Separation Focus

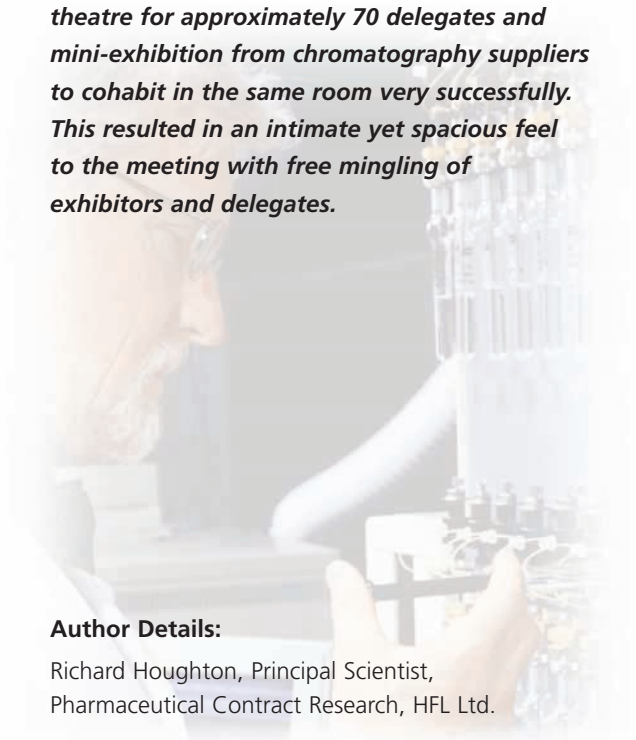
IMPACT OF SEPARATION SCIENCE ON PHARMACEUTICAL R&D AND BEYOND
(15TH NOVEMBER 2007, SUNDERLAND)

Richard Houghton

In November 2004, the Chromatographic Society staged a 2-day symposium entitled "Impact of Separation Sciences upon Pharmaceutical R&D" held at the GSK Research laboratories at New Frontiers Science Park, Harlow. The analytical issues cited, at the time, included the need for techniques that were robust, quantitative, sensitive and allowed short analysis times to cope with the increasing number of samples. The subject of several of the talks described the application of Turbulent flow chromatography and Capillary Electrophoresis. In more conventional HPLC separations, the theme was on the use of short narrow bore columns to improve peak capacity and speed of analysis.

Three years on, the Chromatographic Society organised a 1-day symposium with an almost identical title: "Impact of Separation Science upon Pharmaceutical R&D and Beyond". Interestingly neither Turbulent flow nor Capillary Electrophoresis featured in the program of talks. So what has happened in the intervening years that have taken these topics off the programme? The answer is UPLC and sub-2µm silica particles.

The venue for the symposium was the impressive Stadium of Light (Figure 1), home to Sunderland Football club. The conference facilities at the stadium enabled the lecture theatre for approximately 70 delegates and mini-exhibition from chromatography suppliers to cohabit in the same room very successfully. This resulted in an intimate yet spacious feel to the meeting with free mingling of exhibitors and delegates.



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MINI-EXHIBITION

As an integral part of the symposium, nine companies had tabletop displays of their latest product offerings. In addition there were four product presentations to delegates interspersed with those of the invited speakers.

Dr Hugh Malkin (SGE Scientific Ltd) spoke about on-line micro extraction by packed sorbent (MEPS) for LC & GC. In essence, a syringe packed with SPE sorbent, Dr Malkin showed quantitative data for MEPS used with a CTC PAL autosampler and human plasma samples.

Dafydd Milton (Thermo Fisher Scientific) presented on "Column and method considerations for high speed, high efficiency LC and LC/MS', taking us on a rapid ride through the theory of increased efficiency with sub-2µm silica particles.

Dr K Divan (Dionex (UK) Ltd) gave a presentation on 'Solving Analytical Challenges with a New Detection Technique for HPLC: 3-Deminsional Electrochemical Detection'. Essentially, this is an enhanced electrochemical detector allowing the scanning of the electrode potential and 3-dimensional display of the raw integrated amperometry signal similar to photodiode array data display.

