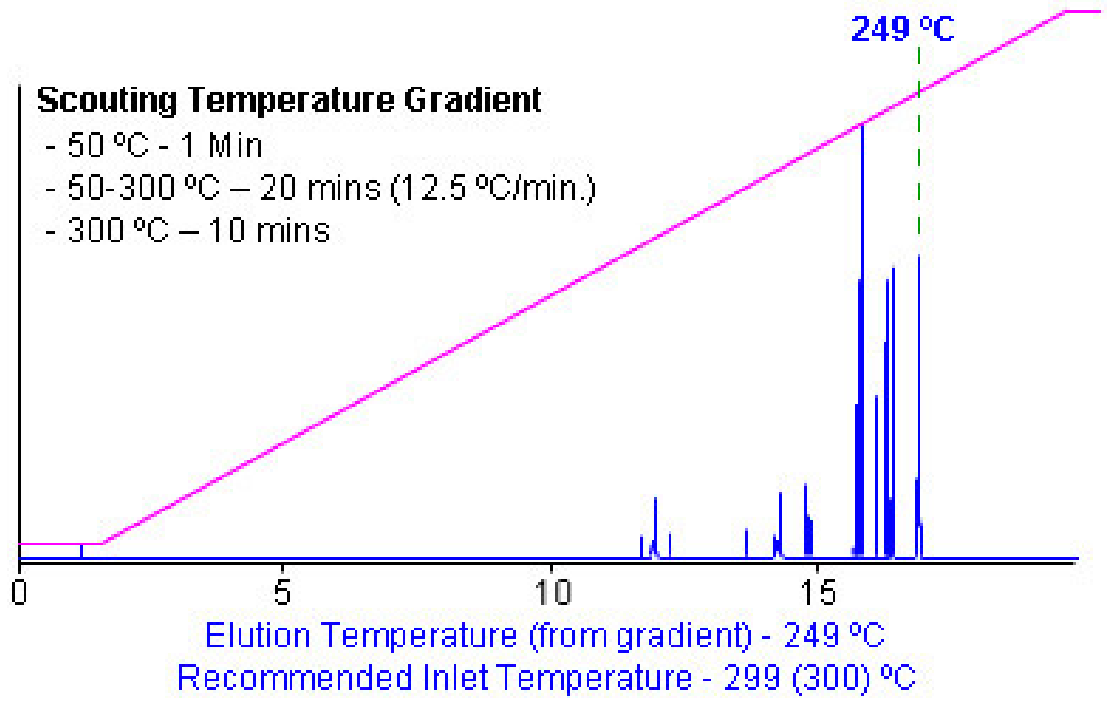
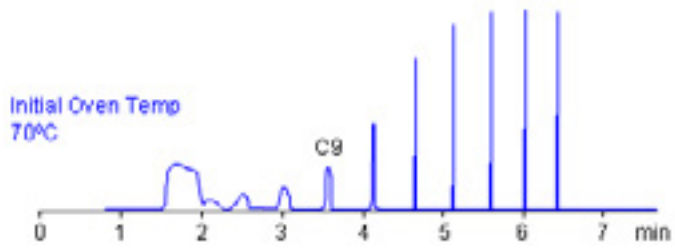


Figure 6: Scouting temperature program.

4) Poor Peak Shape (Early Eluting Peaks)



Poor peak shape for early eluting analytes when you are carrying out a splitless injection is caused by a sample solvent/column polarity mismatch or the wrong initial oven temperature (the oven temperature is too high). In splitless injection the sample transfer from the liner to the column is slow; therefore, to mitigate band broadening effects from this the sample must be refocused at the head of the column. Two focusing mechanisms occur (Figure 7):

1. Solvent focusing, where low boiling analytes remain dissolved in the solvent which condenses on the inner wall of the GC column at low initial oven temperatures. The solvent polarity must match that of the column to ensure a contiguous film is deposited leaving a narrow sample band when the solvent evaporates
2. Cold trapping, where higher boiling analytes are condensed in a tight band in the temperature gradient between the inlet and the column oven. Initial oven temperatures should be set 20 °C lower than the boiling point of the solvent used to dissolve the sample

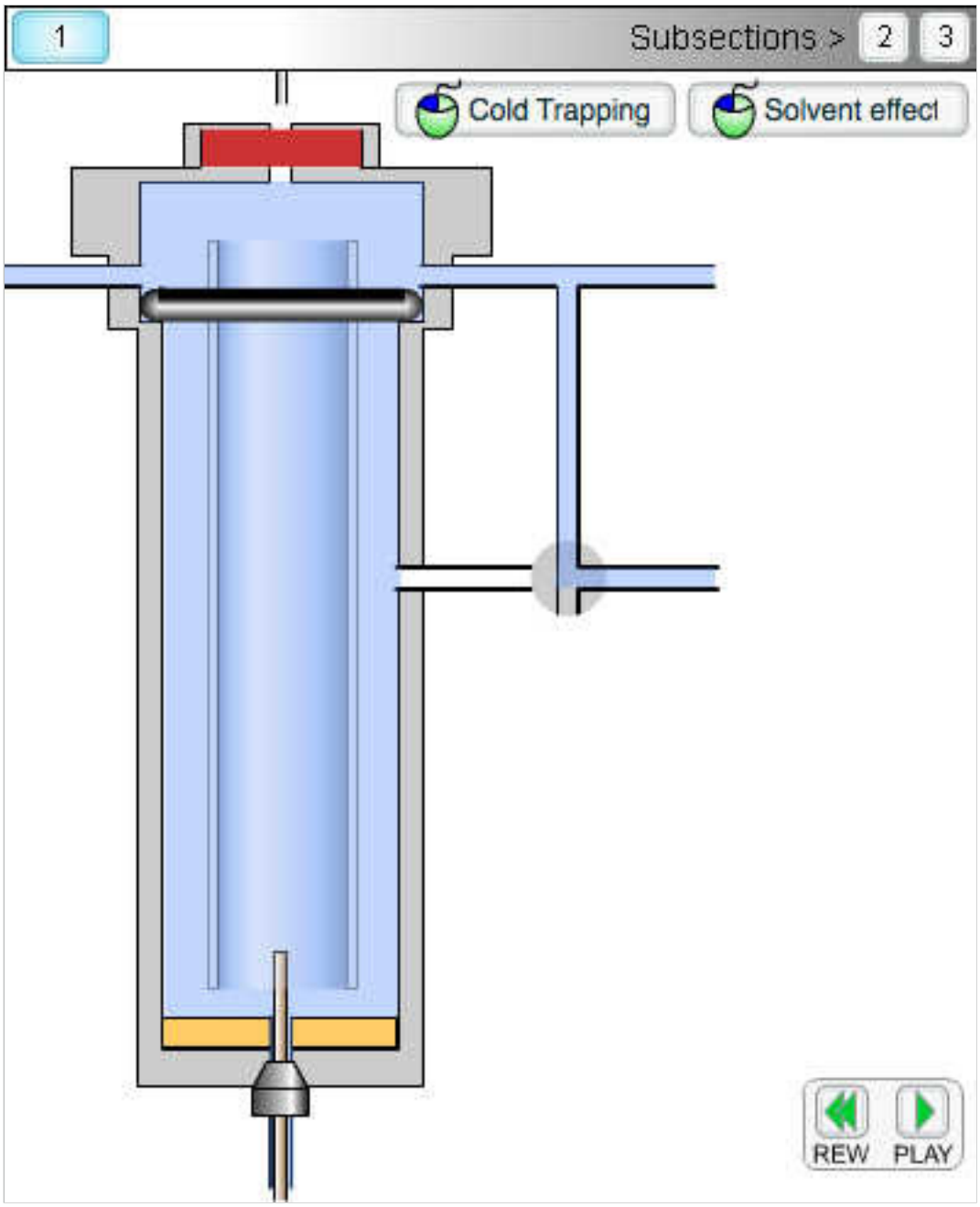
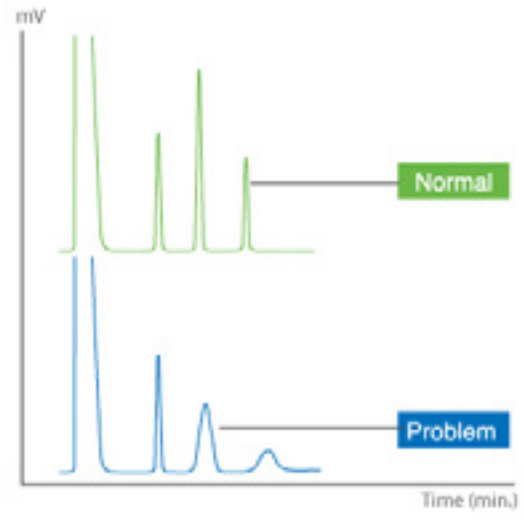


Figure 7: Splitless injection focusing mechanisms.

5) Loss of Resolution From Loss of Efficiency

In order to diagnose this problem carry out a plate count (your software will probably do this) on the peaks. If you are seeing a loss in efficiency the plate count will reduce, in particular for early eluting peaks (Figure 8).



There are several causes of this problem including, poor column cut and installation which leads to analytes being held up at the entrance to the column resulting in a broadened peak, loss or contamination of the stationary phase (this can be resolved by trimming the column), or the incorrect column length or diameter entered into the data system which results in an incorrect carrier gas velocity which in turn affects efficiency (remember each carrier gas has an optimum linear velocity to produce chromatography with the best efficiency).

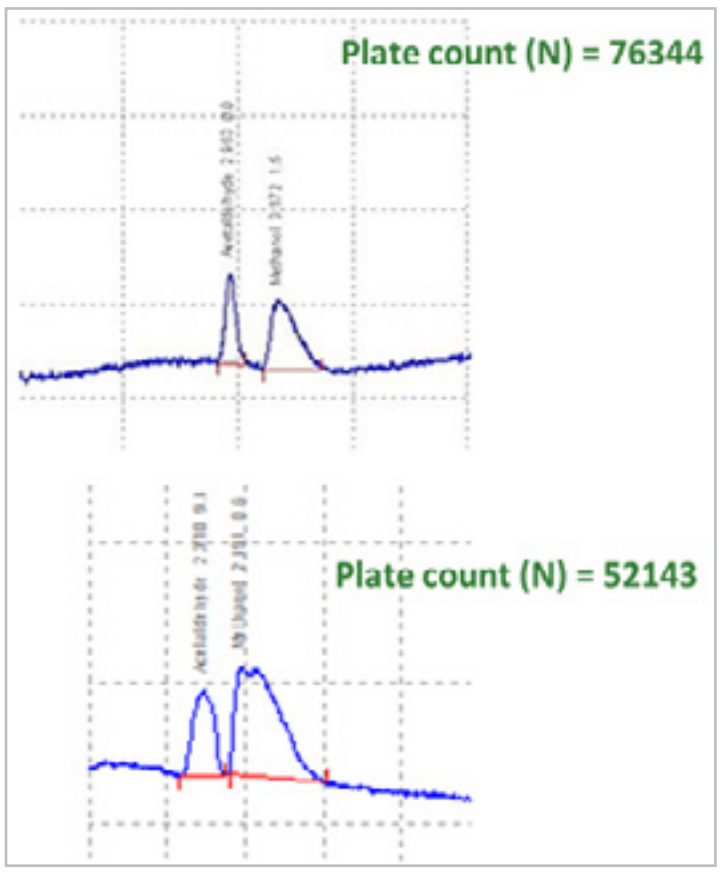


Figure 8: Plate count.

Internal Diameter (mm)	Film Thickness (µm)	Column Length (m)	Theoretical Plates	Theoretical Plates per Meter N/m*
0.18	0.18	20	133200	6660
0.25	0.25	30	138900	4630
0.32	0.32	30	112800	3760

Table 3: Typical column efficiencies. *Measured with a k = 5.

$$N = 16\left(\frac{t_r}{W_b}\right)^2 = 5.54\left(\frac{t_r}{W_{1/2}}\right)^2$$

Where:
t_r = retention time
W_b = peak width at base
$W_{1/2}$ = peak width at half height

GC COLUMN MAINTENANCE
PREVENTION IS BETTER THAN CURE

Using proper procedures for capillary GC column storage and conditioning can have a major impact on column lifetime and the quality of results obtained. This 'Tips and Tricks' instalment covers everything you wanted to know but were never told about proper GC column maintenance.

Column Storage

Correct column storage is necessary to prevent two major occurrences – the ingress of atmospheric oxygen and moisture into the column and the oxidative degradation of the bonded stationary phase through UV catalyzed mechanisms. The following guidelines will help ensure longer column lifetime:

- Remember to seal the column ends when they are not in use to exclude atmospheric oxygen and moisture. The easiest way is to seal the column using silicone septa (cut them in half – it's less expensive!!) or column sealing caps.
- Don't leave the column out on the bench where it can be damaged. Store the column so it will not be scratched. If scratched, the stress to the column may cause it to crack during operation.
- Store the column boxed with the test chromatogram in a dark place. Exposure to high levels of ultra-violet light can initiate oxidization of the stationary phase.

If the column is to remain on the instrument a constant low flow of carrier gas should be maintained with the split flow on. If the split flow is switched off back diffusion of air into the column can occur; this air can then cause damage. In order to prevent a build-up of moisture and air in the oven it should be left on at a temperature of 60 °C.

Oxygen rapidly degrades the stationary phase by cleaving bonds along the back-bone of the column. This is known as a "cyclic backbiting reaction" where the siloxane chain breaks into more thermodynamically stable, but also more volatile, cyclic siloxanes (Figure 1). It is the elution and detection of these cyclic siloxanes which constitutes column bleed. This damage is irreversible. The cyclic structures which are formed during this process have characteristic mass spectra at m/z 207, 281, and 355.

