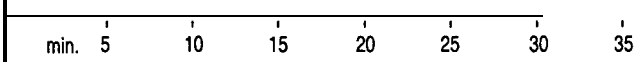
Hints for the Capillary Chromatographer

When is column bleed normal and when is it a problem?

What is column bleed?
Column bleed is the normal elution of stationary phase as the GC column is temperature programed. All columns will show a certain amount of bleed as the oven temperature increases. The question is whether the bleed is normal or excessive for your column and conditions. Column bleed only becomes a problem when it either interferes with quantitation or when it contaminates the detector.

A typical bleed profile for a temperature programmed run is shown in Figure 1. Column bleed is characterized by a gradual baseline rise which reaches a nlateau at the final temnerature of the program. This rise typically begins approximately 20 to 30°C below the maximum operating temperature of the column. Notice that there are no discrete peaks present in column bleed. The type of stationary phase as well as the dimensions of a capillary column will affect the amount of column bleed. For example, a polar phase usually exhibits more bleed than a non-polar phase. In general, the more stationary phase a column contains, the higher the column bleed. A long, wide bore, thick film column has more bleed associated with it than a short, narrow bore, thin film column. Operating at higher temperatures also increases bleed.

Figure 1 - Blank run showing normal column bleed.



30m, 0.2Smm ID, 0.25um XTI@-5 (cat.++ **12223)**
Oven temp.: **30C** (hold 1 min.) to **350°C @**l0"C/min. (hold 5 min.)
Inj./det. temp.: **300°C** **Detector:** MSD
Carrier gas: **helium** **Linear velocity:** **30cm/sec. set @ 35C**
Split ratio: **10:1** **Ionization:** EI
Scan range: 50-400

What are the most common causes of high column bleed?
It is important to recognize that there are different causes of excessive column bleed. Studies have shown (1) that there are several c&nmon GC problems which can cause high bleed. Let's consider the most common causes for excessive column bleed and what steps can be taken to minimize it.

The stationary phases used in capillary columns are susceptible to oxidation. If the column exhibits a high baseline rise, the column may have been subjected to oxygen at a relatively high temperature. This can be from a leak either in the injection port area or in the gas lines immediately preceding the injector. Oxygen can also be present in the carrier gas as a contaminant from the gas cylinder. It is important to prevent oxidation by using oxygen traps on the carrier gas lines and by carefully leak checking the flow system and inlet after column installation.

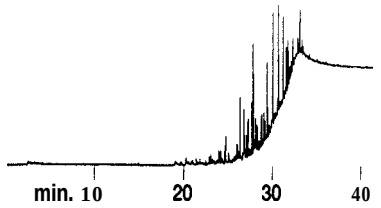
Exposing the column to high temperature without flow or operating the column at temperatures above the manufacturer's recommended maximum will also result in stationary phase damage. This most commonly occurs when a column is conditioned without confirming carrier gas flow, or when a cylinder of carrier gas empties during temperature programming. Restek recommends that flow through the column be verified, before conditioning, by either detecting a non-retained peak or by submerging the detector end of the column in a small vial containing methanol and observing the bubbles (Figure 2).

Figure 2 - Confirm column flow by submerging the column outlet in a vial of methanol.

If the column has been exposed to high molecular weight sample residue, the baseline may exhibit a rise similar to column bleed. When a column has been contaminated, discreet peaks are usually observed in the chromatogram at elevated temperatures. Frequently, solvent rinsing can rejuvenate the column by extracting the contamination. Phase degradation can also result from injecting samples containing strong acids or bases or excess derivatizing reagents.

Sometimes septum bleed is confused with column bleed because the electron impact spectra obtained with mass spectrometry are similar for both. Septa bleed is easily recognized as a distinct pattern of discrete peaks in a chromatogram, whereas column bleed normally does not result in individual peaks. In Figure 3, notice the pattern of multiple peaks just before the baseline begins to rise from the normal column bleed. The best techniques for minimizing septum bleed are using low bleed septa, frequently replacing used septa, using a septum purge, and completing a blank run when the column has been at temperatures below 100°C for several hours.

Figure 3 - Example of septum bleed.

