Is the Art of HPLC Method Development Dead?

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Please believe me when I say that I take no pleasure in asking this question but I feel I must given the experience I had during my ten years working as a method developer in the pharmaceutical industry and last six years as a professional trainer in analytical chemistry.

For me, the main benefit of (reversed phase) HPLC is its selectivity – hydrophobic (non-polar), hydrophilic (polar), ionisable and ionic compounds can all be separated (under certain conditions albeit rarely at the same time). Very similar compounds that only differ in fairly subtle aspects can be relatively easily separated, identified and then quantified. The reasons behind this almost universal applicability are the many factors that can be adjusted in order to affect how a particular analyte will interact with both the stationary and mobile phase. A few factors that can be manipulated in order to affect a specific analytes retention are; polarity and composition of modifying ligand, surface coverage, endcapping, silanol confirmation, monomeric *vs.* polymeric ligand binding of the stationary phase and polarity, type of organic modifier, pH, ionic concentration, temperature for the mobile phase to name but a few. It had been thought (hoped) that with all the variables that could be controlled around the mobile phase that we would not see the proliferation of stationary phases that has blighted GC – where only stationary phase type and temperature can be manipulated to adjust selectivity. As shown in *Figure 1* resolution, the ultimate goal and in most cases pre-requisite of any chromatographic separation, is the culmination of retention factor (*k*), efficiency (N) and selectivity (α).

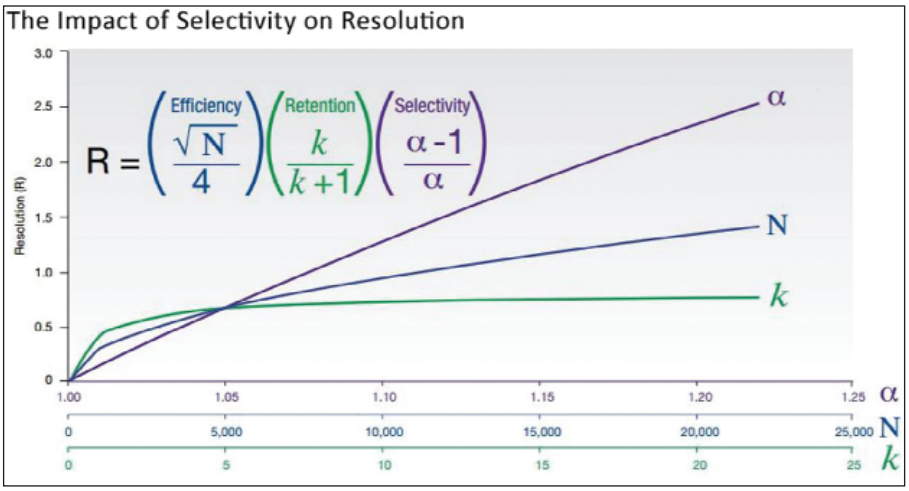

Figure 1 – The Purnell or Fundamental Resolution Equation and Graphical Representations of Each Factor's Overall Effect on Resolution.

At very low values retention factor has the largest impact on resolution but at even very modest values this becomes negligible with efficiency, but predominately selectivity, being the main driver.

The huge inherent efficiency which benefits capillary GC means that even very similar compounds, in terms of volatility or polarity, that posses similar selectivity values can be fully resolved from one another purely down to their efficiencies. A great many GC separations could be carried out on any number of different stationary phases. Even very modern UHPLC separations using state of the art instrumentation and column technologies cannot match the efficiencies enjoyed in GC and therefore a greater emphasis is placed on selectivity. Eli Grushka, then at the State University of New York at Buffalo who in 1974 would seem almost visionary now - discouraging the proliferation of stationary phases; rather than producing many phases all with a particular separation in mind, encouraging the understanding of a few phases that could be utilised for multiple separations [1]. The 1000's of reversed phase columns that are currently available bear testament to his visage not coming true. The vast numbers of stationary phases currently available need to be further characterised and better designated – although there has been recent advances from manufacturers of HPLC columns to adopt are more transparent and industry accepted approach to classifying stationary phases. The Hydrophobic Subtraction Model [2] proposed by Snyder, Dolan and Carr seems to be generating significant interest and is being reviewed by the USP (PQRI approach) currently – over 650 columns, and counting, have been tested, characterised and added to this database. An alternative is the principal component analysis approach postulated by Euerby and Petersson [3-5] based on earlier work by Tanaka [6]. These are just two amongst many approaches which have been proposed to better understand selectivity in reversed phase chromatography and to get an idea which mechanisms are most accurate requires input from ALL chromatographers.

