Chromatography Today Help Desk

Ion Suppression from HPLC Columns

The use of liquid chromatography coupled to mass spectrometry (LC-MS) has become commonplace within the analytical laboratory. The high degree of selectivity that this approach offers coupled to the high levels of sensitivity make it a very desirable commodity. The mass spectrometer has seen substantial developments in resolving power, with introductions such as Time of Flight (TOF) and ion traps revolutionising the mass resolution. This coupled with the improvements in source design to allow the introduction of higher flow rates, and better ionisation of the analytes has seen benefits in the sensitivity and mass resolution obtainable from the detector. The initial introduction of LC-MS into the analytical laboratory was greeted as a panacea to resolving complex challenges with the possibility of injecting a neat sample with little or no separation performed, yet still obtaining qualitative and quantitative data in a matter of seconds. It was soon realised that simply injecting the sample without separating the individual components would result in a loss of sensitivity and data integrity obtained from the detector, due to ion suppression.

Separation of the sample prior to the introduction of individual components into the mass spectrometer does substantially reduce the amount of ion suppression. The rationale for this can be understood by gaining an insight into the ionisation process in electrospray. There are two major processes that are occurring within the electrospray;

- Desolvation – the removal of volatile solvent molecules. Molecules that co-elute with the analyte molecule will affect the desolvation process as it will effectively increase or decrease the amount of energy required to remove the volatile solvent.
- Ionisation – the transfer of charge to the analyte. Co-eluting molecules will compete for charge and so this has a potential to affect the amount of charge that a single type of molecule gets from the process.

Both of these processes are affected by the components that are eluting from the chromatographic column, and so it is necessary to ensure that the sample that is introduced into the chromatograph has as few components as possible, particularly important where analysing samples which are derived from a sample matrix of some description.

It is well known that samples derived from a biological source are prone to ion suppression and pre-treatment of the sample is required prior to analysis to ensure that the qualification and the quantification are not affected. There are a range of different types of sample preparation, that have all been discussed in some detail in previous editions of Chromatography Today, ranging from filtration, to liquid extraction, liquid-liquid extraction and solid phase extraction [1-3].

MS Detection

This would suggest that the coupling of chromatography and mass spectrometry is a good match, with the chromatograph providing the resolving power to ensure that the components eluting into the mass spectrometer are reasonably well separated, thus avoiding the issues highlighted previously. However, there is an issue that most users of LC-MS overlook and that will affect not only the qualitative data but also the quantitative data if care is not taken. To get a better understanding of what is happening, it is necessary to perform a couple of very simple experiments that highlight the issue.

Experiment 1

Infuse the analyte through a syringe pump and tee in the mobile phase without passing it through the HPLC column. The composition of the mobile phase from the infusion pump and also the HPLC should be the same, with ideally the majority of the flow coming from the HPLC pump and not the syringe pump. This will generate a mass spectra of the analyte, which should be the same when the column is added to the system.

Experiment 2

Infuse the analyte using the same arrangement as highlighted in experiment 1, but have the mobile phase going through the column.

A schematic of the experimental arrangement is shown in Figure 1. In all cases the source conditions have been optimised using the infusion pump and HPLC pump only, i.e. mobile phase not flowing through the column. Data is collected over about an hour, although only a short representative 4 minute window is shown.

Results

The data shown in Figure 2, clearly highlights the issue. When infusing just the compound with the mobile phase the signal from the molecular ion (m/z = 195) gives a signal intensity over a 4 minute window of 476,000, compared to 98,000 when the column is in place. It is also very evident that the mass spectrum is different between the two runs, both with and without the column.

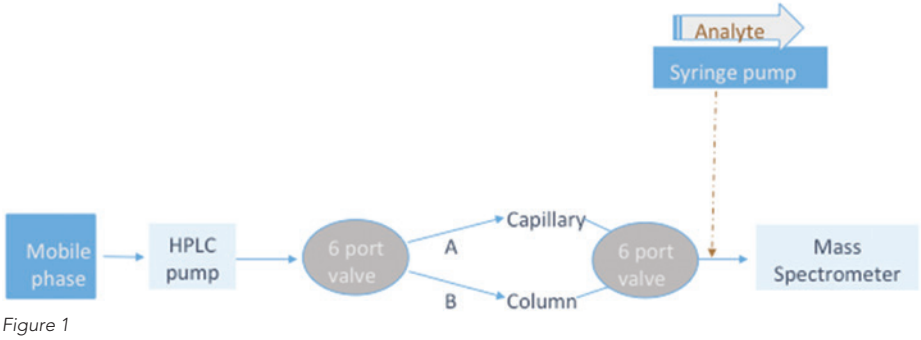

Figure 1

Schematic of the experimental arrangement to determine the effect that the HPLC column has on the qualitative and quantitative performance of the mass spectrometer.

