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The LCGC Blog: HPLC Column Selection - Are You Barking Up the Right Tree?

By Tony Taylor

How do you choose your HPLC columns? I guess the answer to this varies widely and I've seen many strategies being employed including; whatever is on the instrument, first one out of the column drawer, the one that was successful for my last development, from a publication or application, my favourite column from my favourite supplier, we have a set of orthogonal chemistries on our automated column screening system – the list is long.

Choosing the correct column for an application really requires that we consider the many factors which can influence the efficiency and selectivity of a separation including column hardware, support and surface chemistry. On a rainy day a little while ago our technical support group came up with a list of 23 such factors which need to be considered.

But let's return to the original question – how DO you select HPLC columns? Traditionally we may have considered the LogP or LogD of the molecule and perhaps the pKa to give us an indication of the hydrophobicity of the phase required to retain and hopefully separate our analytes. But there really are so many factors and such a wide diversity of columns that this isn't really a scientific approach. Further, I've seen hours and hours of development time wasted in trying to optimise the eluent composition to derive or optimise a separation when the phase chosen is truly unsuitable for the separation at hand and no amount of 'tinkering' or even highly sophisticated computer optimisation will result in a satisfactory separation. So choosing the right column is fundamentally important to our work - when would one use a phenyl-hexyl column, or a pentfluorophenyl phase, or an 'aq' designated phase or a polar embedded ligand. I'm sure you have seen articles written on the fundamental differences in selectivity obtained by a range of columns all designated as being 'C18' and all classified as L7 under the general USP description.

So – is there a more scientific or even better a fast track approach to being able to make more informed HPLC column choices given that we may know the analyte structure(s) or a little about the physico-chemical properties. Or maybe we have tried a column for method development which didn't work out and we need something different (orthogonal). Or we are investigating the specificity of a method for validation and need to prove that we have separated all components of the sample mixture using different modes of analyte / stationary phase interaction.

Well – the major factors which influence analyte / stationary phase interactions and control retention and selectivity are fairly well understood. And, good news, there aren't too many of them and they aren't too difficult to understand. More good news, there are one or two commercially independent bodies which have used test probes to characterise a wide range of columns to help us understand in which type of application they may be useful

Of the many notable groups who have carried out work on column characterisation Tanaka et. al. (1), Euerby and Petterson (2) and Snyder et. al. (3) are those to which I've had particular exposure. I've given reference to their 'seminal' works here, however these can act as a springboard into much wider reading should you have an interest. Of all of these approaches, I personally find the work based on the work of the Hydrophobic Subtraction

model of most utility and helpfully, this model has been adopted by the Product Quality Research Institute (PQRI) in building what (to my knowledge) is the largest independent database of column characteristics available today which can be found on the USP website at the following link http://www.usp.org/app/USPNF/columns.html (note that it's the PQRI database of columns rather than the USP database to which I refer the following explanation).

The Hydrophobic Subtraction model uses various test probe compounds to measure retention or selectivity which indicate the relative extent to which each stationary phase demonstrates a particular property. A full description of each probe is beyond the scope of this discussion – however further discussion on the important properties which are demonstrated is warranted. I've summarise below the descriptors which can be found in the PQRI database and their inference in predicting the performance of a stationary phase.

(H) Hydrophobicity - a measure of the ability of the stationary phase to retain hydrophobic compounds. Traditionally 'carbon loading' was used as a measure of this parameter, but this takes no account of several key effects including efficiency of ligand bonding process, ligand density to surface area ratio, degree and type of end capping etc. The PQRI 'H' value provides a far more quantifiable practical value to this critical feature and provides a measure of 'hydrophobic' retention, but unfortunately, unlike other databases, doesn't include a measure of the ability to separate analytes whose hydrophobicity is similar (typically differing only by one methylene unit).