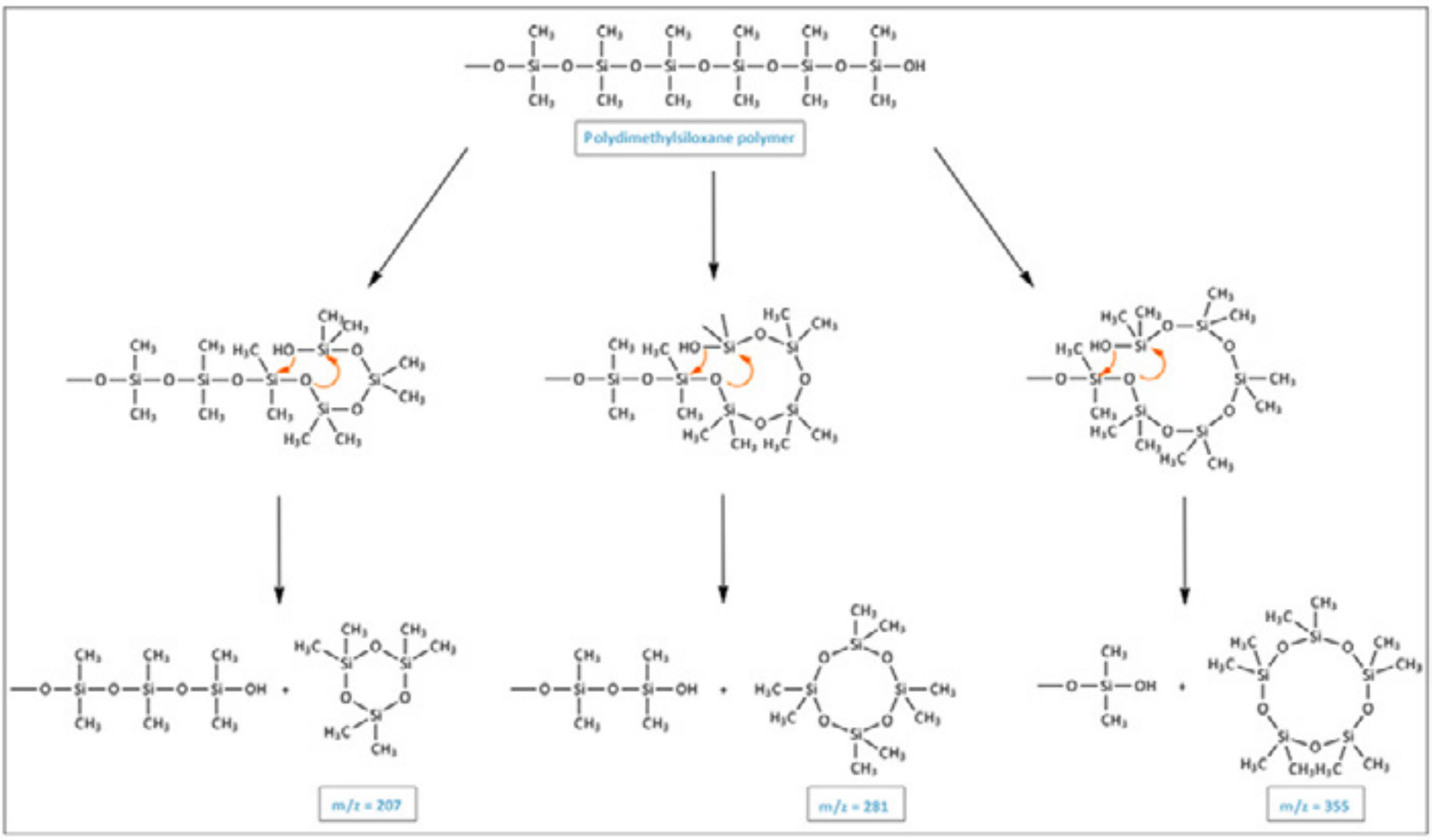


Figure 1: Typical capillary GC column bonded phase 'backbiting' bleed reaction.

The increase in the number of Si-OH groups within the oxidized phase will lead to an increase in the number of secondary silanol (Si-OH) interactions and peak tailing may be observed (Figure 2). This effect is most noticeable with polar and basic compounds.

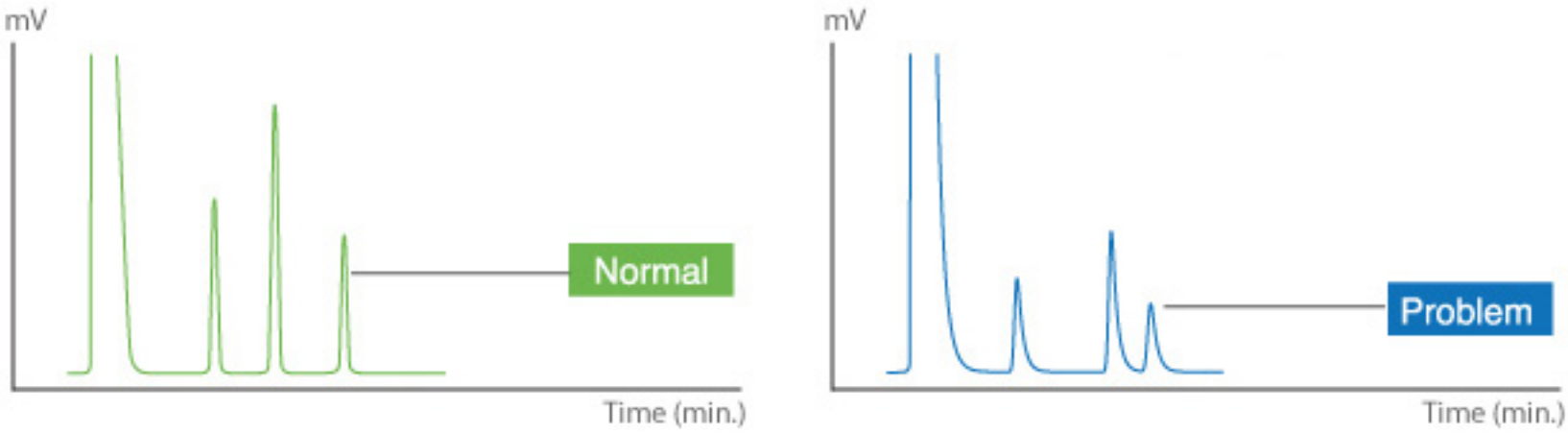


Figure 2: Peak tailing of polar compounds due to secondary interactions with exposed silanol groups in a column showing bleed symptoms.

Column Bleed

Column bleed happens with all columns, all the time. It is the continuous elution of compounds produced from the degradation of the stationary phase as described above (Figure 1). It is important to ensure that the amount of column bleed is minimal and constant, i.e. a flat baseline is achievable at low detector response.

In general, polar stationary phases and thicker films bleed to a greater extent. Bleed is normally seen as an increase in signal at increasingly higher temperatures when operating the GC in constant flow mode and when conditioning a newly installed column (Figure 3). Bleed may appear to be worse when using detectors that are particularly sensitive to the cyclic siloxane bleed products. Examples are; cyanopropyl phases with NPD detection and polyethylene glycol phases with electron capture detectors.

Bleed is best measured as the difference or change in signal at two temperatures, usually around 100°C and at the column's upper isothermal temperature limit. Of course, the signal at both temperatures will have a contribution from background generated from system components that must be subtracted to determine bleed values accurately.

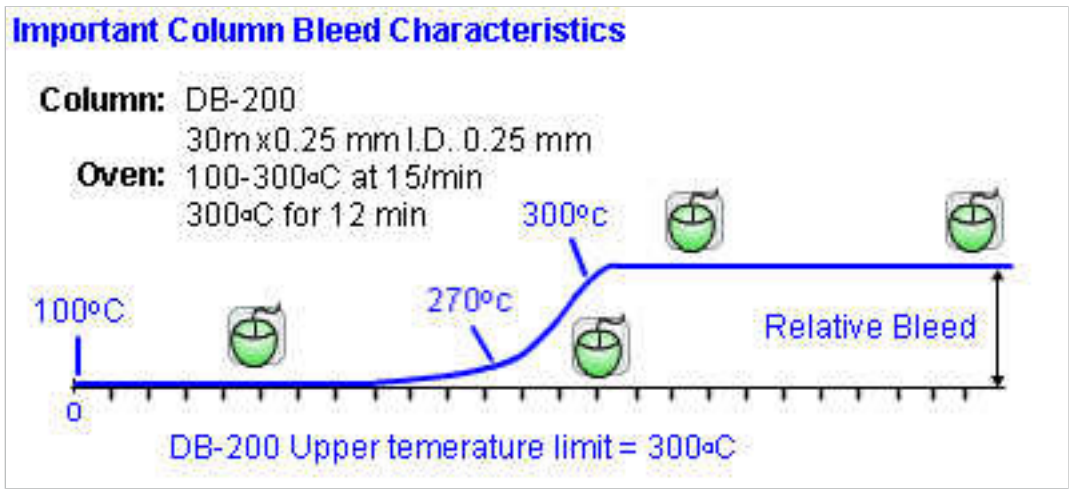


Figure 3: Measurement of relative column bleed.

Excessive column bleed appears as a larger rise in the baseline at the higher temperature regions. There is no absolute measurement to indicate when column bleed is excessive.

Column bleed is best measured as the difference or change in the background signal at two temperatures - Relative Bleed.

Usually the column's upper temperature limit and a lower value around 100oC are used. The absolute background signal is a composite of the background generated by the entire GC system. It is not possible to determine the contribution of column bleed to this total background signal. By measuring the relative amount of column bleed, the other contributors to the background signal are subtracted out.

Most columns are tested using FIDs - the output signal for which is measured is in picoamps (pA). Bleed levels are usually reported as the difference (DpA) in the FID signal at two temperatures (4.3 or 8.6 DpA in the example shown here).

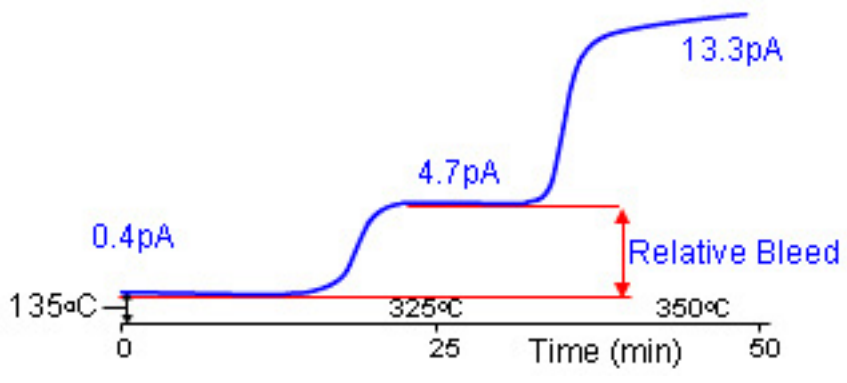


Figure 4: Excessive column bleed.

The bleed profile should be continuous, with no discrete peaks and should begin at around 30-40 °C below the column upper temperature limit, with the profile flattening to a constant signal level. The column will normally have two upper temperature limit values – the lower temperature is the isothermal limit and the column may be operated at this temperature indefinitely without significant degradation of column performance (Figure 5).

The upper temperature limit of the column is the gradient limit and this temperature may be maintained for 10-15 minutes

emperature limits of the column should not be

ygen (usually due to poor conditioning practice or

), using the column at or near the upper gradient

ressive solvents such as tetrahydrofuran, water, or

Figure 5: GC column temperature limits.

Low bleed phases and 'MS' designated phases are now available from many manufacturers that show reduced bleed at elevated temperatures and are especially useful for high sensitivity applications with Mass Spectrometric Detectors. Many phases use an altered 'phenylene' chemistry with phenyl phases which have the functional moiety included in the polymeric backbone rather than as a silicon substituent, resulting in reduced de-polymerization and oxidation of the phase (Figure 6).

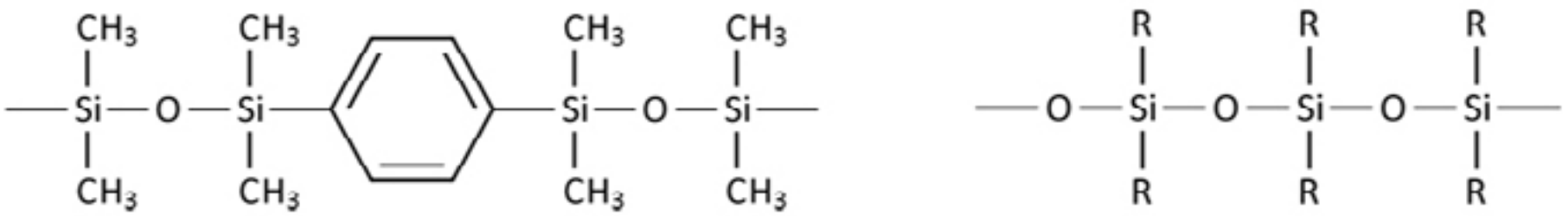


Figure 6: 'Phenylene' type low bleed phase chemistry (left) and a 'standard' polysiloxane backbone (right).
Note - selectivity between traditional 5% phenylmethylpolysiloxane phases and the 'phenylene' equivalent may vary slightly.

It is important to regularly check the condition of self-indicating gas traps or calculate the usage of non-indicating traps (6 bottles of gas maximum prior to a trap change is recommended). It is important to protect the columns from both oxygen and moisture using the correct gas trap. Column fittings should also be regularly tested for leaks using a highly sensitive leak detector.

One major source of column contamination comes from the carrier stream just prior to the bottle running out of gas. Even in gas bottles containing dip tubes there will be excess moisture and other dissolved components significantly above the gas specification levels (typically 0.99999% purity) in the 'dregs' of the carrier. Most manufacturers (and bottled gas suppliers) recommend that cylinders are changed when the bottle pressure (stage 1 of the two stage regulator) falls to about 10% of the original fill value (about 200-250 psi on a 'Size K' cylinder).

Column Conditioning

The column should be conditioned at 20-30 °C above the final temperature of the gradient program or the isothermal temperature in the intended method of use, but the upper gradient programming temperature limit of the column should not be exceeded.

After installing the column purge with carrier at room temperature for around 10-15 minutes at the flow rate required by the analytical method prior to raising the temperature – this ensures the removal of dissolved air within the stationary phase preventing unnecessary oxidation. For most columns a conditioning time of around one hour is more than sufficient (even for polar and thick film columns).

Once the signal plateaus (usually following a sharp increase and shallow decrease), at the conditioning temperature the column may be considered as being conditioned, however for applications that require very high sensitivity (good signal to noise performance), the column should be held at the conditioning temperature for up to three hours (Figure 7).

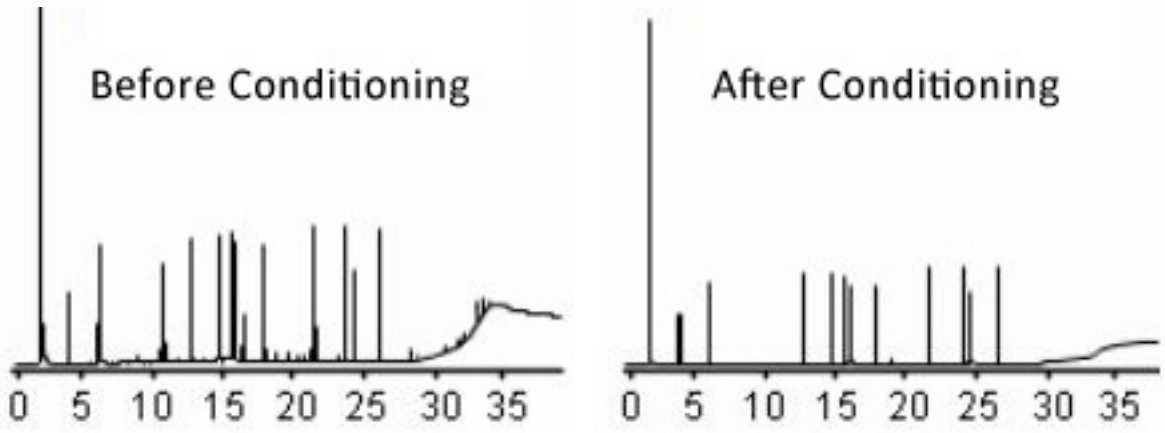


Figure 7: Typical chromatogram before and after column conditioning. Note the lower bleed at high temperatures and the reduction in background noise throughout the chromatogram.