The best definition I have ever heard for defining method development was 'optimum resolution in the minimum time'. Method development is simply the process of making logical and informed changes to a methods conditions based on current knowledge and sound science – essentially a logical and structured 'suck and see' approach. Having worked in method development groups for a number of years and at various organisations, both in the public and private sector, most

method developers, and I include myself, will install their favourite C_{18} column and run a gradient from low organic to high organic at a low pH – usually in an unbuffered mobile phase. When this initial 'method development' does not work, in terms of resolution, peak shape or sensitivity, my heart sinks to think of the number of times analysts have reached into the column drawer or got on the phone to our favourite supplier when our tried and tested column doesn't provide the selectivity we need. Given the vast number of options available to us in order to interrupt and affect particular analytes phase preference why do we not try these in the first instance – especially when they are so much quicker and convenient, not to mention cheaper and when we the mixture in hand contains a number of similar compounds. When I started out in method development roles I can count on one hand the number of times I really looked at my analytes before my initial screening run and selected the most appropriate column and mobile phase pH unless it was glaringly obvious. There are even fewer instances where I have looked to swap my low UV absorbing and low viscosity acetonitrile for methanol; even when prices were soaring.

So, back to the my original question, "why do I have such a downhearted view of the current state of HPLC method development in mainstream laboratories";

1) As I have mentioned above, I feel there is a real lack of understanding and this is compounded by an environment where people see knowledge as power and do not pass on the fruits of their wisdom. More commonly, the senior analysts do not possess the understanding and then go onto to confuse the junior, eager to learn members of staff with fanciful tales of exotic interactions in order to cover up their own knowledge gap. It takes a brave scientist to put their head above the parapet and ask for a theory to be explained to them. As scientists we seem to see asking for things to be explained to us a sign of weakness and somehow indicating that we do not possess the correct knowledge for someone operating in a method development role all this time. The longer we are working in a method development environment the harder it is to ask for concepts to be explained. When I train people in method development I start off by asking what things we can do to affect the analytes phase preference and I usually get some pretty good answers, most of them centred around the polarity of the mobile phase and stationary phase. I then follow up this by asking someone to define polarity for me – in most instances I am stared back at blankly until usually it is the newest, most junior member of staff who volunteers the correct answer, being able to recall their university / college days more readily. I do not ask this question to intimidate people or put them on the spot but merely to show the concepts behind chromatographic retention are based on sound scientific principles which are fairly basic.... when understood and properly explained. Forums are great places for people to ask questions, anonymously if they chose, of their peers without fear of ridicule or being shown up as a charlatan, masquerading in a method development role. There are also various on-line learning tools for people to develop their understanding from whatever starting point they choose at their own pace, and, if they choose, at their leisure.

2) Many companies (initially large pharmaceutical companies from my experience but now this is expanding into most other industry sectors too) have method development systems that incorporate column switching valves, a quaternary pump and are connected to chromatographic modelling software. All the method developer needs to do is vial up the solution and press go. The sample is then screened at a few preselected pHs, sometimes with different organic modifiers, over a mobile phase gradient and this is then repeated through a number of preselected columns. The data is then fed back into the modelling software which will predict the optimum conditions for the separation. Whilst I am always impressed with automation of this process it does leave me wondering what science the method developers in these groups actually do. Surely those of us still interested in the science will look for a role that will challenge us and we will be replaced with people who are satisfied to prepare solutions and press start. I am not belittling this role as it often leads to more robust and better methods being developed in a shorter period of time but what happens when the preselected pHs and columns do not provide the selectivity needed – this is when you need the experienced method developers but they have either moved on for new challenges or have become rusty as they practice the art so infrequently.

