Reversed-Phase HPLC: Columns, Dimensions and Testing
Part 1: Column selection

Ian Mutton and Sean McCrossen, *IEXA*100 Consulting

SUMMARY

This article provides some general principles to apply when considering the purchase of HPLC columns for use in the laboratory. The types of data obtained from column characterisation experiments are highlighted and the relevance of the data to the intended application is discussed. Part 2 will focus on selection of appropriate testing regimes.

Introduction

It has become a cliché to say that the heart of a good HPLC system or method is the column and it requires a well-designed and properly working instrument. The wrong choice of column will define an upper limit to the quality of analysis obtainable. So the analyst tends to ask "what's the best column for my analysis?" This can be a pretty demanding question. Let's just consider analytical work. There are hundreds if not thousands of commercial C18-type column packing materials out there, some obsolete, some still in use because they're needed for certified/registered methods, some well-established materials, and some exciting new ones. They come in a bewildering array of dimensions: lengths from 10, 20, 30, 50, 75, 100, 150, 250mm, internal diameters (i.d.) of 1.0, 2.0, 2.1, 3.0, 4.6mm, and with particle sizes of 1.5, 1.7, 1.8, 2.0, 2.5, 3.0, 4.0, 5.0 and 10.0µm, for example. The novice could be staring at many thousands of potential commercial reversed-phase (rp) products before deciding where to start. It would be good to be able to do better than just choose from something that happened to be in the drawer. Some general guidelines are now discussed.

Getting Started

That initial question "what's the best column for my analysis?" is more readily answered if the analyst is prepared to identify one that's well-suited to its purpose, rather than fully optimised. The optimum needs will depend much on circumstance. An easy separation of two or three compounds will work on numerous column types, dimensions and experimental conditions. If only a few samples need analysing with easily met requirements for sensitivity, accuracy, precision and specificity, then any number of columns will probably prove adequate and it would be pointless to spend time and money seeking unnecessary improvements. On the other hand, a lab facing a high daily throughput of diverse samples would wish to restrict the diversity of its column stock by carefully selecting an efficient packing material of low residual activity and then optimising the column dimensions and method parameters. Another lab, charged with repetitive analysis of a particular product, will wish to ensure that a column is selected that gives adequate resolution of all compounds of interest, and is sufficiently robust to maintain satisfactory statistical performance. As a general point, column robustness is a feature that is frequently underestimated and not fully evaluated. A high-throughput environment is better served by a column that lasts for 1000 injections and costs twice as much as one that only gives 200 injections before starting to fail as evidenced by loss of efficiency, peak tailing, asymmetry or splitting.

Characterising Columns

There are a number of approaches to characterising columns. Manufacturers normally supply test chromatograms designed to demonstrate compliance with their specifications, and advertising literature is full of applications that show the product in a favourable manner. Databases such as the USP Column Equivalence Application are excellent at showing which column packings will exhibit similar selectivities, and can be used to probe aspects of their performance such as carbon loading, H-bonding character and so forth. However, they will not necessarily enable the user to decide if the selectivity will be suitable for the application in hand, nor to distinguish between other important

characteristcs - efficiency, unwanted activity, robustness - of a group of similar columns. The chromatographic literature is full of tests designed to characterise many aspects of a column's performance and the practitioner can compare efficiencies, hydrophobicity, silanol activity (H-bonding, dipoles, ion exchange), metal contents and shape selectivities etc. This may help the user to rule out some columns, but it should be borne in mind that these tests are often done under conditions designed to show best the feature in question. For example, the silanol activity of packings is often compared at pH 7 where the differences may be seen quite clearly. However, many practitioners prefer to avoid phosphate buffers and work with formic or trifluoroacetic acid modified mobile phases at pHs of 2-3, where basic analytes are ionised or exist as ion pairs, and show much less undesired interaction with silanols. This approach is particularly common in the pharmaceutical industry, where the need to analyse numerous novel structures that often contain basic nitrogenous moieties drives development of fast generic gradients at low pH, often with mass spectral detection. This is particularly so in the drug discovery phase. Specific applications given by manufacturers are of limited impact here, as the user will need an efficient and robust column to maximise the overall probability of separating out most likely contaminants. Later in product development and manufacture, separation of specific known compounds is required, and it may well be that the silanol activity suppressed at low pH can be exploited at a higher pH to provide the desired selectivity.

Thus the important point is that users should develop their own tests to challenge the range of columns that are available. A small set of candidate columns should be selected for this purpose. It will certainly be possible to limit the candidates by reference to the numerous comparative tests documented in the literature. By far the most important residual activity encountered with modern rp columns is unwanted tailing of strong bases. Even at pH 2 this has historically been manifested by a number of features: loss of peak area, asymmetry, and peak tailing. With modern phases, this is less apparent, but inclusion of a small amount of one of the triptylines in a test mix is a critical challenge for all rp column materials, and performance ranking with such a test is easily achieved. Other unwanted activities are much less common under these widely used conditions. For example, residual metals on the surface of the silica or leaching from frits or tubing can interact with chelating analytes. Suitable probes such as hydroxylated quinones or anthraquinones have been used to test for this activity, though it is rare to see poor performance in this test with modern columns. Care must still be taken, however, to ensure there is no potential for system-derived metal to contaminate the clean column.