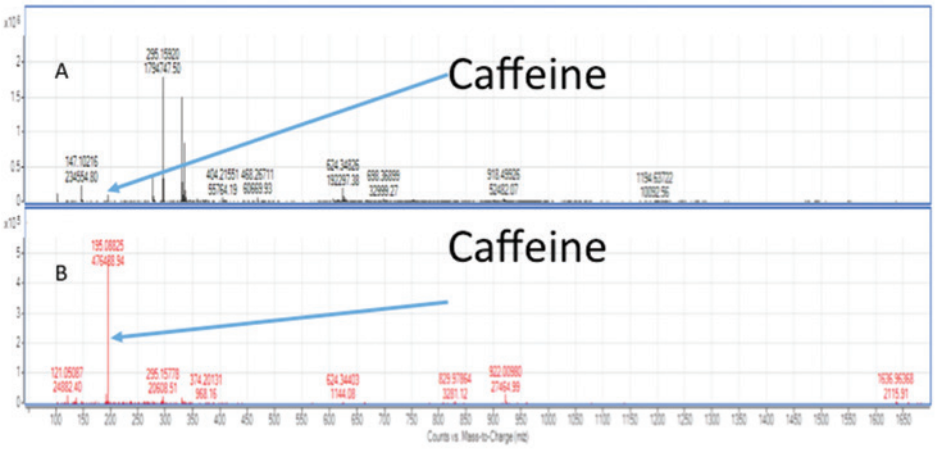

Figure 2

The effect of adding the column to the system, alters both the intensity of the molecular ion for caffeine and the entire mass spectrum.

Top chromatogram (A) The abundance of the caffeine with a column is 97,778 which is 20.5% of the signal as obtained without the column

Bottom Chromatogram (B) abundance of the caffeine peak with no column 476,488

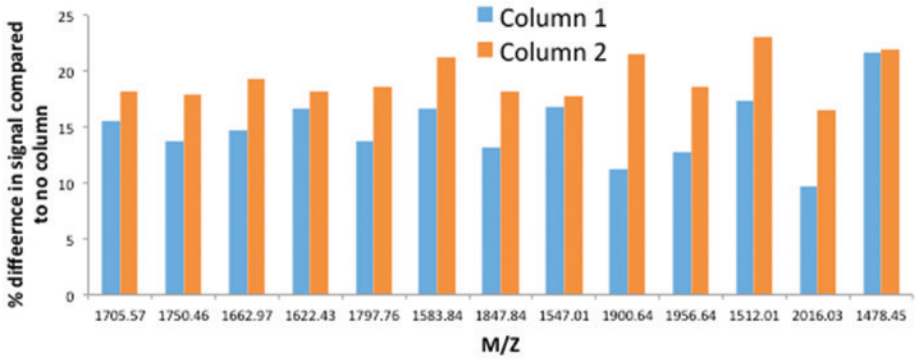

Figure 3

Further experiments, Figure 3, looking at a common protein, bovine serum albumin (BSA), demonstrate the effect that using different columns can have on the relative intensity of individual mass peaks. Figure 3 shows the most intense mass peaks obtained on a Quadrupole – Time of Flight (QToF), comparing the relative amount of suppression observed when using two different columns compared to just the mobile phase. In all cases, there is a drop in the signal compared on the addition of the column to the system, however the absolute difference in signal intensity varies depending on what column is used. The columns used in this case are nominally the same phase but from different manufacturers.

Explanation

The data presented clearly demonstrates that some care needs to be taken when analysing using LC-MS, and that the introduction of the column can have a detrimental effect on the qualitative and the quantitative data. The explanation of why this is occurring is due to column bleed. Closer investigation of the mass spectrum reveals ions are being produced that are not coming from the solvent or from the analyte, and since the difference between the two experimental arrangements is the addition of the column the extra ions have to be coming from the column [4-5]. It is possible to determine the type of column that is being used by looking at the Mass Spectrum and the Helpdesk has found that different columns, both in stationary phase and nominally the same stationary phases but from different manufacturers, have different levels and type of bleed. Although the Helpdesk has not investigated every column, this effect does appear to be widespread, with the mobile phase also affecting the level of column bleed and hence ion suppression.

Conclusion

It is well known that column bleed can and does occur, however the extent to which it occurs and the effect that this can have on the analysis is not often reported for LC-MS. This issue is often overlooked because the coupling of a liquid chromatograph to a mass spectrometer is perceived to be beneficial, and also because most users would not quantify the extent of suppression caused by the column, or indeed try to qualify the ions that are bleeding from the column. In most cases, it probably does not have a significant effect, since a batch of samples will typically be analysed on the same column, however it is clear that the sensitivity of the assay can be affected by using a column that bleeds excessively, and careful selection of an appropriate column could improve the sensitivity of an assay substantially.

Column manufacturers should start to look at this effect and try to address the issue of column bleed, with better bonding of substrates to the base silica or indeed even moving away from using silica as the base substrate, and use more stable substrates that do not bleed. Investigation into the different stationary phases offered by manufacturers already shows that the manufacturing process results not just in perhaps differences in selectivity but also now in differences in sensitivity. The onus is very much on the manufacturers to research into this area and ensure that as the growth of LC coupled to mass spectrometry continues that they provide solutions that can ensure that the separation scientist does not get hampered by the very component that is designed to improve the performance.

References

1. F. Michopoulos, A.M. Edge, Y.T. Hui, T. Liddicoat, G. Theodoridis, I.D. Wilson, Bioanalysis 3 (24), 2747-2755

2. F. Nave, M. J. Cabrita, C. Teixeira da Costa, J. Chrom. A, 2007, 1169 (1-2), 23-30

3. L.A. Berrueta, B. Gallo, F. Vicente, Chromatographia, 1995, 40 (7-8) 474-83

4. Anderson, S. LC-GC North America. Jun2005 Supplement, Vol. 23, p78-78

5. Teutenberg, T., Tuerk, J., Holzhauser, M., Kiffmeyer, T.K,. J. Chromatogr. A. 2006 1119(1):197-201