A Path to Greater Productivity in HPLC

The options facing the chromatographer have multiplied dramatically over the past few years. UHPLC, short fast columns, micro-bore, etc are all possible directions in which to move from the conventional HPLC column. Factor in the need to scale up for semi preparative or preparative use and the options increase again.

In order to make the most cost effective choice, the whole process should be considered to determine the requirements to move from the current position to the required goal. All the available options need to be taken into account to reach the desired chromatographic level in the most cost effective way. The variables of column length, internal diameter, flow rate, particle size and LC system availability should be considered along with temperature and column media options to optimise selectivity and reduce run time. There is a big difference between a 25cm x 4.6mm C18 column run isocratically on a standard HPLC system and a 2cm x 1mm column packed with sub 2µm specialist media run on a UHPLC column with a ballistic gradient or using 10µm media in a 50mm id preparative column.

This article will point the way to the best and most cost effective path to greater productivity in HPLC for an individual analyst or group.

"The options facing the chromatographer have multiplied dramatically over the past few years. UHPLC, short fast columns, micro-bore, etc are all possible directions in which to move from the conventional HPLC column"

Authors Details:

Dr Neil Herbert and Dr Ian Chappell
Grace Davison Discovery Sciences
Carnforth, United Kingdom
www.discoverysciences.com

INTRODUCTION

In today's busy world there are many challenges facing chromatographers who are asked to do more with less, and we need to rise to this challenge. The need for greater productivity leads us to look at faster analyses. The analysis of ever more complex samples leads to the need for greater resolution, and to be more cost effective leads us to reduce costs and spending without compromising quality or reproducibility. We need to look for products or protocols which will produce better results in less time with less cost and, if required, be capable of scaling up to the preparative level.

If we look at our requirements in chromatographic terms, there is often a need to maintain or improve the chromatogram fingerprint, to maintain specified or satisfactory peak resolution and to live within the pressure confines of the HPLC system. We may also need to increase the number of analyses per day or reduce work time, and maybe process more preparative samples. Finally, we need to avoid the need for method development when transitioning between scales and live within the confines of the budget.

Awareness of potential future requirements often dictates our best course of action. Will there be a future prep requirement? Will we need rapid on-line analysis? Will we need UHPLC for very high efficiency impurity profiles and so on?

Different column lengths, ids, flow rates, particle size, mobile phases, temperature, media and equipment need to be considered to make the best choice.

Let us refresh our memory on basic parameters and consider Resolution, Rs,

Resolution $$Rs = 1/4\sqrt{N} \cdot \frac{(\alpha - 1)}{\alpha} \cdot \frac{k'}{(1 + k')}$$

Note the square root function of N

Where: α = Selectivity = k'2/k'1
N = Column Efficiency
k' = Retention Factor

The resolution can be increased by optimising N, α and / or k'.

Changing the selectivity (α) is the most effective approach for a pair of poorly resolved compounds after having first optimised retention. For very complex mixtures, it is often better to increase column efficiency as then the improvement in resolution applies to all peaks in the sample.

Selectivity assumes importance in two scenarios that face the chromatographer:

- First, how do I get a better selectivity to the one I have at present
- Second, how do I get the same selectivity when I change particle size?

In the former case, the maximum selectivity change from the current unsatisfactory column is gained by using a column with orthogonal selectivity. For instance, if a conventional, fully bonded and end-capped C18 column is used initially, then a column with Enhanced Polar Selectivity may be chosen to obtain the maximum change. Typically these materials have a lower carbon load, which reduces the retention of non-polar molecules, but an elevated level of inert silanols, which increase the retention of polar molecules.

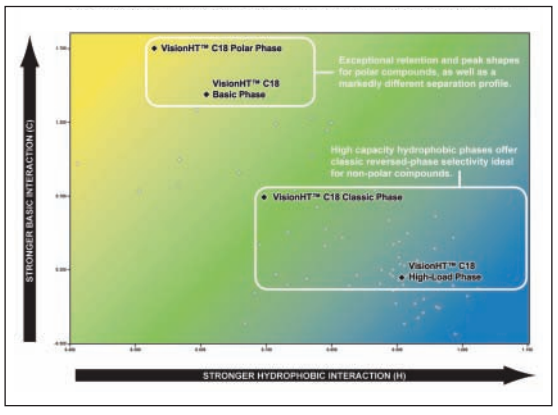

Figure 1. VisionHT™ Reversed-Phase Media Spans the Full Polarity Spectrum.