Phase Fouling and Contamination

Analyte, matrix components, and solvent impurities can all contaminate the column. The contaminants may either be involatile, in which case they will be deposited onto the stationary phase, or semi-volatile, and will elute over extended periods causing baseline disturbances. Involatile impurities will tend to accumulate at the head of the analytical column giving rise to many chromatographic problems including, broad, tailing, and split peaks especially when using splitless injection (Figure 8). Peak shape problems usually arise due to an interference with the gross separation and focusing processes that occur at the head of the analytical column.

As fouling normally occurs at the head of the column it is possible to trim the column and, therefore, restore optimum performance. Trimming up to 5% of the column length is normally adequate; however, if this does not improve any peak shape problems then a further 5% can be trimmed. It has been postulated that trimming the column will affect resolution; although it should be remembered that resolution is not directly proportional to column length and doubling column length only provides a 1.4x improvement in resolution. Any concerns can be alleviated by revalidating with a known method.

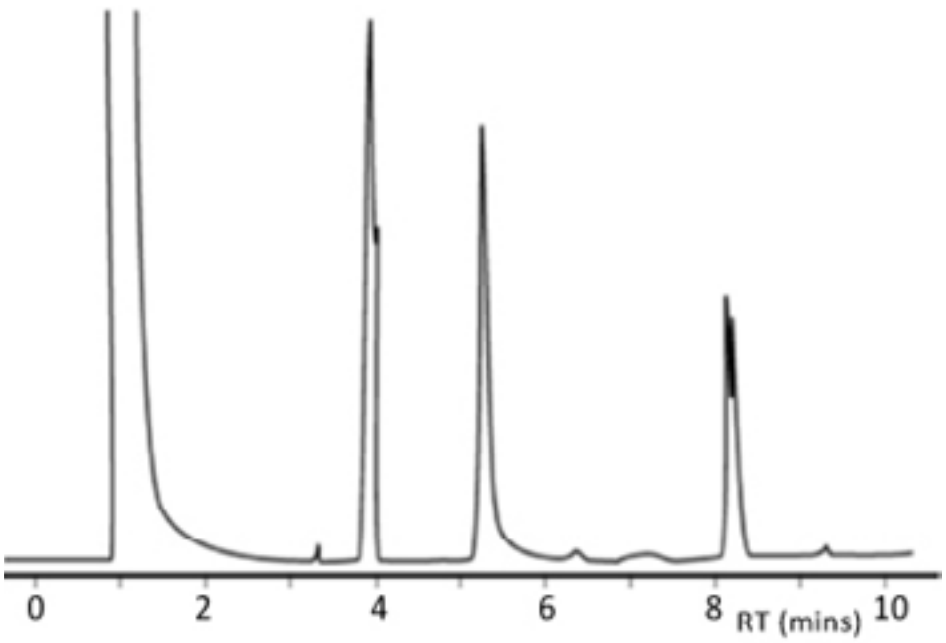


Figure 8: Distorted peak shapes resulting from contaminated stationary phase (disubstitued aromatic amines, 14% cyanopropylphenyl stationary phase).

Chemical Damage

Most GC columns will have good chemical resistance except towards inorganic acids and bases; H_2SO_4, HCl, NH_3, KOH, NaOH, H_3PO_4, HF etc. When a GC column has been exposed to these types of compounds it will exhibit column bleed, a lack of inertness (evidenced by peak tailing), and a loss of retention and resolution.

The use of perfluoroacids, such as trifluoroacetic, pentafluoropropanoic and heptafluorbutyric acid can damage the stationary phase, however, they have to be present in high levels, 1% or higher.

Cutting 0.5-1 m from the front of the column can remedy the effects of damage. To prolong the column lifetime a guard column or retention gap can also be used, although these may also be damaged by harmful substances and trimming or replacement will be necessary; this, however, is a lot more cost effective than replacing a GC column.

Peak Fronting caused by column overload

An old or highly oxidized column is likely to show symptoms of 'overloading' due to the loss of phase at the head of the column resulting in lowered capacity and efficiency. Fronting peaks may be observed (Figure 9).

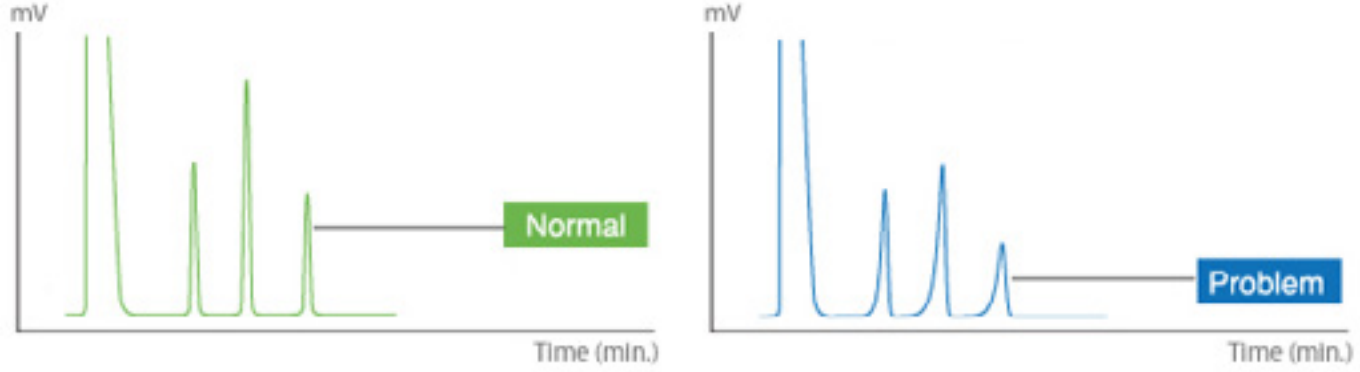


Figure 9: Peak fronting due to column overload ultimately caused by loss/occlusion of stationary phase.

Retention Gaps

Up to 5 meters of deactivated silica tubing known as a Retention Gap may be connected via a simple push-fit union to the analytical column. Retention gaps are used to:

- Trap the non-volatile residues from samples that may potentially contaminate the analytical column. The retention gap acts to retain the non-volatile materials but does not interact with the analyte species. Regular trimming of the column inlet may take place to avoid peak shape problems caused by contaminant build up.

- Alleviate the problems associated with polarity mismatch, as well as some of the peak shape problems associated with on-column and large volume injection. The deactivated uncoated retention gap material allows the formation of contiguous films with most solvents that will tend to focus the analyte on the stationary phase at the head of the analytical column. Solutes eluting closest to the solvent front or those that most closely match the polarity of the solvent will show the greatest peak shape improvements when a retention gap is used.

Leak Checking

It is important to check for leaks to minimize the ingress of air and moisture which can damage the GC column. Regular leak checking should be part of the routine maintenance schedule, especially after any new connections have been made (i.e. changing the column, routine maintenance etc.). Leak detectors can be used or if a mass spectrometer is connected as the detector the leak detection function can be employed. A leak will produce a distinctive mass spectrum with peaks at m/z 18, 28, 32, 44 or 14, 16 which correspond to H_2O, N_2, O_2, CO_2 or N, O. Usually if m/z 28 is larger than m/z 18 there is a leak.

Column Rinsing

A capillary GC column must have a bonded and cross-linked stationary phase to be compatible with solvent rinsing.

Solvent rinsing kits can be purchased from any column manufacturer (Figure 10). The process of rinsing involves passing millilitres of solvent through the column.

An injection of a large volume of solvent will NOT have the same effect and contaminants will not be removed from the column.

Before rinsing 50 cm should be cut from the inlet end of the column. The inlet end of the column is then inserted into the vial of the rinse kit which is attached to a pressurized gas source (N2, He). Successive solvents are then passed through the column by applying 10-15 psi of pressure from the pressurized gas source (a flow of 1 mL/min is desirable). If viscous solvents are being used longer rinse times may be required. After the last solvent has been passed through the column the pressurizing gas is allowed to flow for 5-10 minutes before the column is properly re-installed into the GC system and conditioned as normal.

Solvents which are appropriate for column rinsing and will provide good results are methanol, dichloromethane, and hexane which are applied in series. Other solvents will also work (and may be required depending on the samples which have been analysed), however, the following criteria should be met:

- A polar and non-polar solvent should be used.
- The most polar solvent should be used first followed by the other solvents in order of decreasing polarity.
- Using the injection solvent is advisable as the sample components should be soluble in it.
- Each successive solvent should be miscible with its predecessor.
- Water followed by methanol should be used if aqueous-based samples have been analysed (i.e. biological extracts, waste water, soil etc.).
- Halogenated solvents should be avoided as the final rinse solvents if the detector installed is an electron capture detector (ECD). Acetonitrile should not be used if a nitrogen phosphorous detector (NPD) is installed.

Suggested solvent volumes for different column diameters are detailed in Table 1.

Column I.D. (mm)	Solvent Volume (mL)
0.18-0.2	3-4
0.25	4-5
0.32	6-7
0.45	7-8
0.53	10-12

Table 1: Solvent volumes for rinsing columns.
Using larger volumes will not damage the column.

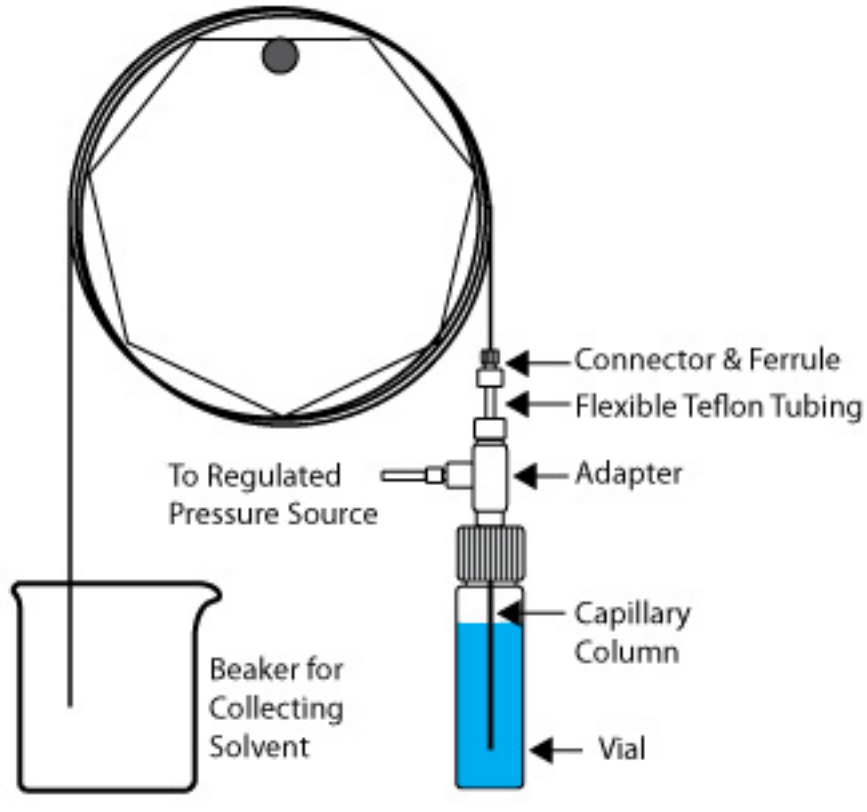


Figure 10: GC column rinsing kit.

Extending GC column lifetime

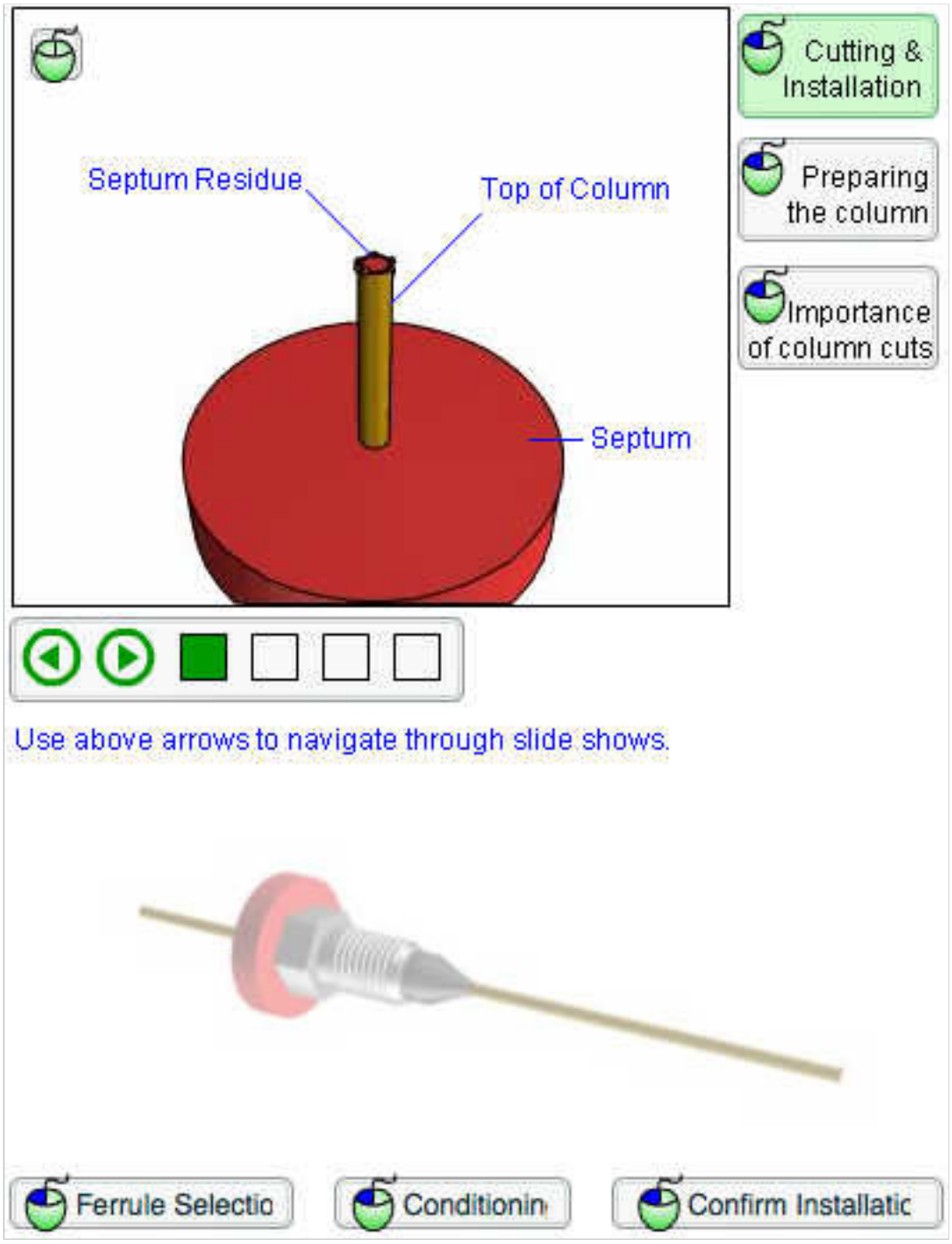
Once the GC column has been correctly fitted it is desirable to achieve the best possible results for as long as possible, so here are some final tips for extending column lifetime.

