Protecting your GC Column

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The use of guard columns within chromatography, both HPLC and GC, is not as widely used as it could be. Many analysts see guard columns as an unnecessary fuss and an extra complication within the whole analytical arrangement. There are many reasons for this, and understanding these reasons gives an insight into how to improve the productivity of many commercial laboratories.

Within gas chromatography, the analysis of samples which have involatile components can lead to a gradual deposition of material at the inlet end of the column, which does not migrate from the column due to inherent high boiling points of these compounds. To try and remove these compounds will require a substantial period of time at very elevated temperatures and relies on the compound very slowly partitioning down the column. Generally speaking it is highly unlikely that it will be possible to bake the columns long enough and at a high enough temperature to effectively remove them, and to all intents and purposes these compounds remain irreversibly trapped at the head of column.

There is another source of possible contamination from samples containing semi-volatile components. These compounds will elute from the column if the correct temperature is applied for a long enough period, however due to their lack of volatility it may require several hours to effectively bake out the column to remove these contaminants. Also, it may not be practical to do this as there is potential to damage the column irrevocably, as at elevated temperatures stationary phase is lost and the column will start to lose structural integrity.

Contamination can come from a variety of sources, and the specific injection technique used will determine exactly the degree of contamination on the column. Clearly injecting samples directly onto the column or using large volumes will result in a greater degree of contamination of the column. Typically contamination arises from dirty matrices, such as biological fluids, soil, wastewater, food matrices, where there has been minimal sample preparation.

The contaminants can cause a range of difficulties for the chromatographer, as they will interfere with the partitioning of the analytes, since the stationary phase effectively becomes the contamination. Depending on the nature of the contamination there may also exist a possibility of peak tailing due to non-desirable interactions occurring between analytes which contain hydroxyl or amine functionality. It is therefore very desirable to reduce these effects to a minimum.

For gas chromatography it is quite common to remove part of the inlet end of a GC column as this will remove the bulk of the involatile and many of the semi-volatile contaminants. Reducing the length of the column by 30 – 50cm will typically not affect the performance significantly, however repeatedly performing this operation will obviously have a detrimental effect of the ability of the column to effectively resolve between critical pairs. Another option is to use a guard column as was suggested at the start of this article. These can come in two formats, either as an integrated part of the column or as a separate entity which is then coupled to the main column using an appropriate sized coupling.

Separate guard columns will typically be a length of fused silica which can suffer from unwanted activity and introduce dead volume into the system, both of which can adversely affect the peak shape of the analytes. There is also the potential with some unions that small leaks can be introduced into the system which will have a detrimental effect on the chromatographic performance. Unlike with HPLC where the coupling strength between a guard column and the main analytical column is governed by some form of screw thread, with many press fit couplings it depends substantially on the technical proficiency of the operator.

In this article two significant comparisons will be made. The first will investigate the effect of removing the front part of a column when there is an integrated guard present and when there is not one present. A separation of a series of PAH's will be used to determine the effect that both of these approaches has on the integrity of the separation.

The second comparison will look at the difference observed with another test solution derived from toothpaste. In this example the difference between using an integrated guard column and the same analytical column that is fitted with a guard column using a press fitting to determine if there is any difference in performance.

Method

The first method uses a series of PAH's as a marker of column performance. In both cases an equivalent analytical part of the column is used, allowing a direct comparison of the two approaches.

The PAH test mixture contained 16 test probes which were;

acenaphthene, acenaphthylene, anthracene, benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(ghi)perylene, chrysene, dibenzo(a,h)anthracene, fluoranthene, fluorine, indeno(1,2,3-cd)pyrene, naphthalene, phenanthrene, pyrene

Experimental

Instrumentation:

In both experiments the instrumentation used was a Thermo Scientific TRACE GC with DSQII Mass Spectrometer detector (Austin, USA).

Experiment 1 – Removing the front part of the column

Column(s):

1. Thermo Scientific TraceGOLD TG-5SILMS 30m x 0.25mm x 0.25µm (ThermoScientific, Runcorn, UK)

2. Thermo Scientific TraceGOLD TG-5SILMS 30m x 0.25mm x 0.25µm with SafeGuard (10m)

The experimental conditions used were as follows;

Carrier gas: Helium, set at a constant flow rate of 1.2mL/min.

The injection mode was splitless at a temperature of 300°C, with a splitless time of 1.5 minutes, after which the split valve was opened at a ratio of 100:1. The injection volume used was 1µL.