A column selectivity plot for one commercially available range is shown in *Figure 1* with a chromatographic example in *Figure 2*. By choosing a column in a different part of the graph, the chances of obtaining a more favorable selectivity change and an increase in resolution are greatly increased. This data is generated using a standard set of protocols. The Snyder / Dolan / Carr protocols are one such set[1]. They use a range of analytes to determine the key phase parameters of hydrophobicity, shape selectivity, hydrogen bond acidity, hydrogen bond basicity, and ion exchange characteristics at pH 2.8 and 7.0. The values refer to selectivity only and are a characteristic of the phase. *Figure 3* shows the selectivity difference of a family of different C18 bonded phases using these protocols.

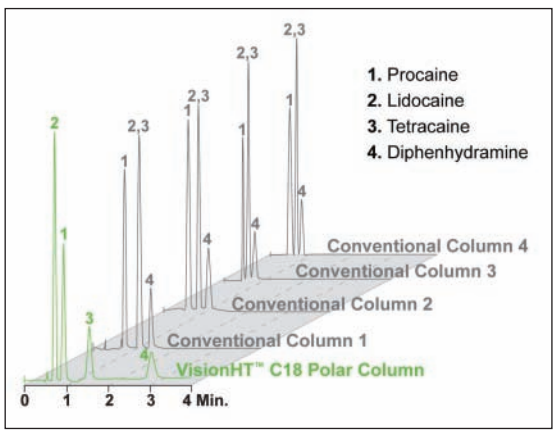

Figure 2. VisionHT™ C18 Polar Phase Vs Fully Bonded and End-Capped C18s.

In the second case, for each phase, uniform media using the same base silica and identical bonding technology for all particle sizes should be used.

Historically however, changes in specification, for ostensibly identical bonded silica's of varying particle size, have been seen. *Figure 4* shows the chromatographic selectivity behaviour of the VisionHT™ C18 Basic phase with different particle sizes. It can be seen that reproducibility is very good for the various parameters. This means that one can transition up and down in scale and particle size with the confidence that selectivity will not change.

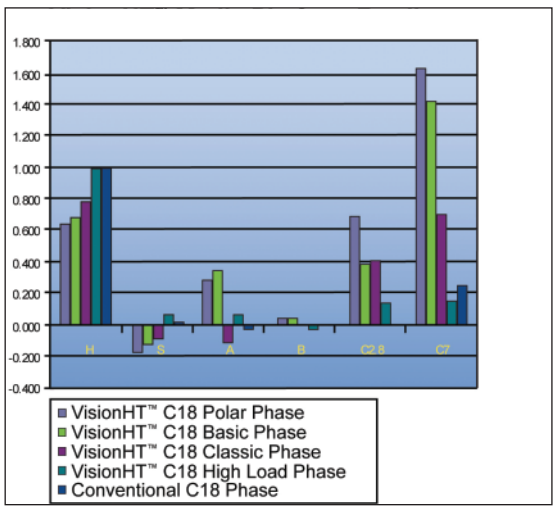

Figure 3. Snyder-Dolan-Carr Coefficients for VisionHT™ Media Platform Family.

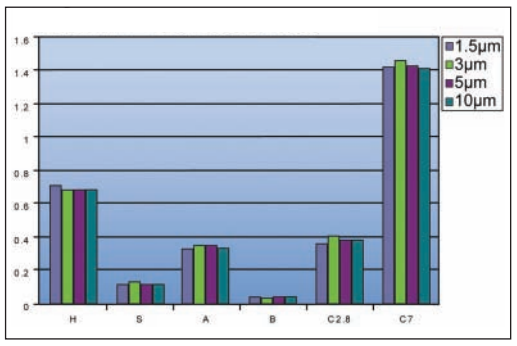

Figure 4. Snyder-Dolan-Carr Coefficients for VisionHT™ C18 Basic Phase.