1. Fit carrier gas traps close to the instrument (to remove oxygen and moisture) this is a great way to improve column lifetime and detection limits.
2. Use the plate count of a test analyte to monitor column efficiency and set a column discard limit based on your knowledge of required plate counts for your types of analysis.
3. Columns can often be miraculously restored to life by trimming 5% of the total column length from the inlet end — note that retention times may shift slightly after this operation, but efficiency may increase and peak shapes will improve. Retention time changes can also be mitigated by using the instrument software to accurately calculate the length of the cut column. This typically involves inputing the column dimensions, as denoted on the column ID tag, and the retention time of an unretained peak - volatile gases such as propane or butane or volatile solvents such as methanol at higher temperatures with an FID.
4. Consider using a guard column if your samples are particularly dirty.

10 COMMON GC MISTAKES

1) Not Taking Enough Care at the Inlet

Any leak in GC will cause significant problems with chromatography and the inlet is the most likely area. Don't take the risk of having to troubleshoot a leak, change the septum regularly and make sure the column is correctly installed and leak-free.



2) Turning the oven on before the carrier gas

If there's one thing that will permanently damage a column more quickly than anything else it's heating the column without any carrier gas flowing. Get into the habit of making sure the carrier gas is flowing before you switch on the oven AND make sure the oven is cool before switching the carrier off. When switching the oven off, set the oven temperature to 35oC before switching the oven off as this will cool much quicker.

3) Not programming in column dimensions

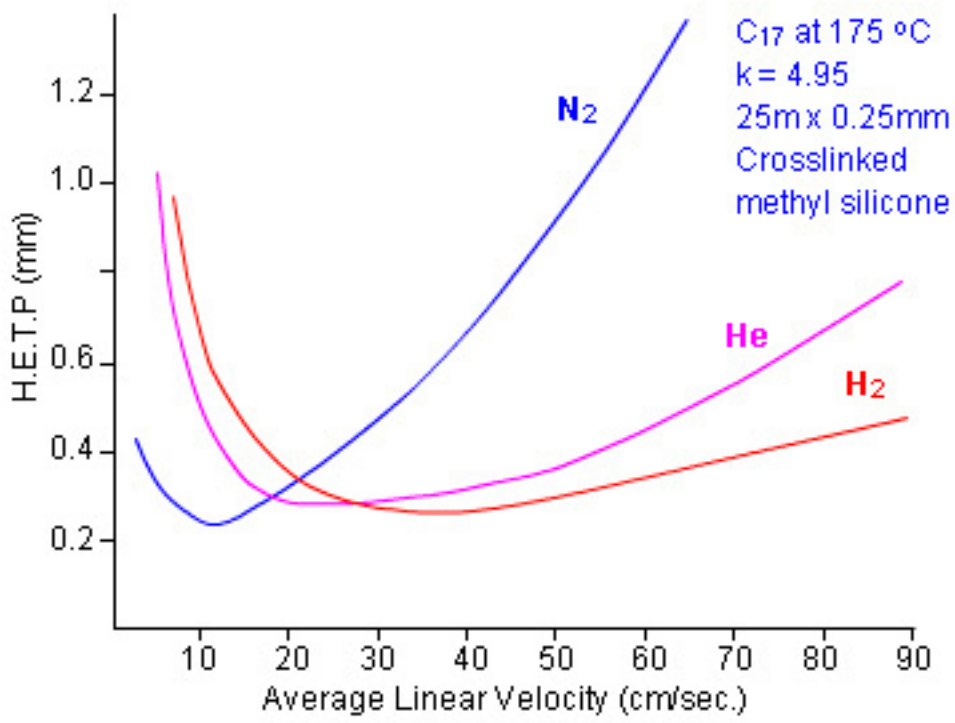
Most modern GCs use an EPC (electronic pneumatic controller) to accurately deliver carrier flows and this relies totally on the correct column dimensions being entered by the operator. Make sure anytime you change the column you enter the correct dimensions into the GC or else your flowrate and split ratios will be wildly inaccurate.

4) Not using optimal flowrate for carrier

Each carrier gas has an optimal linear velocity, which gives the best efficiency (Nitrogen c.12cm/sec, Helium c.35cm/sec and Hydrogen 40 - 50cm/sec).

To obtain this optimal linear velocity the flowrate is matched to the internal diameter of the column in use. e.g. for a 0.32mm ID column using helium the optimal flowrate will be about 2.0ml/min.

If the optimal flowrate is not used, column efficiency can be significantly reduced.

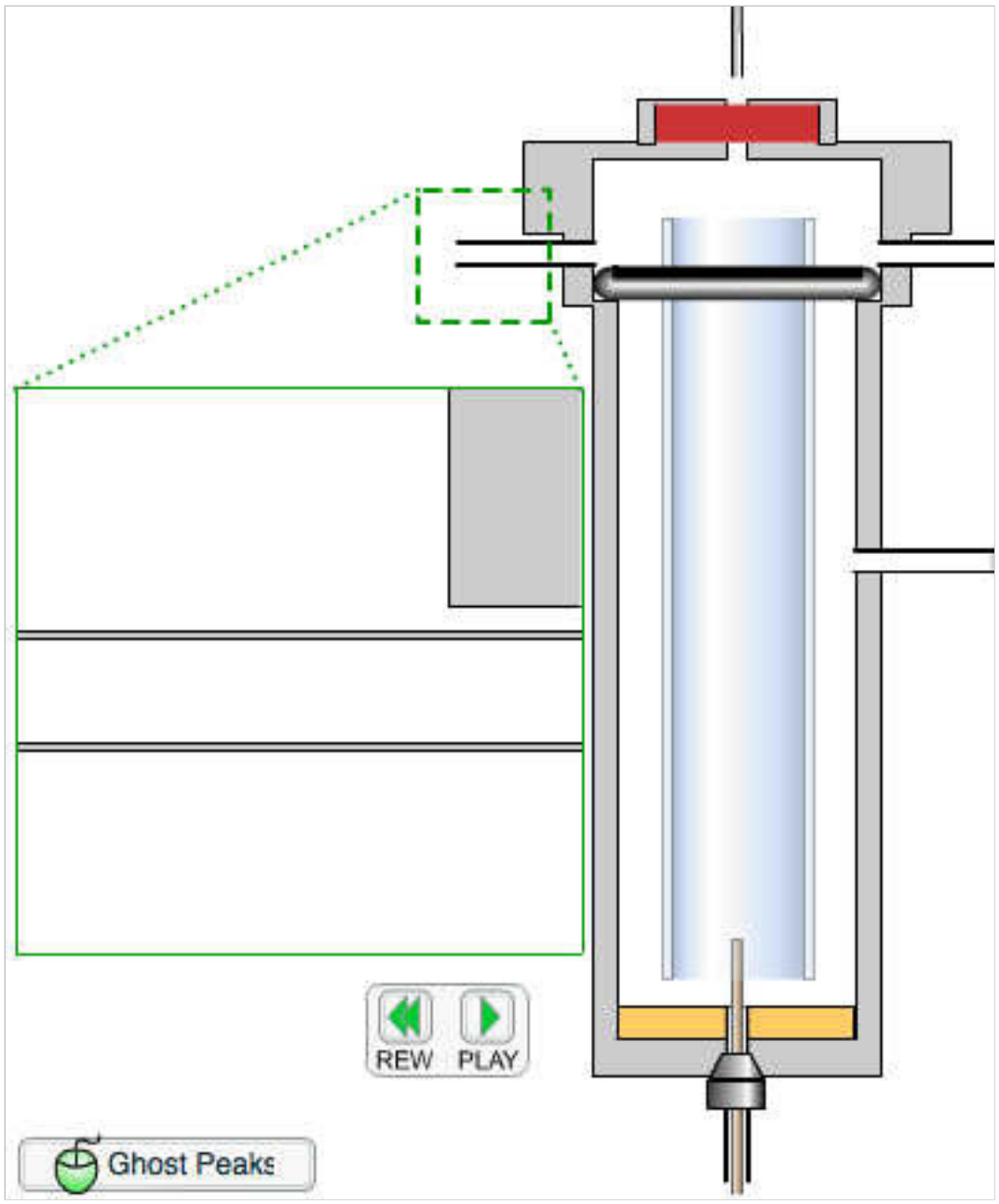


5) Using too large an injection volume

When a liquid sample is injected into the GC inlet, this vapourises into a much larger volume of gas. The size of the vapour is dependant on the solvent being used and the injection volume.

If this volume exceeds the size of the liner a condition called backflash occurs.

Using a higher split ratio does not reduce this affect, you must either reduce the injection volume, use a different solvent or use a larger liner.



Optimising Injection Volume

You can follow the instructions opposite to use this handy backflash calculator.

Try entering these values in the boxes provided.
Typical Experimental Values:

Liner Length :	75 mm
Liner I.D. :	4 mm
Head Pressure :	10psi
Injection port Temperature :	250°C
Solvent :	Hexane

You can alter the injection volume
NB : stick to whole or half microlitres

Then click the '**Inject**' button to show the expansion volume of the solvent vapour.

Once you are familiar with it --
Click the **'Start Experiment'** button below to answer some questions on Backflash in GC.

Start Experiment

Backflash Calculator

Key Learning Points

Enter **liner Specifications**, **Inlet Conditions** and select the **Solvent.**
Then press Inject.
Any value in the **green** or **amber** region of the liner is OK – any value in the **red** region exceeds the liner dimensions and risks Backflash

Solvent

select one...

Density (g / mL)

MW

Conditions

Injection Port Temp. (°C)

Head Pressure (psig)

Injection Volume (µL)

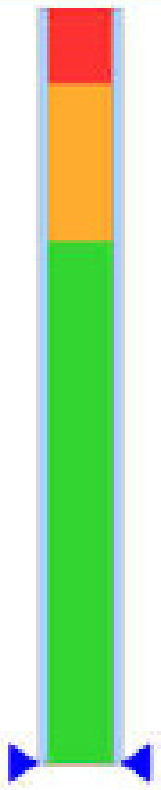
Liner Specifications

Length (mm)

I.D (mm)

Liner Volume (µL)

Vapour Volume µL

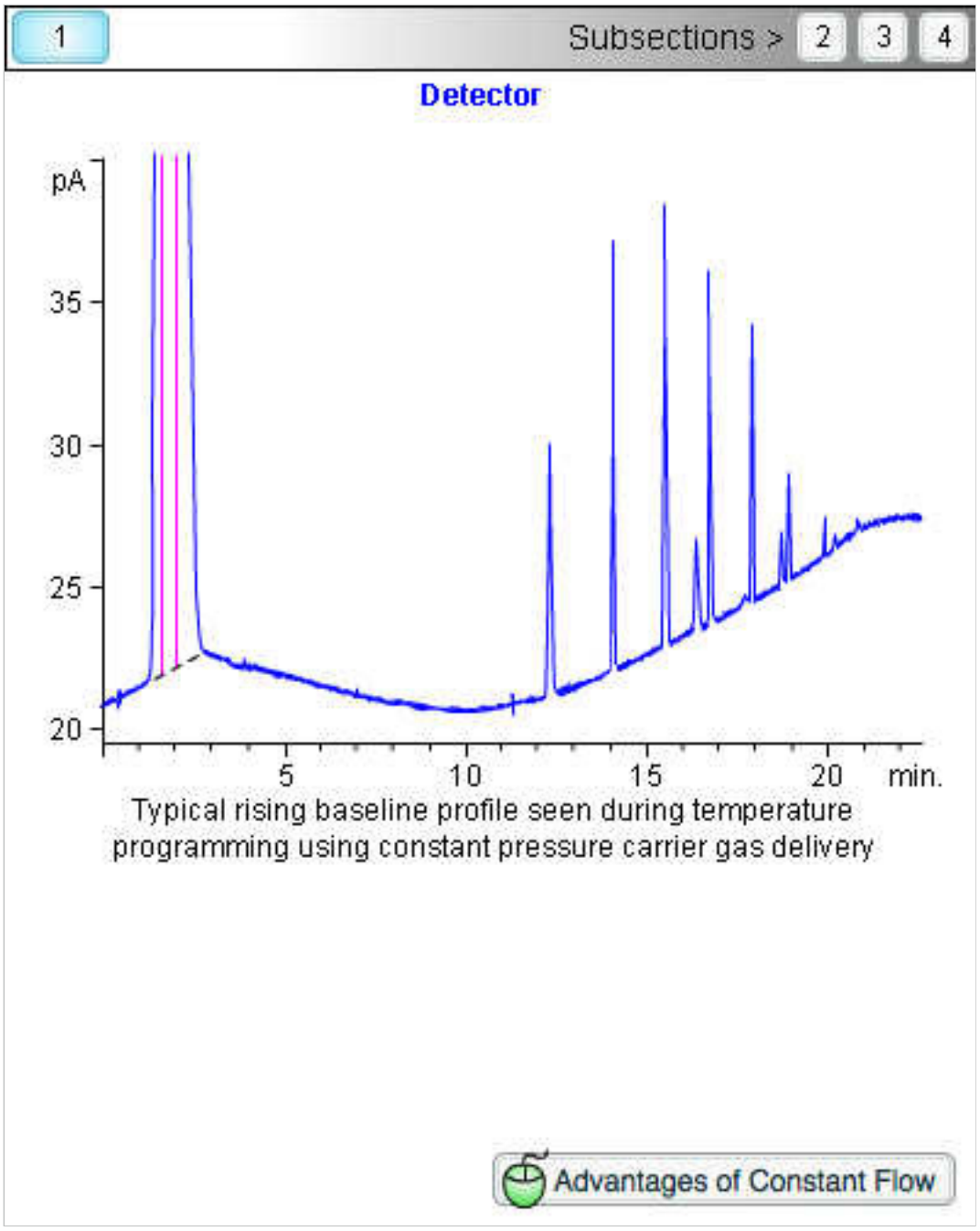


Inject

Reset

6) Not specifying constant flow / constant pressure

As the oven temperature increases, the pressure within the column increases. In a constant pressure system, flowrate will decrease to maintain a constant pressure. This increases retention time and decreases efficiency as the flow moves away from the optimal linear velocity. In constant flow mode, the instrument maintains a constant flow (with optimal linear velocity) by increasing pressure. Retention times are shorter and efficiency maintained. Constant pressure and constant flow modes will give different retention times and peak shapes, so make sure you specify which mode should be used.