Dr Terry Nicholson (Crawford Scientific) spoke on 'Fast LC & GC for the masses – factors affecting speed and resolution'. The majority of his talk was aimed at to those who weren't able to afford to upgrade their hardware to cope with the increased back-pressures associated with UPLC.

THE PROGRAM

Dr Lough opened the symposium with the presentation of the Chromatographic Society Silver Jubilee medal to Dr Mel Euerby for his contribution to separation science. Dr Euerby has an international reputation in the field with over 90 publications and 80 conference presentations to his name. His main areas of interest include electrodriven separations, stationary phase characterisations, computerised method development and fundamental research into retention mechanisms of analytes in all aspects of chromatography. He is a Principal Scientist within the Pharmaceutical and Analytical R&D function at AstraZeneca, Charnwood with special responsibility for evaluating and implementing new chromatographic techniques for use within the analytical departments. Immediately following the presentation, Dr Euerby gave the opening lecture on 'Does UPLC make me a better chromatographer?'

Figure 1. Venue for the meeting - The Stadium of Light, Sunderland (www.freefoto.com)

The manufacturers claim that UPLC is 20 times faster than conventional HPLC *(Figure 2)* – is this really achievable? Some of the improvements seen with UPLC systems are not just down to the sub 2 µm particles. Conventional HPLC systems probably only achieve 65% of the potential column efficiency. However, if you run the same column on a low dispersion system it may achieve up to 95% of the potential efficiency.

Dr Euerby likened UPLC to a formula one car, it gives high performance when it is working but is not yet as reliable as HPLC which he likened to the Ford Fiesta. The new UPLC systems have demanded the optimisation of the detector optics which has made a significant improvement to peak resolution.

He urged caution when comparing column selectivity. The selectivity of the sub 2 µm C18 phase is very different to the equivalent 3 & 5 µm phases citing the comparison of the Acuity HSS T3 versus and Atlantis T3 columns.

COMPANY	PRODUCTS	CONTACT
Hichrom	ACE Columns; Chiral Technologies and most brands	www.hichrom.co.uk
SGE	MEPS	www.sge.com
Shimadzu	Recent Developments in Column and Instrument technologies	www.shimadzu.co.uk
Thermo Fisher Scientific	Hypersil Gold UPLC columns	www.thermo.com
Grace Davison	VisionHT UPLC columns	www.discoverysciences.com
Thames Restek	Pinnacle DB UPLC columns	www.thamesrestek.co.uk
Dionex UK	Ultimate® 3000 Rapid Separation LC (RSLC)	www.dionex.co.uk
Varian	High Performance Instruments plus columns	www.varianinc.com
Crawford Scientific	Zorbax RRHT columns and Training software	www.crawfordscientific.com

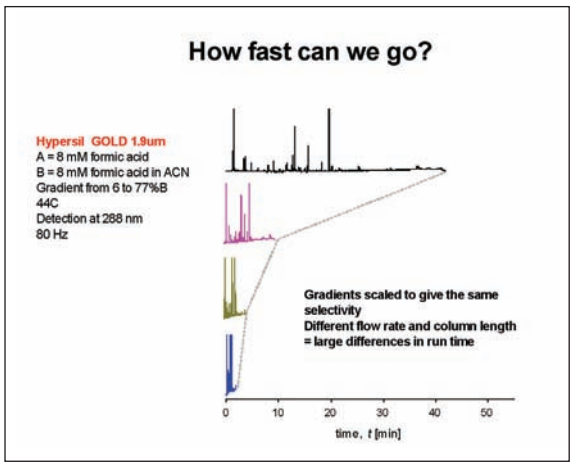

Figure 2. Potential impact on run-time from the use of higher flow-rates and shorter columns with a sub 2 µm stationary phase