(S) Steric resistance or shape effect - the ability of the stationary phase to discriminate between molecules with different 3D 'shape' in solution and hydrodynamic (Stokes) volume. This can have a significant effect on analyte selectivity due to the importance of inclusion or exclusion from narrow pores within the silica substrate, or more importantly, between the bonded phase ligands on the silica surface. The S value tends to increase with longer stationary phase ligands or more densely bonded phases, as is intuitively obvious from the preceding discussion. In some cases for example, this parameter can be exploited to allow resolution of isomers or molecules of different chain length.

(A) Hydrogen Bond Acidity – a measure of the level of analyte secondary interaction via low activity (energy) non-ionised silanol groups on the support surface or functional groups on the bonded phase ligand. This influences selectivity, particularly with retention and separation of weakly basic molecules, without suffering excessive peak tailing.

(B) Hydrogen Bond Basicity – a measure of the phase's ability to hydrogen bond with weakly acidic analytes which is proposed to be via water sorbed within the stationary phase structure. This is also a desirable feature built into certain stationary phases (e.g. polar embedded phases), which promote water adsorption, to provide significantly modified selectivity. Larger 'B' values can have considerable effects in enhancing retention and selectivity of weakly acidic analytes.

C (2.8) Silanol Ionisation at pH 2.8. – a measure of the ionic interactions between the analyte and silanol or other groups capable of ionic interactions on the solute surface at pH 2.8. At this pH, all residual silanol groups should be in the less active (non-ionised , vicinal) form. Any acidic silanols that have been activated (e.g. by metal ions in the stationary phase support) remain charged and can have significantly detrimental effects, particularly giving rise to excessive tailing of bases. More traditional phases (based on type I (A) silica) tend to have high C (2.8) values, with resulting poor peak shape, due to the higher degree of acidic silanol groups present. It should be noted here that ionic interactions can play a major role in defining the selectivity of a stationary phase, especially with regard to the separation of analytes which have a common structural scaffold but differing functional group chemistry. If the column possess this ability whilst maintaining good peak shape for polar and ionisable compounds, this can be significantly advantageous.

C (7.0) Silanol Ionisation at pH 7.0 - a measure of the ionic interactions between the analyte and sinlanol or other groups capable of ionic interactions on the solute surface at pH 7.0. At this pH, all residual silanol groups should be fully ionized and the C (7.0) value gives a measure of the total amount of ionized silanol groups available for secondary interaction with analytes. High values indicate that the degree of effective end-capping is low or that the silica surface is higher energy, containing a higher degree of lone (acidic) silanol groups. Whilst this may be seen as disadvantageous in terms of peak shape, a higher degree of ionic interaction with an analyte may result in a favourable outcome in terms of the selectivity between ionisable analytes.

So – how can all of this be practically useful? Well – it may take some time for you to fully understand the inference for each of the terms outlined above, but Figure 1 shows a simple way in which these parameters can be visualised to usefully compare columns. I prefer this approach to the mathematical model which is often used to describe a 'column difference factor' in which all terms are combined to compare the overall similarity or

orthogonality of two phases in a single number.