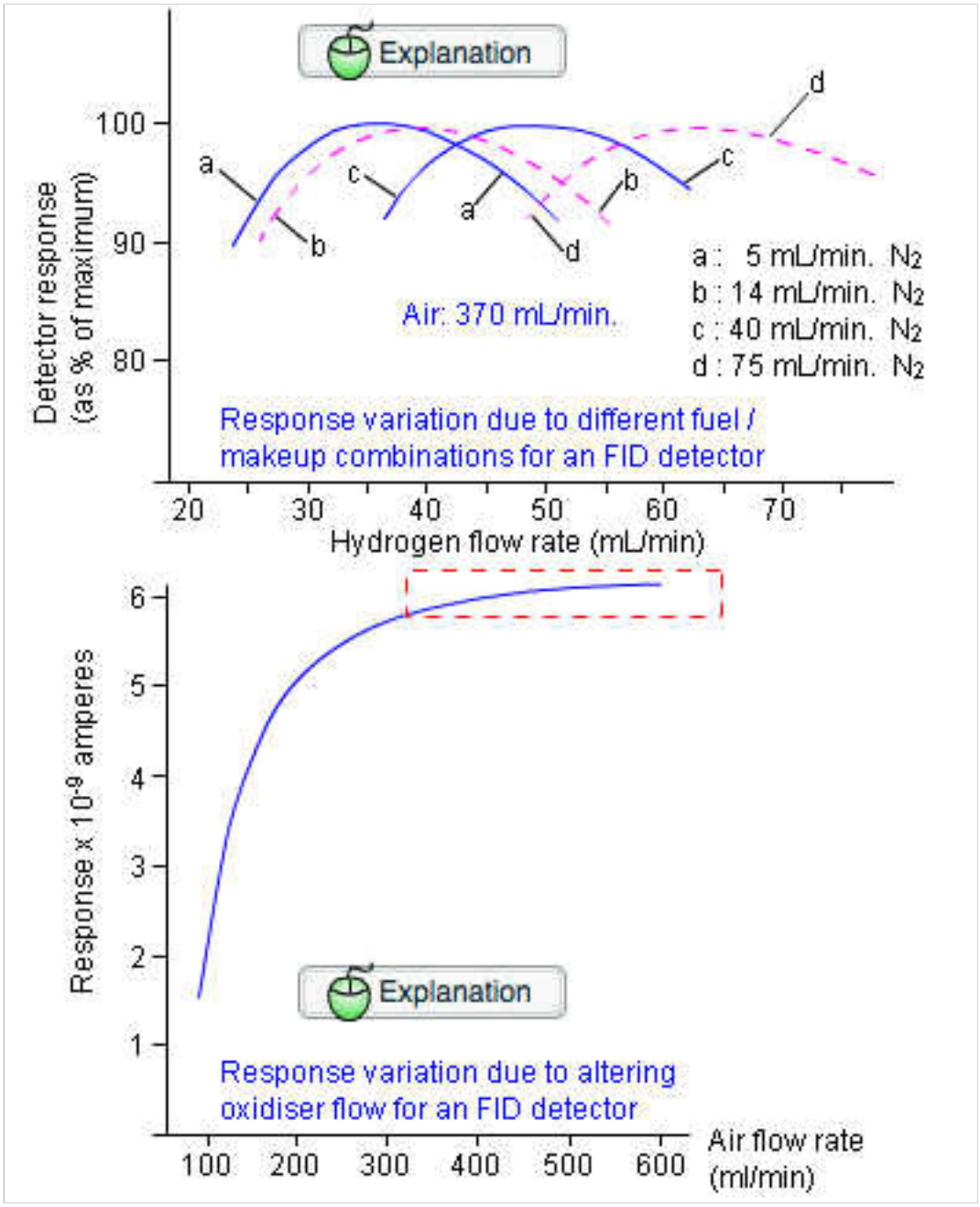


7) Water on non-polar column

With some sample types it can be un-avoidable to inject the sample in water. Be sure that the column you use is suitable for water injections. As the column phase becomes increasingly non-polar (e.g. a DB-1), it loses the ability to adsorb the water sufficiently and chromatography breaks down. For water injections try to use the most polar phase you can (e.g. Wax) and accept that column life will decreases and bleed increase.

8) Not using the correct flame gas ratios

Lighting and maintaining a hydrogen / air flame relies on an optimal flow and ratio for all the gases in the detector. See the advice in 'My Detector Flame Won't Light'.



9) Not capping the column during storage

The bonded stationary phase in a GC column is easily oxidized, particularly in the presence of UV light. Be sure to cap the ends of the column to seal in inert carrier gas and stop air getting in. Store the column in a box in a dark cupboard to minimize the amount of light energy reaching the column. This is important to stop the column degenerating on storage.

10) Not checking you have enough gas

In many labs, the gas supply is remote from the GC. Although it isn't always easily accessible, it's a whole lot less inconvenient than having to repeat a run of samples because either the carrier or detector gasses ran out before the original run was complete. Make checking the gas supplies one of the first things you do.

SOLVENT CHOICE FOR GC INJECTION A CRITICAL METHOD VARIABLE!

I guess we all have our 'go to' solvents for our work in GC, and most of the time this will be based on the availability of the solvent and the chemical nature of our samples. Polar samples (analytes) generally need a more polar solvent (methanol for example) and less polar analytes a less polar solvent (n-hexane or heptane for example). Sometimes we might even use an intermediate polarity solvent when we aren't sure of the analyte composition, ethyl acetate is a popular choice in this instance.

I talk to many folks involved in GC and the conversations around sample preparation and the choice of ultimate sample diluent are almost always around the chemistry of the sample (matrix) or the analyte and the requirements to properly solubilise the sample.

But there are so many more issues to consider when choosing the appropriate sample solvent and setting some of our critical method variables. I've given a short treatment of these considerations in this instalment so you might be better informed when developing, optimising, transferring or troubleshooting your GC methods.

Sample Solvent (Diluent) and Injection Volume

The sample diluent has a direct effect on the amount of sample which can be injected into the GC under a given set of inlet conditions.

As the sample is rapidly introduced into the GC inlet via the autosampler syringe, there is a rapid (explosive) evaporation of the sample solvent which transfers the sample molecules into the gas phase. The degree of expansion will depend upon the nature of the sample solvent (i.e. its expansion co-efficient), and the inlet temperature and pressure, which is dictated by the combination of the liner size and the carrier gas flow rate through the inlet.

If the sample size (the number of microliters of sample you chose to inject) is too large, and other operating variables are not favourable, the gas phase sample will exceed the volume of the inside of the inlet liner, and the gas phase sample will contact the underside of the septum and 'spill' over into the carrier gas inlet and septum purge lines. As these lines are not heated (other than the portions directly inside or next to the inlet), then the gas phase sample will condense, leaving some of your analyte deposited within the pipework of your sample inlet system.

If one were to then inject a 'blank' solvent, under the same circumstances, the same overfilling phenomenon would occur, and just as a wave 'laps' the seaweed and other detritus from a beach, some of the previously deposited sample components within the pipework may be drawn back into the inlet and make their way into the column. We would be presented with a mini version of the previous chromatogram which we describe as 'carry-over' and which is a major cause of quantitative irreproducibility in capillary gas chromatography.
Chromatographers call this phenomenon 'backflash' (Figure 1).

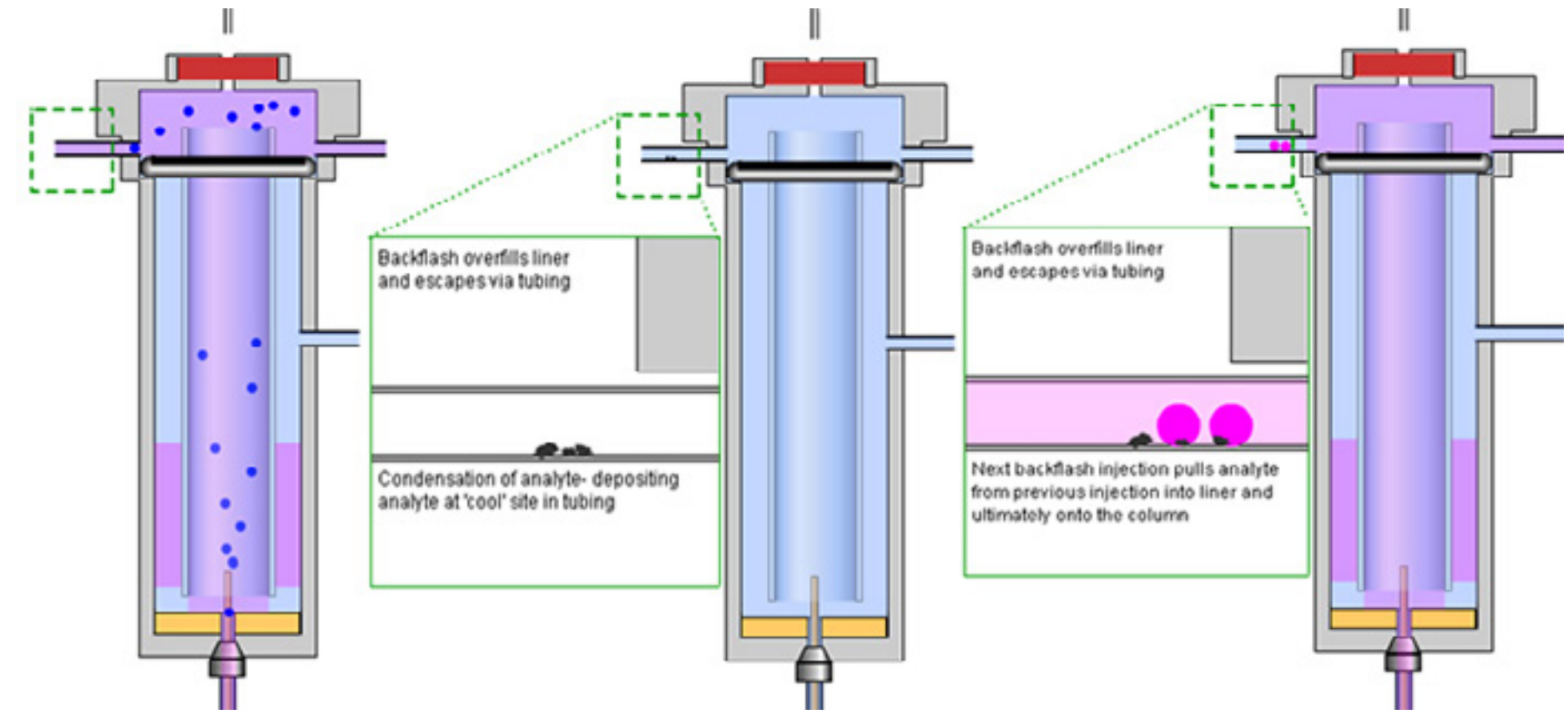


Figure 1: The principles of sample 'backflash'.

A – the solvent volume is too large to be contained in the inlet liner and 'spills' over into the inlet tubing.

B – The analyte condenses in an unheated region of tubing.

C – the next backflashed injection solvent vapour solubilises the deposited analyte and carries it back into the inlet and subsequently on to the column – resulting in carry-over.

So – how does one decide on the proper injection volume or the operating variables which will avoid this insidious carry-over effect?

Well we typically use software calculators to assess the correct amount of sample to inject and the appropriate inlet temperature and pressure.

The calculator needs to know the nature of the solvent and its expansion co-efficient in order to calculate the volume of gas created under a given set of conditions, from a given volume of solvent injected into the system.

It will also need the liner volume (easily calculated or requested from suppliers) and the temperature and pressure of the inlet, which of course you can get from the GC system.

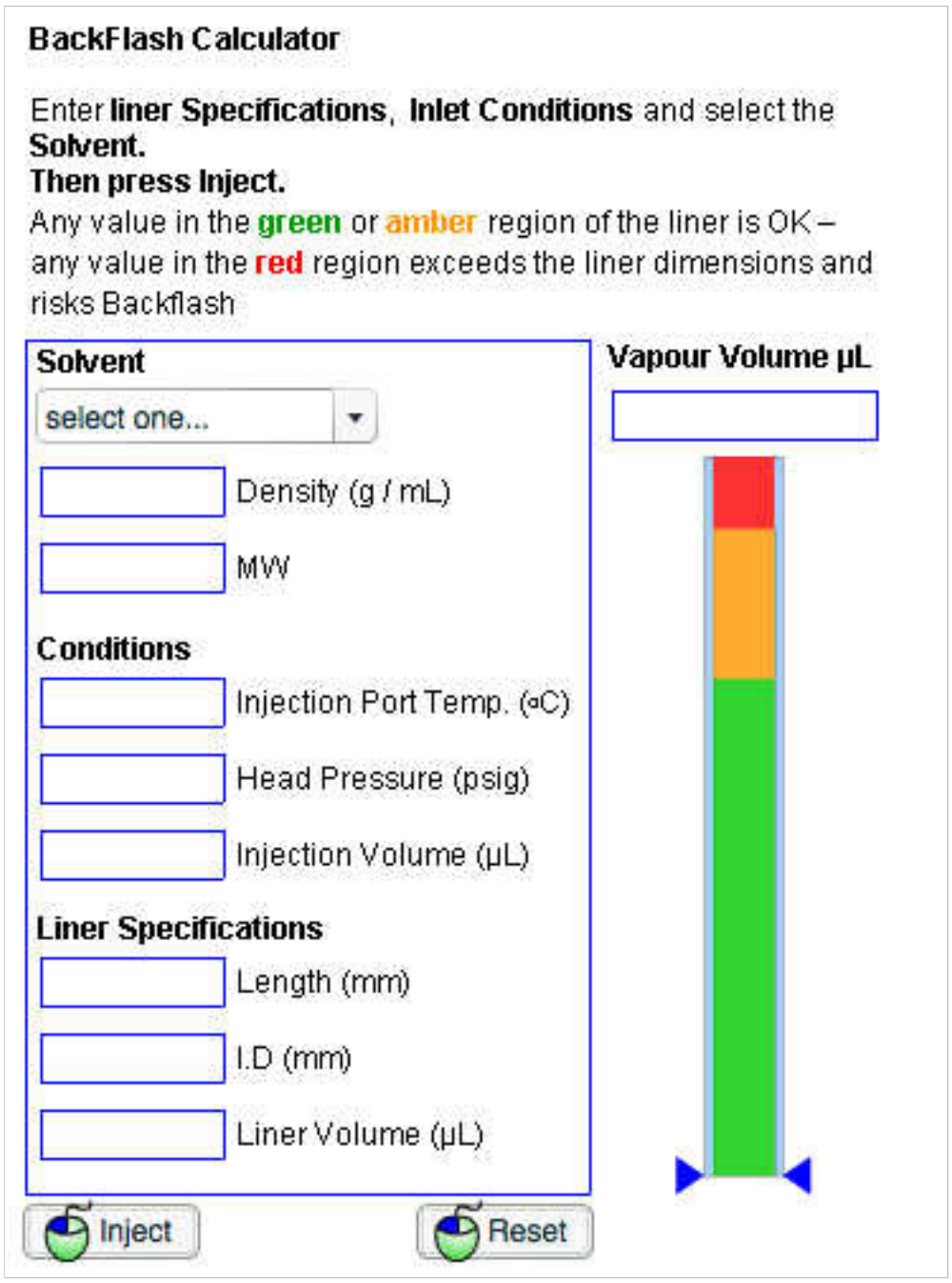


Figure 2: An example Backflash Calculator.