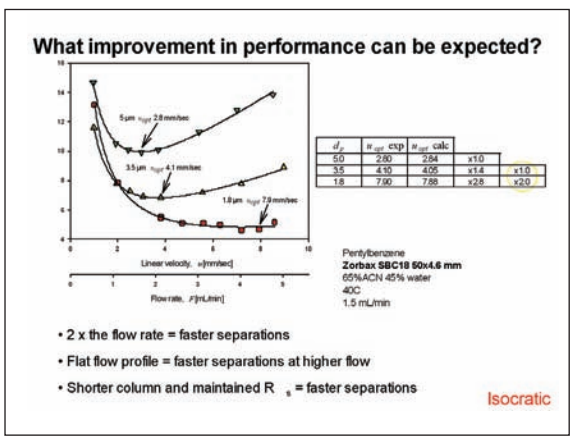

Figure 3. Impact of reduced particle size on the van Deemter plot

He presented a very favourable comparison of the reproducibility of quantitative data generated on both HPLC and UPLC, commenting that "UPLC is rock solid". Throughout the talk he repeatedly urged people to use high mobile phase flow-rates, pushing the system hard to deliver a real difference in chromatographic performance. In future, he is looking for hardware that can withstand much higher pressures and improvements in detector technology to cope with the narrower peaks i.e. mass spectrometers that can scan faster.

He questioned whether the manufacturers have yet mastered packing smaller particle columns as there is still significant variability in columns *(Figure 3)*. In his summary, he returned to the title of his talk – 'Does UPLC make me a better chromatographer?'. He answered the question by saying that it hasn't made him a better chromatographer but it has allowed him to do the things he already did well, much faster.

The Implementation of High Speed and Resolution UPLC Systems in Pharmaceutical Development by Dr. Melissa Hanna-Brown (Pfizer Global Research and Development Labs, Sandwich, UK) described the recent advances in instrumental technology for liquid chromatographic separations with particular focus on increased pressure and temperature parameters for routine liquid chromatographic separations. The beginning of this talk was dedicated to explaining what higher pressure can actually offer us in terms of increasing speed or resolution.

To demonstrate how to define the limitations of conventional versus higher pressure systems, an explanation was given about van Deemter plots when compared to kinetic plots. From the kinetic plots (constructed with real data in the Pfizer Analytical Research Centre, Ghent, Belgium), it was clear how increased pressure systems offered enhanced speed for all particle size formats. Furthermore, the plots clearly help define the regions within which highest efficiencies can be achieved and results were shown for different particle size format columns.

The presentation then went on to cover real examples of impurity profiling and dissolution analyses from pharma development applications where UPLC is being used for increased productivity and throughput. The latter part of the talk focused on UPLC and method development where example schemes for method development akin to those commonly used for HPLC with in silico simulation/optimisation software were shown. Method development was essentially shown to be no more difficult than that for HPLC and in fact – in most instances faster (< 5 days) using an orthogonal LC-MS column screening, followed by temperature/gradient optimisation scheme. Finally, the talk was rounded off with some indications of how temperature can be used as a routine and useful method development tool when high resolution is an important pre-requisite with examples of serially coupled sub 2µm columns operating at temperatures circa 90°C shown for pharmaceutical impurity profiling. Again – kinetic plots clearly aided the interpretation of how temperature can be used to dramatically increase speed when in combination with sub 2µm particles resulting in greater throughput and productivity.

Dr John Lough (University of Sunderland) – 'The other Two Factors in the Resolution Equation – in the context of Discovery, Development and Cleaning Validation'. As chromatographic resolution can be expressed as a function of efficiency, selectivity and capacity factor, John's talk was aimed at the latter two parameters. He made the point that increasing retention beyond a k´of 2 yields little increase in resolution but often selectivity is a better way of improving peak resolution. However, improving selectivity should be more than just targeting different C18 phases, indeed phases providing truly orthogonal retention mechanisms may be the answer to some difficult separations. Examples he gave were the use of ion-exchange and Hypercarb™ phases.

Janet Hammond (AstraZeneca, Macclesfield) – Analytical Challenges posed by the CHMP Genotoxic Impurities Guideline'. Potential genotoxic impurities (PGIs) are a hot topic in the pharmaceutical industry with the CHMP guidelines coming into effect in January 2007. The guidelines apply a threshold of toxicological concern (TTC) estimated to be 1.5 µg/person/day. Examples of impurities that are causing concern are residual synthetic reagents and solvents, related impurities to the active pharmaceutical and counter ions. The analytical challenges can be exacting as many of these PGIs, by their very nature, are reactive and unstable. They are usually present at trace levels and the matrix can often have an effect on accurate quantitation. Janet presented a number of methods they have developed in their laboratories which included tosyl esters, an ames positive epoxide synthetic intermediate, methanesulphonates and chlorobutanol.