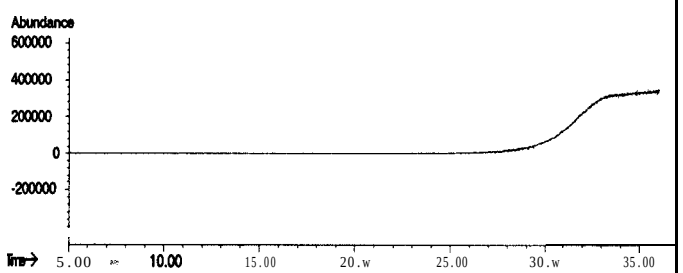


ISm, 0.53mm ID, l.Opm Rtx@d (cat.# 10252)
Oven temp.: 40°C (hold 15 min.) to 3OOT @ lSOC/min.
Inj./det. temp.: 250°C/3000C
Carrier gas: hydrogen Linear velocity: 4Ocm/scc.

How can column bleed be accurately measured?
To determine how much column bleed is acceptable for an analysis, you must have an understanding of the necessary detection limits, the type of column being used, and the detector and signal sensitivity during operation. When analyzing trace components with very sensitive detectors, even a small amount of column bleed can interfere with the analysis. If using long length, thick film columns, more bleed will be experienced than with short length, thin film columns. The combination of stationary phase type and detection system used can have a profound effect on how much column bleed is exhibited. For example, nitrogen sensitive detectors (TSDs or NPDs) would exhibit a higher baseline signal from a cyanopropylphenyl stationary phase than Flame Ionization Detectors (FIDs).

It is important to be careful when interpreting the chromatogram obtained with a blank run. Some data systems use an autoscale feature which normalizes the intensity axis to the largest peak in the chromatogram. If there are no peaks, then the chromatogram is drawn with the baseline at full scale, giving the illusion that the column has high bleed (Figure 4).

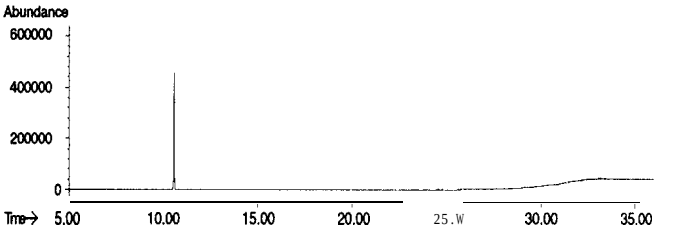
i'igure 4 - Autoscaling can give the appearance of high bleed.



30m, 0.25mm ID, 0.25um Rtx-1 (cat.# 10123)
Oven temp.: 35°C (hold 1 min.) to 340°C @ 10Cmin. (hold 5 min.)
[nj./det. temp.: 300°C Detector: MSD
Carrier gas: helium Linear velocity: 3Ocm/sec. set @ 35T
Ionization: EI Scan range: 50400

A simple way of accurately measuring column bleed is to inject a known concentration (i.e. 25ng on-column) of a component that shows good response on the detector being used and temperature program the column to its maximum temperature. Measure the peak height of the component and compare it to the baseline offset from the bleed. Although the relative intensities of these two values depend upon several factors, these values can serve as a reference point to compare with other columns and systems. Figure 5 shows the bleed level on the same column shown in Figure 4, however, a 25ng injection of naphthalene was included as part of the blank run. Notice that Figures 4 and 5 have the same absolute amount of bleed (500,000 counts), but the bleed level in Figure 5 appears much lower because the plot is scaled relative to the 25ng naphthalene peak. Without the naphthalene injection, an analyst can be fooled into believing that the bleed level is much higher than it actually is.

Figure 5 - Baseline bleed compared to 25ng naphthalene.



Sample: 25ng on-column injection of naphthalene
See Figure 4 for other conditions.

How can column bleed be minimized?
To minimize column bleed, there are several precautionary measures. All systems should be installed with oxygen and moisture traps on the carrier lines. When installing a column it is important to check the entire system for leaks. This includes any column connections, injection port fittings, and carrier lines. All columns should be conditioned following the manufacturer's recommendation. Additional routine conditioning may be required to remove high molecular weight residue, depending on the type of samples you are running. If the column becomes extremely contaminated from dirty samples, rinsing the column may be necessary in order to rejuvenate it. Routine replacement of the septum will eliminate leaks resulting from coring and/or cracking. On GC/MS systems, it is very easy to monitor for air and water leaks. Acceptable levels of air and water vary from system to system, so,check with the manufacturer for the recommended limits.

Once there is a leak free system and the column is conditioned, make an injection of a standard sample and program the column to its maximum temperature. The relative height of the peak to the height of the maximum baseline will give a fair assessment of the column bleed. ■

1) M.A. Hayes, J.J. Harland, H.D. Rood and K.T. Klatt, "Proceedings of the Tenth Int. Symp. on Cap. May (1989).