The oven temperature was initially held at 80°C for 1 minute, then ramped at 40°C/min to 200°C where the ramp rate was reduced to 15°C/min until the temperature reached 325°C where it was finally held for 3 minutes.

To determine the effect of cleaning the column by removing the front end, 13 metres was removed from both configurations. This is clearly an excessive amount of column removed in one go, however it replicates what would happen in many laboratories where over a period of time the column will be gradually reduced in length by removal of the front part of the column. The performance of both columns was then compared to identify which approach would give the better data, either using just the column or using the column and the column with integrated guard.

Method 2 – Integrated vs press fitted

Experimental

Columns:

1. Thermo Scientific TraceGOLD TG-WAXMS 30m x 0.25mm x 0.25µm

with SafeGuard 5m

2. Thermo Scientific TraceGOLD TG-WAXMS 30m x 0.25mm x 0.25µm

with TraceGOLD capillary guard column 5m x 0.25mm connected with a column union

The experimental conditions used were as follows;

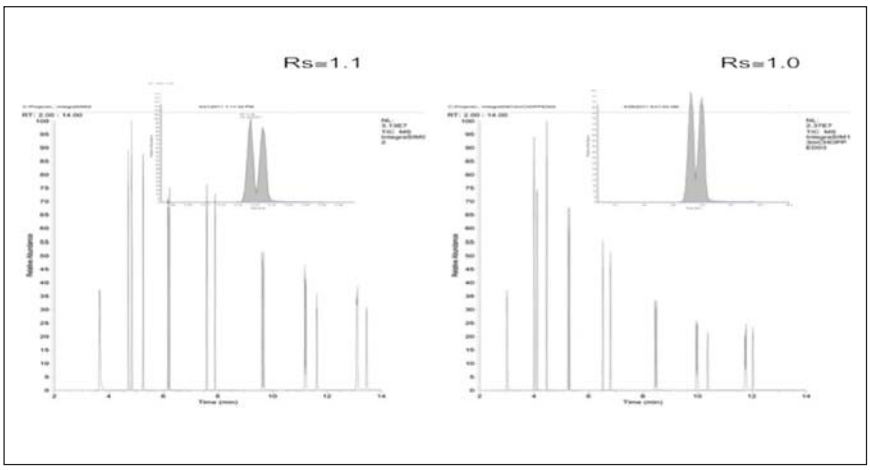
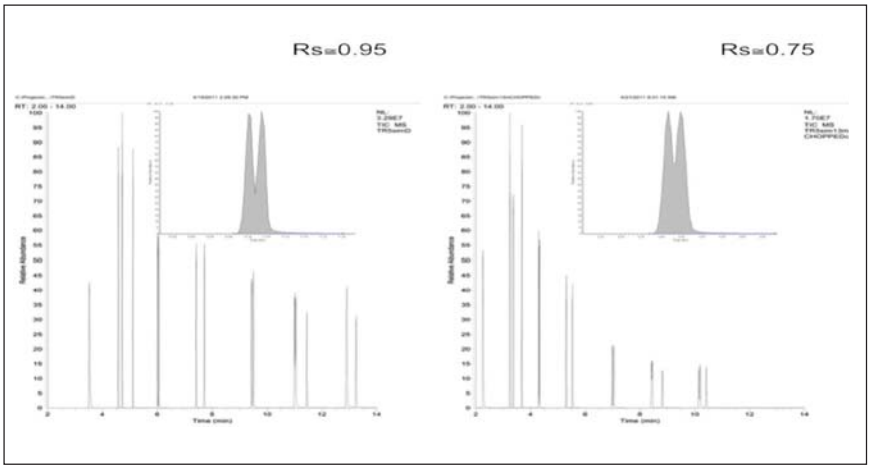


Figure 1 & 2: The effect of cutting the top of the column to simulate removing contamination with (figure 1) and without (figure 2) an integrated guard column.



Carrier gas: Helium set at a constant flow rate of 1.0 mL/min.

The injection mode was split, using a ratio of 20:1, with the injector temperature set at 250°C. 1 µL of sample was injected onto the system for each arrangement of columns used.

The oven temperature was initially held for 1 minute at 100°C and then increased at 10°C/min until the temperature reached 250°C where it was held for a further 4 minutes.

Results

Experiment 1

Figures 1 and 2 show the resultant chromatograms obtained after 13 metres of each column had been removed. Initially the column performance was exactly the same, as was to be expected. However, once a substantial part of the columns had been removed there was a difference in the performance of the two columns. The actual amount of column removed is equivalent to more than 40% of the initial 30 metre column. In this experiment a 21% loss in resolution was observed when monitoring the critical pair of phenanthrene and pyrene (Figures 1, 2).