Dr Ghulam Shabir (Abbott Diagnostocs Ltd.) – HPLC Method Validation – Best Practicies for Regulatory Compliance in the Pharmaceutical Industry. Dr Shabir took the audience through a step-by-step process for validation of a chromatographic method to meet the ICH regulatory requirements. He focused particularly on pre-validation requirements, those of analytical equipment qualification, stability of analytical solutions and the establishment of a system suitability test to ensure adequate chromatographic performance. He recommended that the capacity factor (k') should generally be >2.0, the resolution (Rs) >2 and a Tailing factor (T) of ≤2. He emphasised that attention to detail in setup and during the pre-validation stages will lead to greater success in the method validation phase.

Phil Borman (GlaxoSmithKline, Ware) Analytical Methods – Quality by Design Approaches for the 21st Century. Phil described the drivers for change in the way GMP analytical methods are developed and validated within GSK as both internal and external. Within the company there has been a recognition that, in the past, there had been instances where methods were being operated that had no obvious role in ensuring product quality, 'validated' methods didn't work well in routine QC environments and significant resource was being invested in 'analytical technology transfer' which did little to ensure methods worked throughout their life-cycle.

There was also no formal risk assessment/operational efficiency tools being applied to the method development and validation process to ensure a method was fit for purpose. Externally, the FDA was urging for innovation and continuous improvement and the application of a risk based approach to ensure safer pharmaceutical products.

As a result, GSK has introduced "Quality by design" to its analytical method development i.e. a systematic approach to development that begins with predefined objectives and emphasises product and process understanding based on sound science and quality risk assessment. Phil then described the four stage design process applied to analytical methods, illustrating this with a number of method mapping techniques and a strategy for robustness testing.

He summarise by saying that an Analytical Methods Quality by Design approach represents a paradigm shift in the way methods are developed, validated, transferred and controlled *(Figure 4)*. This approach will result in:

- More robust and rugged methods that are designed with the end user in mind
- A leaner science and risk based approach to method validation and transfer
- A method change control process based on structured risk assessments and reference to existing method understanding
- Significantly increased regulatory flexibility in relation to introducing method improvements

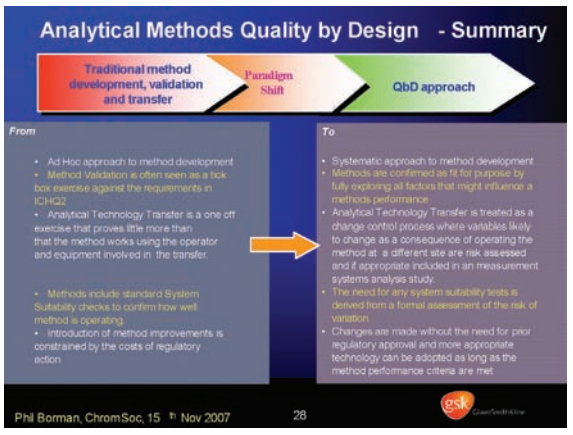

Figure 4. Summation of QbD pathway

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A lot of ground was covered in a single day with some truly excellent presentations, particularly those from Drs Mel Euerby and Melissa Hanna Brown. The various trade presentations were also of a very high standard providing useful information.

The day was rounded off for those who were interested in a tour of the stadium taking in the changing rooms and pitch side. But the final question of course is - will UPLC still be on the agenda in 3 years time?

Certainly from my perspective as a bioanalyst, the use of sub 2 µm stationary phases has had a big impact in speeding up analytical run-times and that is without us "putting our foot to the floor" (using the car analogy)!

There is clearly more to be had as improvements in the manufactures hardware allow higher temperature and pressure separations, improving resolution and reducing potential matrix effects. But the only certain way to find out is to join us in November 2010.