Efficiency too is an important parameter, and this should be independent of column diameter and proportional to column length. This may not always be so. For many years the standard i.d. of an analytical column was 4.6mm. With the advent of high throughput environments for many applications, many users wished to move to 2mm diameter columns in order to save on solvent purchase and disposal costs. However, due to packing difficulties largely related around wall effects on the more exacting specifications of the smaller bore, the efficiencies of the narrower columns were often not as good as those of their larger counterparts. For example, the isocratic efficiency of naphthalene run in a neutral system with a retention factor of between 5 and 10 can be expected to be between 2 and 2.5 when expressed as reduced plate height h, where h = H/dp, H = L/N and dp, L and N are respectively the particle diameter, column length, and observed number of plates. Often careful measurement in a low dispersion LC with a fast response detector would show this to be true for 4.6mm columns, whereas h for the 2mm version was often 3-3.5. Manufacturers are steadily improving the packing efficiency of 2mm columns so in a good modern product the difference is much less marked. However, the user should characterise the value of h with a simple test at a flow rate close to the van Deemter minimum (e.g.0.5ml/min for a 4.6mm column and 0.1ml/min for one of 2.1mm). Additionally, h will be at a minimum (i.e. most efficient) for columns of intermediate length (50-150mm). Very short columns may show up the effects of dead volume and packing irregularities around the frits, and longer columns can exhibit some minor efficiency loss due to packing difficulties.

The recent trend towards particles of smaller size also influences the column testing strategy. Efficiency improves with decreasing particle size, albeit at the cost of higher pressure, and the flatter van Deemter curves obtained (see **Figure 1**) permit use of less steep (i.e. faster) gradients using shorter columns.

Here the impact of extra-column volume can be quite important and may be similar to that of the liquid in the column (a 20 x 2mm column only contains about 25µl of mobile phase). It then makes sense for one of the application-relevant tests to be a measure of the gradient peak capacity of the system as a whole, since the same column may show a different performance in different instruments that have not all been optimised in every respect of tubing dimensions and detector geometry and detector time constant.

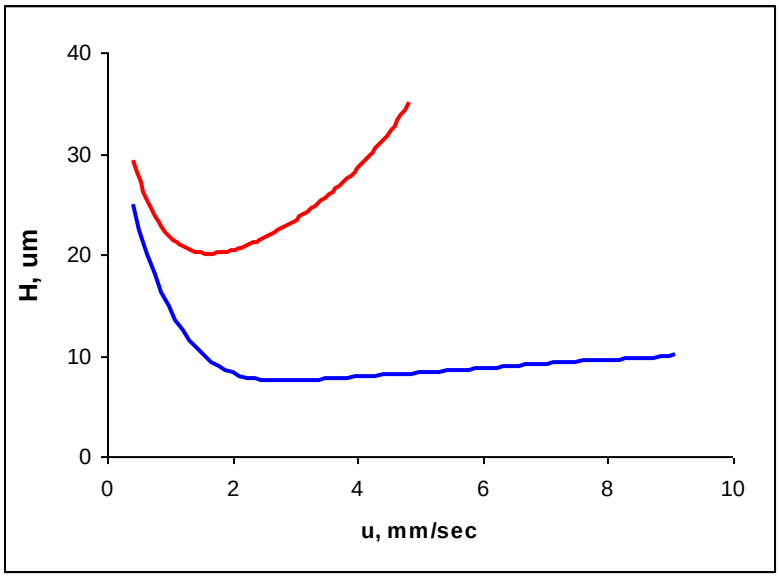

Figure 1: Hypothetical van Deemter Curves for Columns of 10um (Red) and 3um (Blue) C-18 Materials

Conclusion

There are numerous column test procedures available, and all have some relevance to the analyst's task. However, many of the tests are not relevant to the requirements needed in the lab. Users should seek to modify established procedures, taking those aspects from them that are best placed to deliver application-relevant data on efficiency, residual activity, and robustness.

*IEXA*100 is an expert group of chromatography and analytical scientists. Chromatography expertise is the mainstay of the group with broad experience in other analytical areas of spectroscopy and pharmaceutical analysis, including regulation and quality. *IEXA*100 works with end-users, chromatography groups, and manufacturers on diverse projects including patent litigation, training, analysis strategy and troubleshooting analysis issues. Particular expertises cover HPLC column testing, characterisation and the application to method development and validation.
www.iexa100.co.uk
info@iexa100.co.uk