These calculators are very useful as they allow one to assess the required changes to the operating conditions in order to avoid backflash, or on a more positive note, to assess how much sample can be injected without risking carry-over, if one wants to improve the sensitivity of a method.

Further, the calculator can be used to make informed choices on the detector temperature setting required for a particular method, alongside the general information that one might have about the boiling points of the higher boiling sample components.

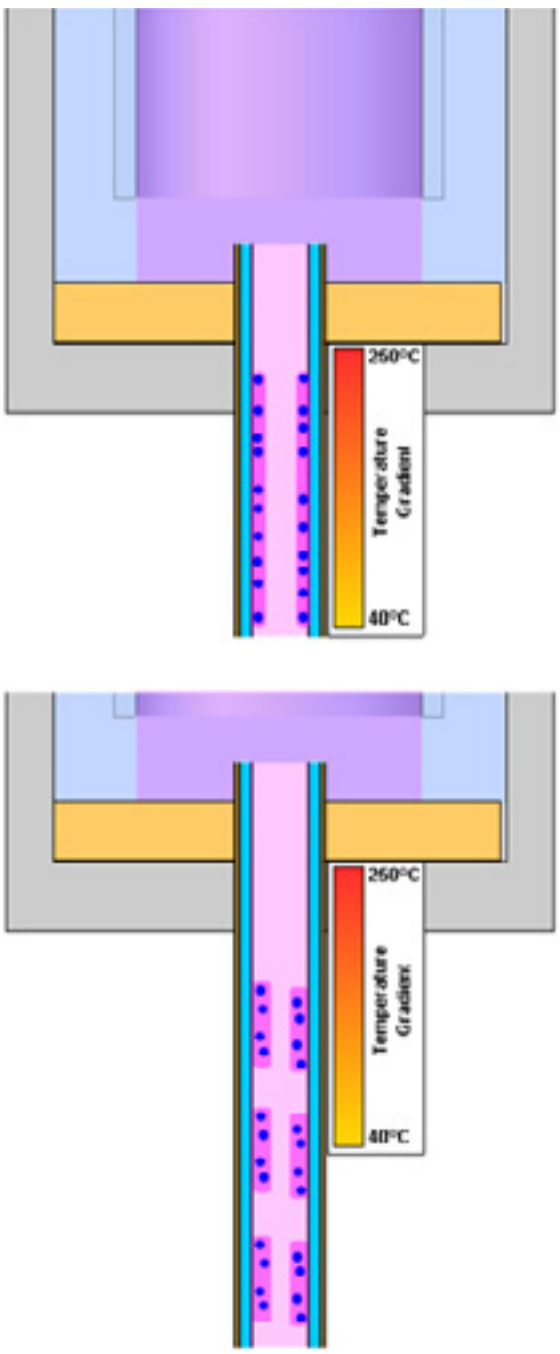
The calculator can also be used to assess the effects of increasing the head pressure (carrier gas flow), during the moment of sample injection which constrains the expansion of the sample solvent plug, which is known as 'pressure pulsed injection', a strategy which is also used to increase the volume of sample injected without risking backflash and hence analyte carry-over.

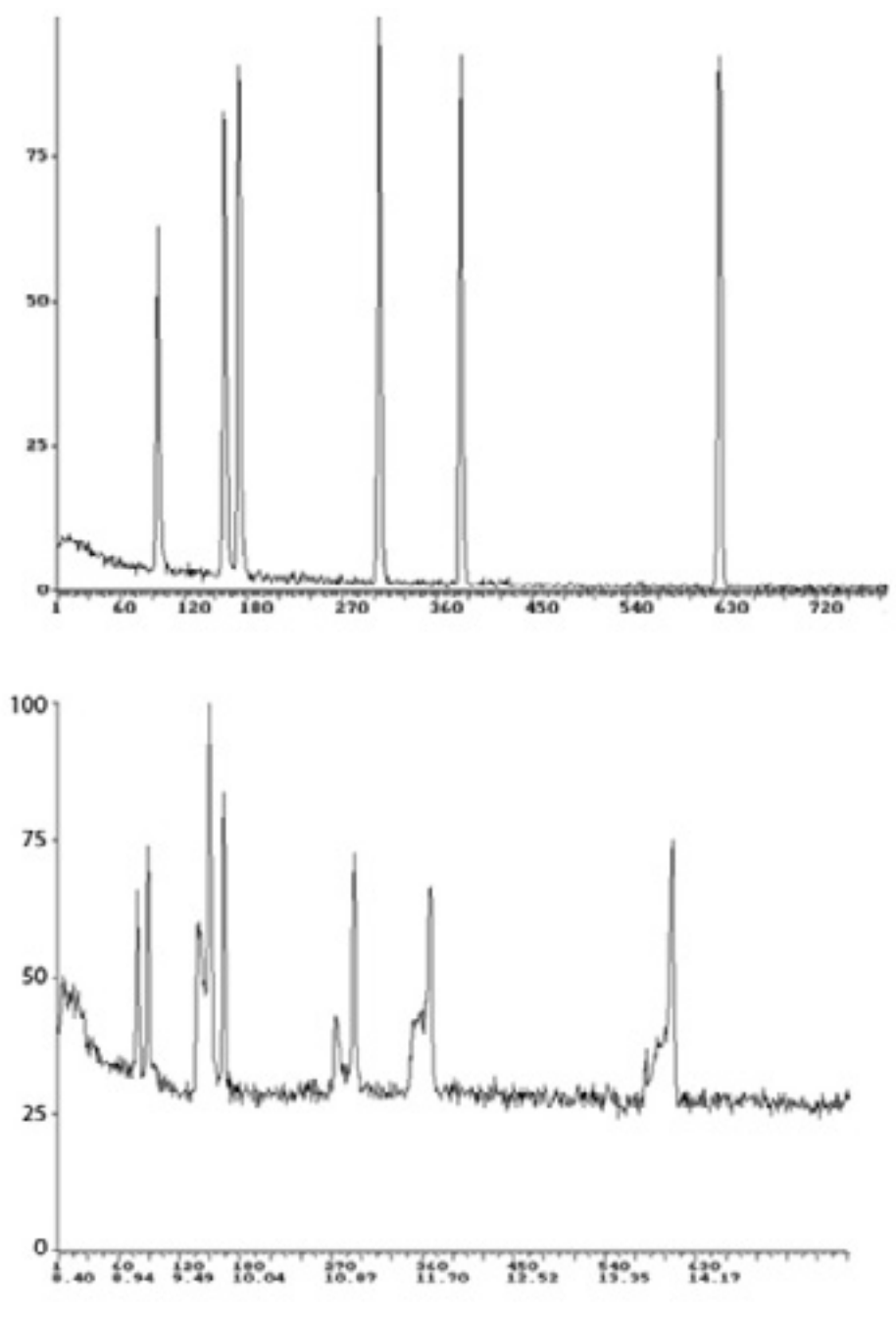
Diluent effects on peak shape

During the initial phase of a splitless injection, the column is cool (40 – 70°C typically) and the sample solvent is allowed to condense on the stationary phase on the inner wall of the capillary column. Due to the reduced vapour pressure caused by the flowing carrier gas, the solvent evaporates and 'concentrates' the dissolved analytes into a sharp band. This effect, known as solvent focussing, helps to overcome the very broad peak shapes associated with splitless injection into a hot oven, caused by the prolonged time taken to move the sample vapour from the inlet to the column under splitless conditions.

The peak focussing effects rely upon the solvent forming a contiguous (uninterrupted) film on the inner wall of the capillary column. If the polarity of the column stationary phase is not well matched with the polarity of the sample diluent, this film formation can fail and 'pooling' of solvent can occur which results in a non-contiguous film and very poor peak shape.

These effects along with the resulting chromatograms can be seen in Figure 3.





These effects can also occur to a lesser extent in split injection, however they are typically much less severe.

Typically one should avoid extremes such as using methanol as a sample diluent with non-polar GC stationary phases such 100% polydimethylsiloxane or n-hexane as a sample diluent when using polar phases such as waxes. However, minor effects can also be seen when using intermediate polarity phases or solvents and one should take care to match the solvent polarity with that of the stationary phase.

So, the next time your reach for the solvent bottles to dissolve or reconstitute your sample prior to loading it into the GC vial – just stop a moment and run through the considerations above. Make an informed solvent choice – your chromatography will be all the better for it!

GC INLET MAINTENANCE... HAVE YOU REALLY HEARD IT ALL BEFORE?

Many troubleshooting investigations in chromatography often don't lead to a single causal factor. Often, the reason for problems or lack of method robustness are related to many small 'contributory factors' and this is particularly true of the problems associated with sample introduction in capillary Gas Chromatography.

I often find that, whilst most folks have a fair idea of what constitutes a good routine maintenance program for GC Split / Splitless inlets, the reasons for the routine are poorly understood. So, to add some deeper understanding to the problems associated with poor inlet maintenance, here is a quick multi-media blog on GC Inlet Maintenance.

Septa:
Allows the sample syringe to enter the inlet and make the injection without depressurising the inlet or interrupting the carrier gas flow.

Problems:
Out gassing - Septa are made from plasticised rubber or silicon and the plasticiser materials or silicon components 'out-gas' giving rise to increased baseline noise or the 'hedgehog' baseline appearance one associates with the elution of a homologous series of analytes. This is typically overcome by a gas which flows across the underside of the septum inside the inlet to carry away these outgassing products. This is called the 'septum purge' or 'septum flush' gas flow, and has a typical flow rate of a few mL/min.

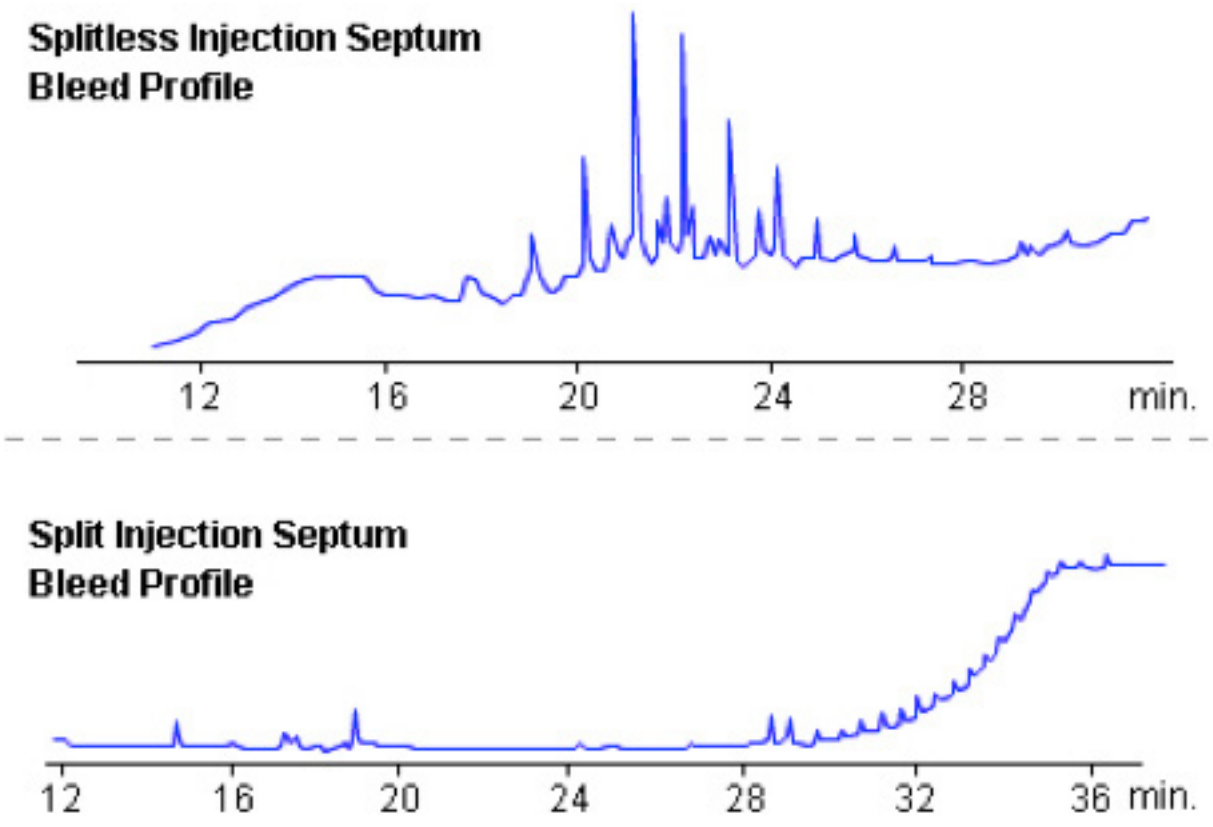


Figure 1: Typical septum bleed profiles from splitless and split injections.

Septa 'core' or split through prolonged use, if the incorrect syringe point style is used or if the septum nut, which holds it in place, is over tightened, is badly fitting or if the septum is the incorrect size.