Coupled with a family of silica phases of differing particle size, there is a requirement for a range of column configurations to best suit the key areas of application. In *Table 1*, we have assumed that there is a critical pair of components, from a resolution standpoint and we used the values shown.
The minimum required resolution is defined as 1.9 for a robust method. The plates required to perform our separation were calculated to be 10,064 per column from the Resolution equation. The table shows a selection of our analytical options.

Note that where the id has been changed the flow rate has been adjusted to maintain the same linear velocity as the 4.6mm id column. Pressures are calculated based on the 25cm x 4.6mm, 5µm example.

Various column options can give the same efficiency and resolution. The reduction in run times and increase in column back pressure should be noted when we use small particle sizes and enhanced flow rates. This can be offset, to some extent, by a reduction in column length. Small particles give their optimum efficiency at higher flow rates and, more importantly, particularly for sub 2µm materials, the Van Deemter profile is much flatter, allowing much faster flow rates to be used without sacrificing column performance. The down side however, is high back pressure. Above 6,000 psi we need a high pressure HPLC and above 10,000 psi a UHPLC system.

If a column heater is available, temperature can be used to reduce back pressure by reducing solvent viscosity. It can also improve column efficiency and change selectivity.

In *Table 1* we used a selectivity value of 1.1; if we change our selectivity value by changing mobile phase conditions or using a different phase chemistry, these parameters change and *Table 2* shows other options.

Table 2. Effect of Selectivity on Required Column Efficiency

Defined Values	
h =	2.5
Retention Factor	5
Resolution	1.9
Particle Size	5µm
Selectivity	Required Column Efficiency
1.5	749
1.3	1562
1.2	2994
1.15	4889
1.1	10064
1.05	36680
1.01	848462

Table 3. Effect of Column Length on Column Efficiency

Column Length	Plates / Column
250mm	20,000
150mm	12,000
100mm	8,000
50mm	4,000
30mm	2,400
20mm	1,600
10mm	800

Where α becomes smaller, selectivity is reduced and life becomes much harder; but where it increases, much shorter columns can be used with reduced run times and lower pressures. *Table 3* shows the effect of column length on available plates per column.
Mixing data from *Tables 1, 2* and *3* to give tables directly relevant to ones own situation can be a valuable exercise.

It is an advantage in all these deliberations if the same phases are offered in all the particle sizes to enable easy transition between HPLC methodologies from UHPLC to Analytical to Preparative Column format examples are shown in Figure 5.

Because of the range of HPLC equipment, there is a need to transition from one type of system to another to make optimum use of the advantages of each type. The availability of a single family of identical phases across the particle size range makes this an easy task. Both isocratic and gradient transitions can be performed. Additional considerations are:

1. Change in Column ID; we need to maintain linear velocity down the column.

2. The use of smaller particles leads to an increase in column efficiency per unit length. To maintain column efficiency for the assay, the column length may be reduced, resulting in reduced run times.

3. Adjust Injection Volume to prevent column overload.

4. Adjust Gradient times to suit the new column dimensions. When we move from isocratic systems to a gradient situation, the gradient segment times need to be reduced as a function of column length.

5. Factor in the Extra-Column Dead volumes so that we are considering equivalent systems. These are the dead volumes associated with the injector, connecting tubing, fittings and detector. Also, in gradient systems, we need to take the system dwell volume into account (volume from the gradient mixer to the top of the column).