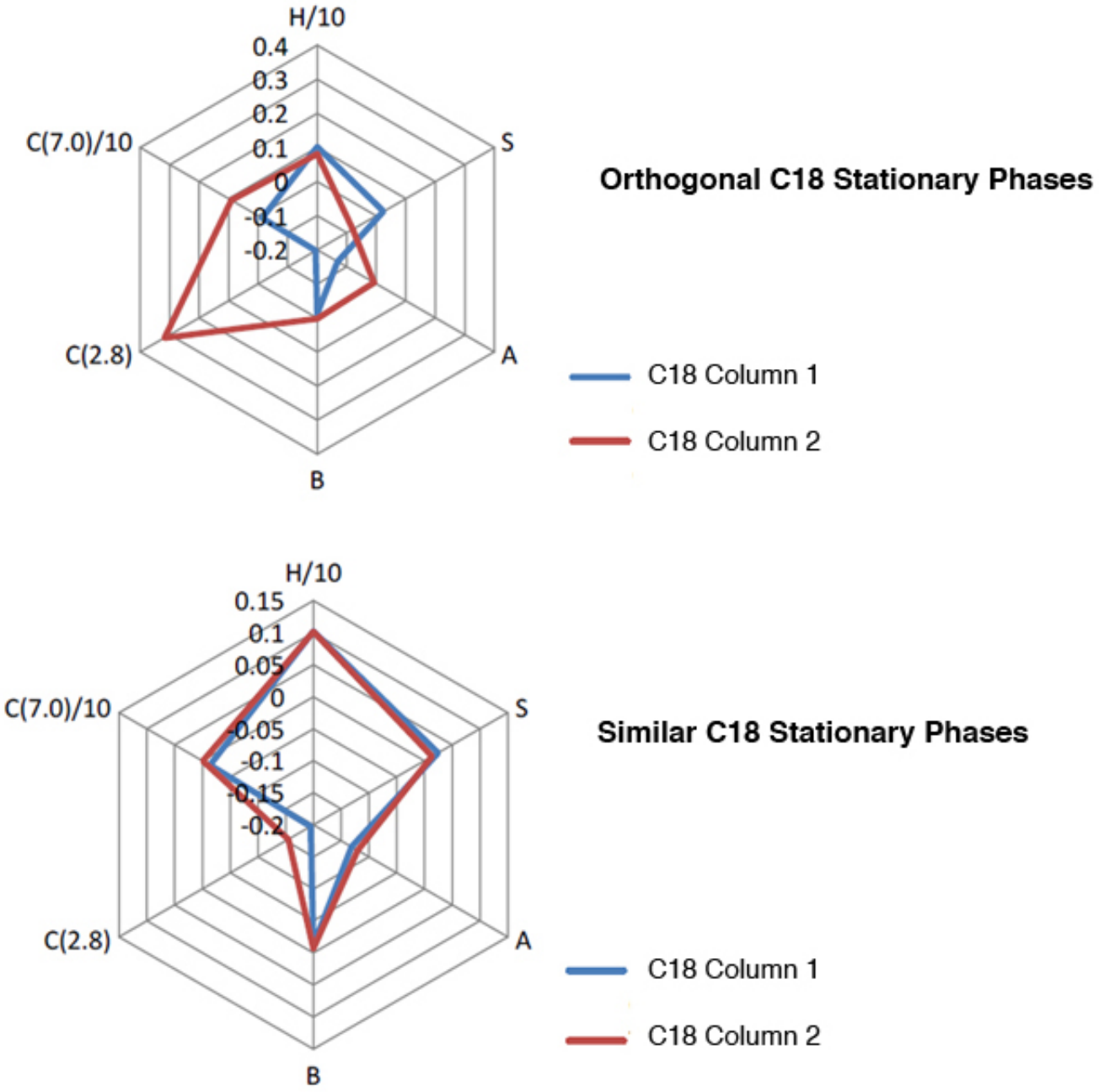

Figure 1: Radar plots of PQRI column classification data showing similar and orthogonal C18 phases

So from Figure 1, if my supply of Column 1 had somehow been interrupted and I needed to find a very similar phase, a search through the database would reveal that Column 3 would be highly likely to give me similar chromatography, given that all the major descriptors of retention and selectivity are virtually overlaid on the radar diagram. Further, if I'd been unsuccessful in developing a method using Column 1, and I wanted to use a C18 column, I would consider Column3 as being a suitable (orthogonal) alternative. I could also tell from the plot that Column 2 would be likely to differentiate analytes based on polar functional groups that may differ between analytes, without giving peak tailing, but it would be less shape selective than column 1. With use and experience, it is possible to become familiar with the descriptors such that a correlation between each individual parameter and analyte structure / properties is possible, so that a column with a particularly dominant shape selectivity factor, for example, can be sought for method development.

At the very least, I suggest that you take a look at the database and see what values are thrown up for your favourite columns. I can guarantee that this will bring an enlightenment regarding why a particular column works for your particular application. And just maybe, the next time you need to select a column, you may visit the column database first, just to see if you really are barking up the right tree – and more importantly, you know why!

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For more tutorials on LC, GC, or MS, or to try a free LC or GC troubleshooting tool, please visit www.chromacademy.com

References

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