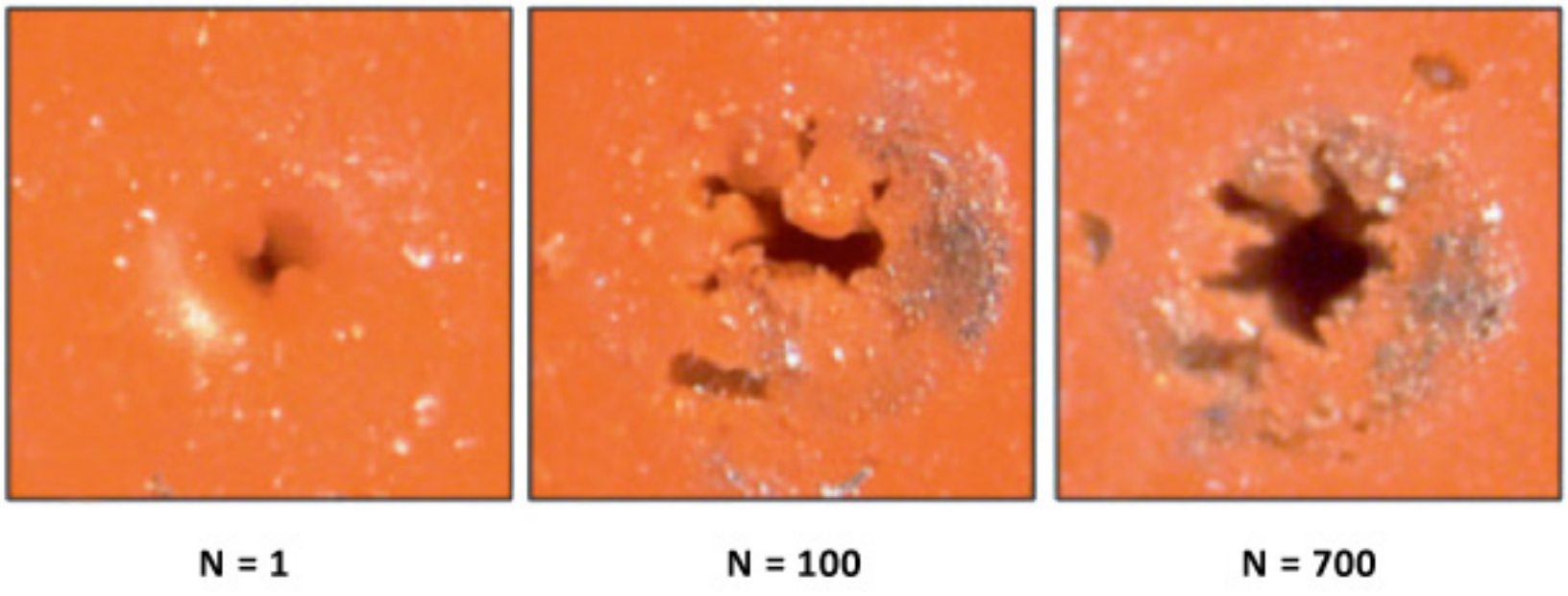


Figure 2: Coring of septa following repeated injections.

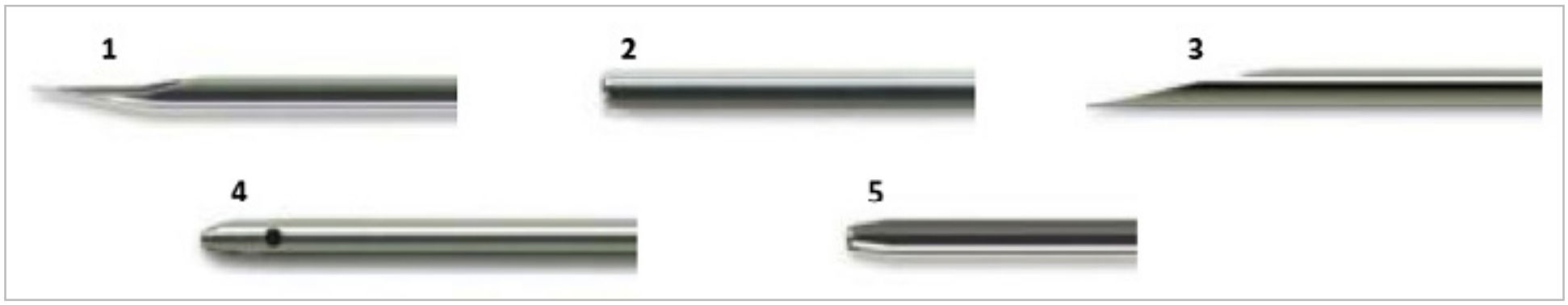


Figure 3: Needle tip styles such as type 5 cause less coring than type 3.

If the septum is split or cored, then the inlet may leak during the injection phase. With very badly cored septa, the carrier will leak as the inlet pressure is increased to maintain constant flow during the thermal gradient.

Septa 'stick' to inlets leaving behind material on the metal surfaces of the inlet, causing inlet sealing problems and bleed.

What you see:
From shards of septum which have fallen into the inlet or from a system where the septum purge flow is too low or not working, you will see the out-gassing products of rubber and plasticiser which are usually homologous series which cause noisy, rising and 'hedgehog' type baselines. The same goes for septa which leave residues on the inlet metal surfaces if they have become 'stuck' within the inlet. If you are using an MS detector – look out for the following ions in the baseline / background which are indicative of septum bleed:

m/z 73, 207, 281, 149, 167, 279

Note that the ions at 73, 207 and 281 m/z can also arise from column bleed (polysiloxanes), however the ions at 149, 167 and 279 m/z are due to plasticisers and are particularly indicative of septum bleed. Ions at 296 and 429 indicate septum breakdown products (rather than bleed) – usually from septum shards in within the liner which are in contact with hot quartz or metal surfaces.

From a system which leaks during the injection phase you will see a noticeable shift in baseline position across the solvent peak and poor peak area reproducibility.

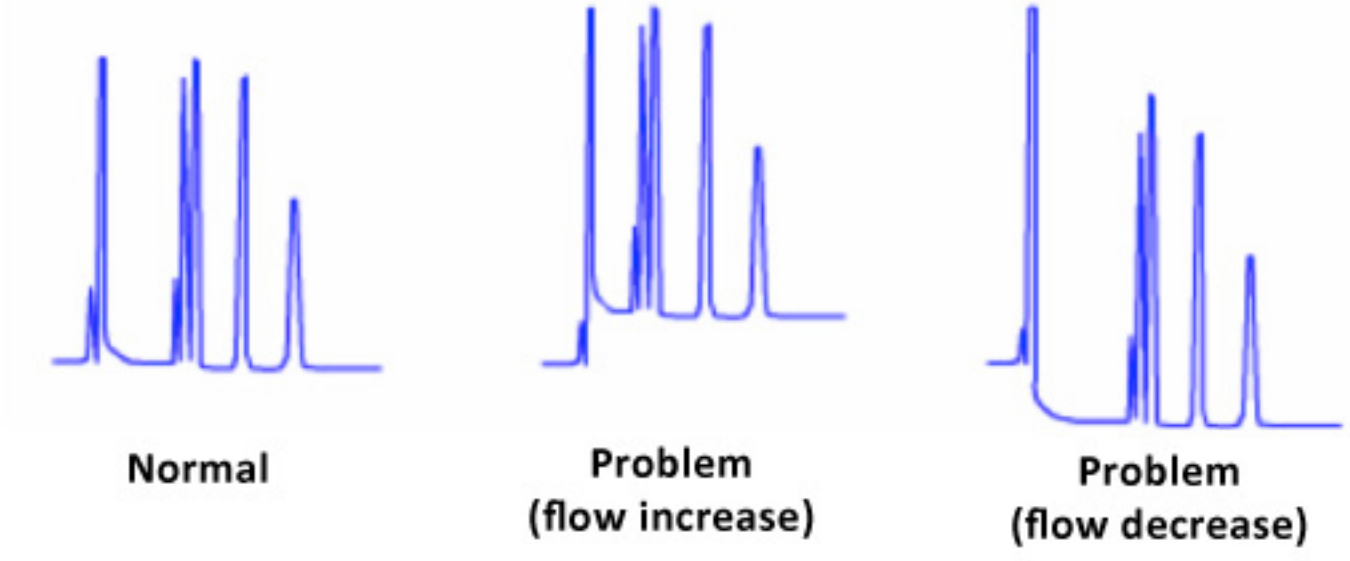


Figure 4: Shift in baseline position after injection, indicating a leak through the septum during the injection.

Where the system cannot achieve the carrier pressures required at higher oven temperatures, the system may enter safety shut-down mode or you will experience a large baseline shift during the analysis with widely varying retention times.

How to stop it happening:

Ensure you have the correct septum material for the inlet temperature. Inlet temperatures above 350 oC generally require a special septum material. What temperature are your septa rated to? Why not go and check right now!

If you overfill the inlet with sample gas (sometimes called backflash), sample components will deposit onto the lower septum surface. Over time these bleed into the inlet, and onto the column, causing 'ghost peaks' or 'memory effects'. What old timers like me would call 'carry-over'. You should check that the conditions of the GC injection are compatible with the volume and solvent used for sample injection and that the volume of gas plasma formed on sample volatilisation, doesn't exceed the inlet liner volume. See here for a nice downloadable tool to calculate sample gas volume (http://www.chem.agilent.com/en-US/Technical-Support/Instruments-Systems/Gas-Chromatography/utilities/Pages/GCCalculators.aspx)
The hole within a 'pre-drilled' septum acts as a centre guide and prevents coring provided that the septum is held under the correct torque.

Figure 5: Pre-drilled septa contain a needle guide which help to prevent coring / splitting.

Where the system cannot achieve the carrier pressures required at higher oven temperatures, the system may enter safety shut-down mode or you will experience a large baseline shift during the analysis with widely varying retention times.

Some septa have 'non-stick' coatings which can bleed into the column and cause baseline noise and discrete low level interfering peaks. Use only high quality septa.

Install the septum according to your manufacturer's recommendations – paying special attention to the torque of the retaining nut. Too tight and the septum will split and core much more easily. Too loose and the nut may loosen further after repeated injection, causing loss of carrier pressure and instrument shut down. Change the septum for each major batch of analyses, for critical or trace analyses. Over a very short time you will have a positive financial payback.

Figure 6:
Badly fitting or lower quality septa leave residues on the inlet and 'cookie cutter' residues.
Image courtesy of Agilent Technologies, Santa Clara, CA, USA.

Check that the actual septum purge flow from your instrument matches the method set point or instrument readout. Since the inception of highly reliable Electronic Pressure Control (EPC) systems, flow meters have become much less commonplace. However EPC systems can fail, become blocked, give rise to variable or incorrect flows just like annual valves – it really is worth checking the flow manually from time to time (perhaps as part of your PM or OQ/PV schedule).

O-rings:

Some instrument designs feature an o-ring to isolate the carrier and spilt flow from other instrument flow paths. These are typically fitted around the inlet liner or as a sealing ring around the underside of the septum cap and are made from fluorocarbon or graphite.

Figure 7: Fluorocarbon o-ring seals used in certain instruments.
Other instruments will use PTFE or graphite inlet seals.

Problems:

Depending on quality, fluorocarbon o-rings can out-gas contaminating materials at higher temperatures

- The o-ring can deform and cause leaks if not held under the correct torque.
- The o-ring can stick to the liner or inlet metal surfaces, causing sealing problems.

What you see:

Discrete peaks on the baseline – typically due to the focussing of contaminants at lower over temperatures and subsequent elution of triphenylphosphine oxide, the principal outgassing component

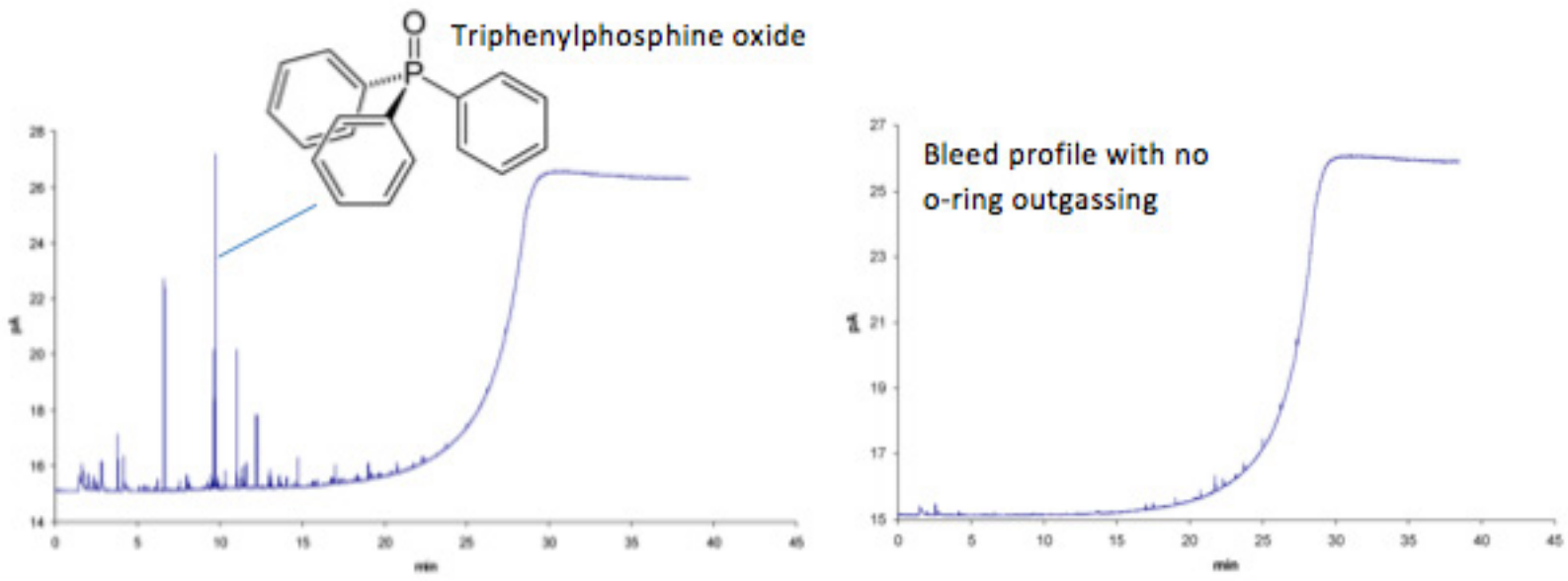


Figure 8: Outgassing products from o-rings can lead to discrete peaks within the GC bleed profile, primarily due to the release of triphenylphosphine oxide

Look for 277 m/z in the mass spectrum of your background or any interfering peaks!