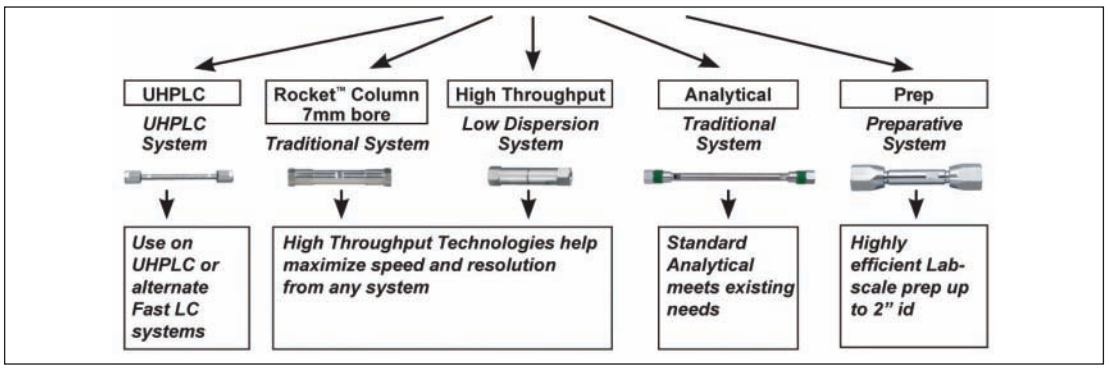

Figure 5. VisionHT™ Media Platform - Available Column Formats.

Table 1. Alternative Column Options for Similar Resolution

Defined Values							
h =	2.5						
Selectivity	1.1						
Retention Factor	5						
Resolution	1.9						
Plates Required / Column	10064						
Run Time, 25cm column, A	15 mins						
Plates / Column Available	Length	I.D.	Particle Size	Flow	Flow Equiv 4.6mm I.D.	Run Time	Pressure Drop Based on A
10,000	250mm	4.6mm	10µm	1mL/min	1	15 mins	400psi
20,000	250mm	4.6mm	5µm	1mL/min	1	15 mins	1600psi
12,000	150mm	4.6mm	5µm	1mL/min	1	9 mins	960psi
13,333	100mm	4.6mm	3µm	1mL/min	1	6 mins	1778psi
13,333	50mm	4.6mm	1.5µm	1mL/min	1	3 mins	3556psi
13,333	50mm	7mm	1.5µm	2.3mL/min	1	3 mins	3556psi
12,000	150mm	4.6mm	5µm	2mL/min	2	4.5 mins	1920psi
13,333	100mm	4.6mm	3µm	2mL/min	2	3 mins	3556psi
13,333	50mm	4.6mm	1.5µm	2mL/min	2	1.5 mins	7111psi
13,333	50mm	4.6mm	1.5µm	3mL/min	3	1 mins	10667psi
13,333	50mm	2mm	1.5µm	0.57mL/min	3	1 mins	10667psi
13,333	50mm	1mm	1.5µm	0.14mL/min	3	1 mins	10667psi

In conclusion, there is an optimum column for a given application which takes into consideration column packing, run conditions, and hardware configuration. We need to know exactly what we need to do, and be keenly aware of what systems / resources are available. We can then choose the optimum column and LC conditions to fulfil these requirements. We need to move in stages to the ultimate goal – although the posts may have moved by that time!
Good luck.

REFERENCES.

[1] L. R. Snyder, J. W. Dolan, P. W. Carr, J Chrom A, 1060 (2004), 77-116

Note:

A more detailed version of this paper can be obtained by contacting Neil Herbert at neil.herbert@grace.com.

New Chromatography Product Pages

The integration of new **Viscotek** product pages within the **Malvern** website highlights how gel permeation chromatography/size exclusion chromatography (GPC/SEC) technology enhances the company's ability to deliver insightful, information-rich material characterisation solutions for the broadest range of industries.

Market-leading Viscotek GPC/SEC systems provide size and absolute molecular weight distribution, structural information and, for copolymers/conjugated proteins, compositional data, for a diverse range of natural and synthetic polymers, bio-molecules and proteins.
For protein scientists the fit with Malvern's Zetasizer dynamic light scattering technology is especially productive, while for the polymer industry, synergy with the rheology portfolio brings added value. Application experts support users in exploiting complementary techniques to maximise the return on analytical spend.

Far more than simply a show case for the company's extensive range of analytical instrumentation, the Malvern website has a collection of industry pages that illustrate how different techniques address the issues of specific user groups. It is also the hub that underpins the company's exemplary customer support. With a comprehensive webinar programme, brainshark presentations that explain and educate, and case studies that demonstrate deliverable benefits Malvern leads the way in application focused service. Testimonials relating to every aspect of the company's performance provide compelling evidence of the success of this approach.