However, when the same amount of column length was removed from the column with the integrated guard there was only a 9% loss in resolution between the critical pairs. It is worth noting that removing 13 metres of the integrated column actually removed all of the guard column and 3 metres of the active part of the column that is coated with stationary phase.

Experiment 2

Figure 3 shows the resultant chromatogram obtained from analysing the toothpaste sample using the two different columns under investigation. It can be seen from these chromatograms that the performance of the integrated column is better than that obtained using the separate guard column as there is a significant improvement in the signal to noise ratio, by almost a factor of two.

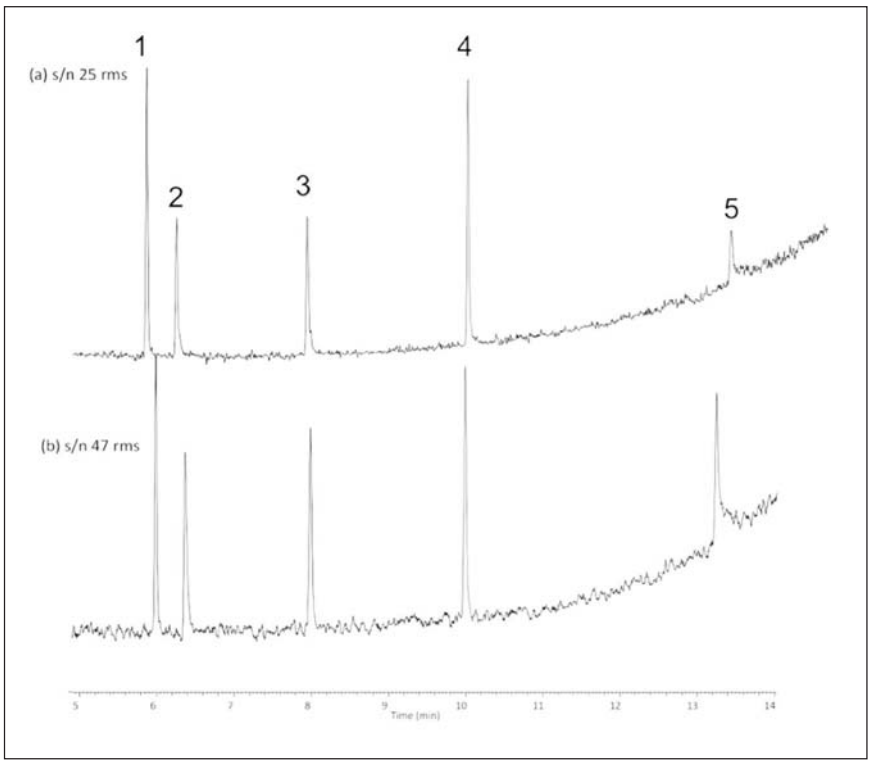


Figure 3: Comparison between a press fitted guard and an integrated guard column showing the improvement obtained when a single unit is used

Conclusions

The use of an integrated guard column has advantages when compared to a press fitted guard and also a situation where a guard column is not used. In the first experiment where a length of the column was removed and a comparison made of the resultant efficiencies, it can be predicted that removing 13 metres of the column would result in a loss of 25% in resolution which was very close to the data obtained for this experiment. Comparing this loss in resolution with the integrated guard column where only 10% of the stationary phase was lost (3 metres out of 30 metres), it can be seen that there are advantages to using an integrated guard column. Theoretical predictions suggest that the integrated guard column should lose about 5% of its resolving power, and the data in this case gave less than 10%.

The loss of signal to noise that was observed when comparing an integrated guard with a press fitted guard column was attributed to the extra dead volume with the press fit connector. An integrated column does not suffer from this extra dead volume and so as a result the signal to noise is better.

The use of guard columns both in HPLC and also in GC is still not optimal. There are many opportunities to reduce costs and to improve the quality of the data that is being produced. Using an integrated guard column in a GC environment ensures that not only is the performance optimised for that specific column, resulting in a better signal to noise ratio, but also that the column lifetime is improved. As was demonstrated with the separation of the PAH's it is possible to get better resolution over a longer period of time.

Correct use of guard columns can substantially reduce costs and also improve the performance of a laboratory. Many users see the addition of another connector to attach guard columns particularly within the field of GC, as being fraught with difficulties, and a potential solution to this is to use the integrated version of the columns being used.