If residues of the previous o-ring remain on the liner or on the inlet metal surface, this may lead to gas flows within the inlet leaking into one another. At worst this will lead to a safety shut down, however, the problem may be more insidious and cause poor quantitative reproducibility.

How to stop it happening:

- Use high quality o-rings.
- Change the o-ring every time you change the liner (see below).
- Do not re-use liners or o-rings which have been stuck to the liner or inlet inner metal surface.

Liner, Packing and Inlet seals:

The liner is typically a tube of quartz glass which is used to constrain the gaseous sample so it does not contact the hot metal surfaces inside the inlet. In some instrument designs it acts to separate the column and split flows and is designed to be a replaceable consumable which will become contaminated with involatile sample residues over time. To facilitate sample mixing, provide a large surface area to allow volatilisation of high boiling analytes and to prevent column contamination, sometime the liner is packed with a plug of glass wool.

Figure 9: Quartz glass liner packed with deactivated quartz wool.

Some instrument designs have a lower seal within the inlet to allow efficient splitting of the sample, to act as a coupling device for the analytical column and to present an inert surface to the sample, especially during splitless injection where the analyte may be held within the liner for prolonged periods.

Figure 10: Metal liner seal.

Problems:

Over time, the inlet liner, packing material and seal becomes contaminated with involatile sample material, which acts as a surface to adsorb analytes through inter-molecular physico-chemical interactions such as hydrogen bonding between polar sites.

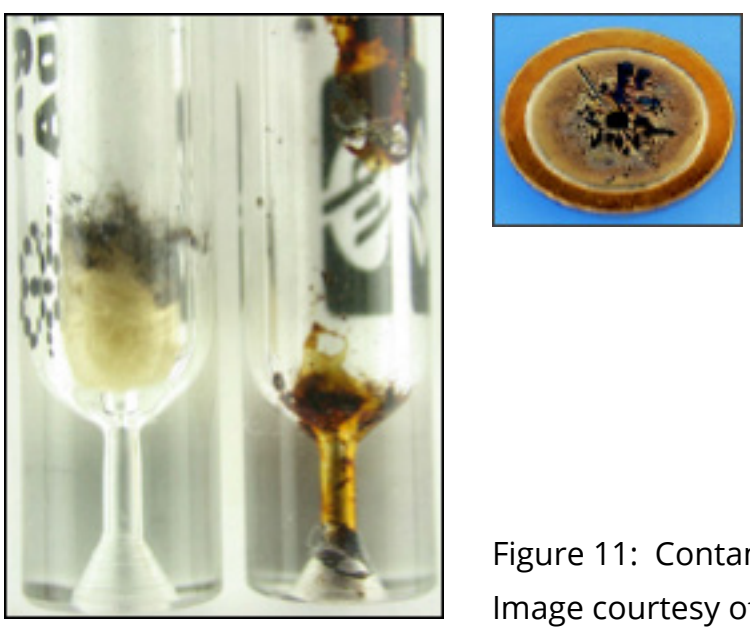


Figure 11: Contaminated liners (left) and inlet seal (right)
Image courtesy of Agilent Technologies, Santa Clara, CA, USA.

Typically, the liner and packing materials will be constructed from quartz glass which contains a large number of surface polar (silanol) groups. To prevent unwanted secondary interaction with the analyte, these groups are chemically 'derivatised' to present a much less polar, less 'active' surface. Over time, the increased temperature and exposure to moisture will hydrolyse the derivatising species to the polar silanol groups and unwanted polar / polar interactions between the analytes and the liner and packing surfaces will be possible.

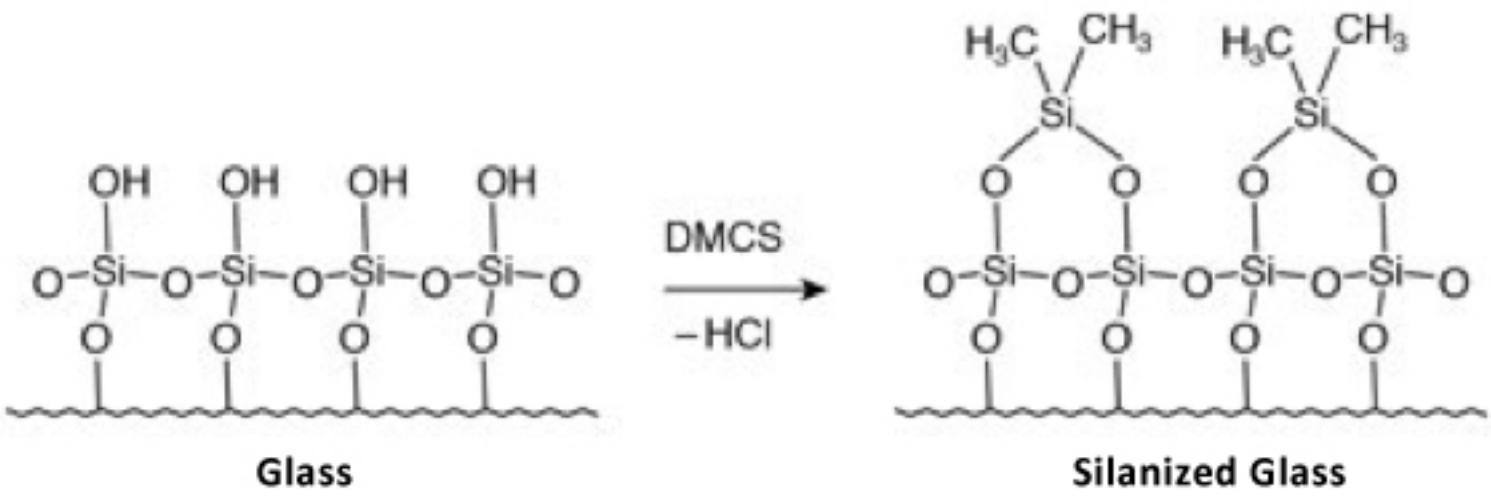


Figure 12: Typical liner / packing silylation reaction.

Typically the metal seals within the inlet will also be deactivated – and the hydrolysis described above is also possible.

What you see:

Poor quantitative reproducibility and peak tailing are the most common forms of problem when inlets become active.

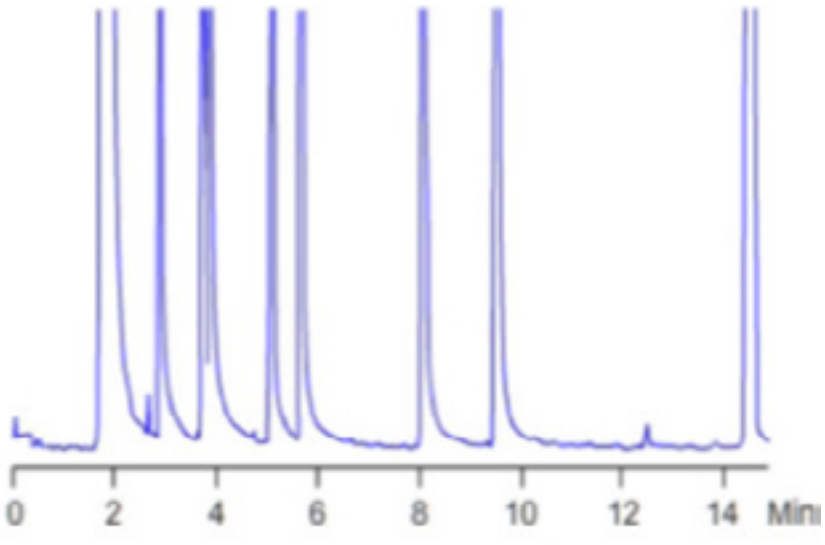


Figure 13: Analyte peak tailing caused by unwanted secondary interactions with active sites within the liner, packing material or inlet seal.

In extreme cases, analytes may not appear within the chromatogram if they are quantitatively adsorbed within the inlet.

Sometimes, analytes are not thermally stable (pesticides, carbamates, explosives, brominated flame retardants etc.) and will degrade with prolonged exposure to higher temperatures. The presence of glass wool within the liner, and especially in cases where this glass wool is active in terms of polar / polar interactions, can exacerbate the thermal degradation of labile compounds.

How to stop it happening:

- Ensure you use good quality, deactivated, liners and packing materials.
- Ensure the liner design, packing amount, density and positioning are suitable for your application.
- Ensure the liner, packing and seal are deactivated with modern deactivation process which will guarantee the highest levels of inertness.
- Change the liner as often as is necessary. This will depend on your analytes and applications, and most importantly on the nature of your sample matrix and the amount of sample clean-up that you do prior to analysis. Most manufacturers recommend that the liner is changed 'regularly' – and you should satisfy yourself that the liner is clean and deactivated whenever you are running large campaigns of samples, critical analyses or trace level determinations. Remember that the o-ring should be changed with each liner change and any metal seals are also changed with every or every other liner change, especially where analytes are thermally labile, peak tailing is occurring, analytes are particularly polar or trace level determinations are being undertaken.

The split line:

A large bore (typically 1/8”) tube which carries the split gas away from the inlet. The split line may also contain a filter or trap filled with adsorbent material such as deactivated charcoal, which acts as a trap for volatile species and reduces the emissions from the instrument split line vent.

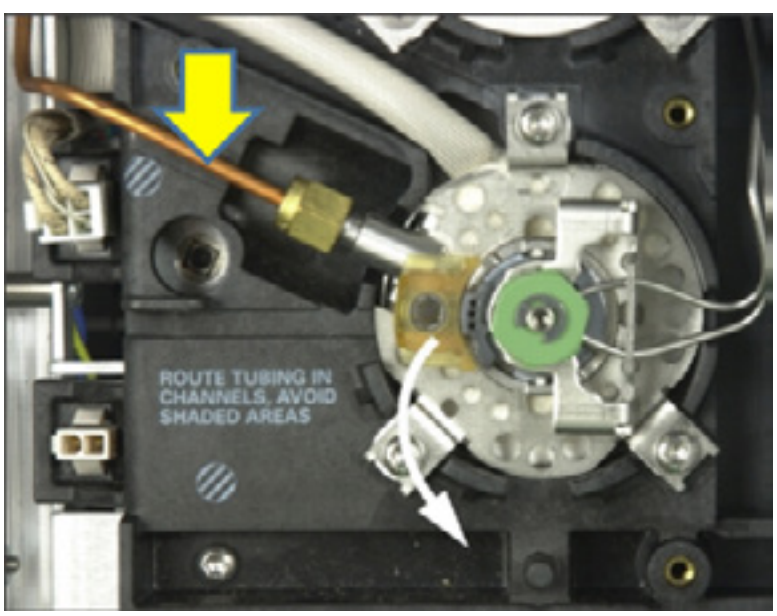


Figure 14: (Left) Split line indicated by the yellow arrow. Typically a wider bore tubing which allows split gas to escape from the inlet. (Right) Typical in-line split vent filter. Image courtesy of Agilent Technologies, Santa Clara, CA, USA.

Problems:

The split line and filter (trap) become blocked over time with an accumulation of involatile material. The speed at which the tube and trap becomes blocked will be dictated by the number and cleanliness of samples.

What you see:

Contamination of the split line will result in 'ghost peaks' – broad baseline disturbances before or after all or certain analyte peaks. If a blank solvent is injected some or all of the peaks of the previous injection will be present, with low response and pronounced peak broadening.

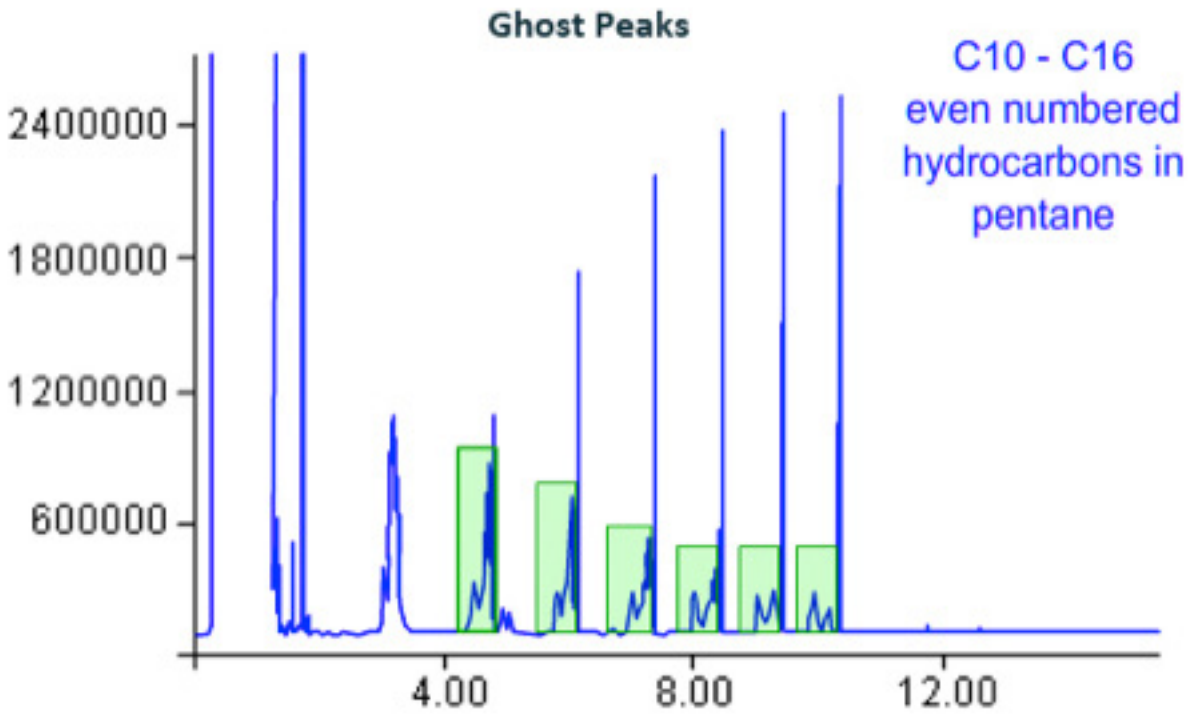


Figure 15: Ghost peak deformations caused by, amongst other causes, re-injection of contaminants from the split line.

In extreme cases, retention times may vary, this is due to the EPC unit struggling to control and balance the various applied pressures and resulting flows against the back pressure created within the inlet by the blocked split line.

How to stop it happening:

Most preventative maintenance routines should contain a check / clean / replace action for the split vent filter and the split line should be removed, inspected and rinsed or replaced as necessary. For instruments with heavy usage or when analysing large numbers of dirty samples, this action should be carried out every 6 months or more frequently if required.

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