3) My main reason for some of the shoddy methods I developed was due to the time constraints given to me by my manager, which were taken from the overall company strategy which seemed to have been put in place to satisfy the shareholders. Sometimes it was an excuse, I admit, but in most instances it was because I had to develop a validation ready method containing a host of related and retained substances from the manufacturing process, degradation products and post formulation matrix in under a week. I invariably found that managers didn't appreciate that not all methods would take the same amount of time to develop – 'The last method only took you a couple of days, why is this one taking so much longer?' was a rhetorical argument thrown back at me on numerous occasions. It stands to reason that the more time spent developing a method, the better chance it has of standing the test of time. An extra week or two in the method development phase is inconsequential compared to the time it would take to re-develop the method post validation, especially if the method had been filed with a regulatory body. My latter experience of Pharmaceutical Development was a prime example of time being a major factor on both, the quality of the methods developed and the calibre of people attracted to my method development group. We had adopted a milestone driven submission strategy where certain critical points had to be met at predefined time periods, irrespective of the potential impact on the quality of the method.

4) The last reason for my assumption that the art of method development is dying is due to the advent of high efficiency columns. This may sound a little contradictory, but now much higher efficiency separations are capable by employing sub- 2µm fully porous particles or modern core-shell particles of varying diameters (1 - 5µm), we seem to be relying on super efficiencies to separate complex mixtures and forgetting about selectivity. Let us not forgot that the main driver behind resolution, above a very moderate retention factor (***k***), is selectivity. If I was still practicing method development on a regular basis I'm sure my strategy would have only differed in that I would be now employing my favourite C_{18} but in a smaller particle size or as a core-shell. Let us not forget that these advances should come as no real surprise as Martin and Synge [7] described how increased efficiencies could be enjoyed by reducing the size of the

particle in their pivotal plate theory paper over 70 years ago. Core-shell (then called pellicular) particles, and their benefits, were first described by Hovarth and Lipsky [8,9] over 50 years ago (there is still some debate as to exact reason for their higher efficiencies but the benefits are real and can be enjoyed by anyone – even those of us without the budget for UHPLC instrumentation [10]).

In summary I am, dare I say it, excited about the amount of work being undertaken to more accurately understand the vast number of stationary phases we currently have. With a better understanding we will be able to optimise our column selection strategies and at the very least make an informed choice as to which stationary phase to choose next after our favoured C_{18} has failed us. I am also pleased to see the continued trend, in the main, where the majority of new columns introduced are designed for specific applications such as proteins/peptides and other bio-molecules, PAHs and so forth and not just 'me too' phases clogging up an already congested column marketplace. I am slightly concerned about the knowledge which currently seems to being lost from method development groups as people rely on automation/modelling software and increases in efficiencies to solve all resolution problems. In addition, the current trend seems to be move away from method development groups and have a few skilled scientists working cross-functionally – whilst there are clear project based benefits to this approach I can not help to think that in isolation certain skills will become a little esoteric and I for one always benefited form bouncing ideas off others. Contrary to how it may have come across I am a huge fan of high efficiency stationary phases and in particular how the (re-)introduction of core-shell particles has brought high efficiency separations to the mass market who cannot afford to upgrade all their conventional HPLC instruments. I am not some Luddite who shuns all advances, I am just a firm believer that we should all have at least a basic understanding of what is happening in our magical cream or black box, especially the column, and our role should not purely be to prepare samples and standards and place them into the vial rack. I sincerely hope we do not follow the well trodden path of automotive industry whereby car engines are now shrouded in matt plastic covers and the days of actually seeing the internal workings, and therefore garnering a limited understanding and interest in how they work are a thing of the past. All I ask from column manufacturers is that selectivity is not forgotten – I am pleased to see that most manufacturers who supply the popular hybrid pH resistant, core-shell and sub 2µm stationary phases offer much more than a C_{18} or C_8.

So, to answer my title question, do I think that the art of HPLC method development is dead? No, it's fair to say that it's not in the best shape but I still encounter enough chromatographers who share my passion and quest for knowledge and understanding of the subject to leave me feeling we're not in dire-straits just yet. Working as an analytical method development chemist is not purely a job title and you do not suddenly become well versed in the practice and understanding of method development by moving to the function. You learn from experience, trial and error and from ensuring you know enough about your subject to make these logical and pragmatic decisions which essentially all method development is. As to its condition in 5 or 10 years, let's wait and see. I would like to finish with a request / challenge, next time you're developing a method, or even just running you next routine analysis, have a look at your analytes structure and think if the C18 column you have in your hand is the best stationary phase for the separation…. and… if in doubt….. ask.

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