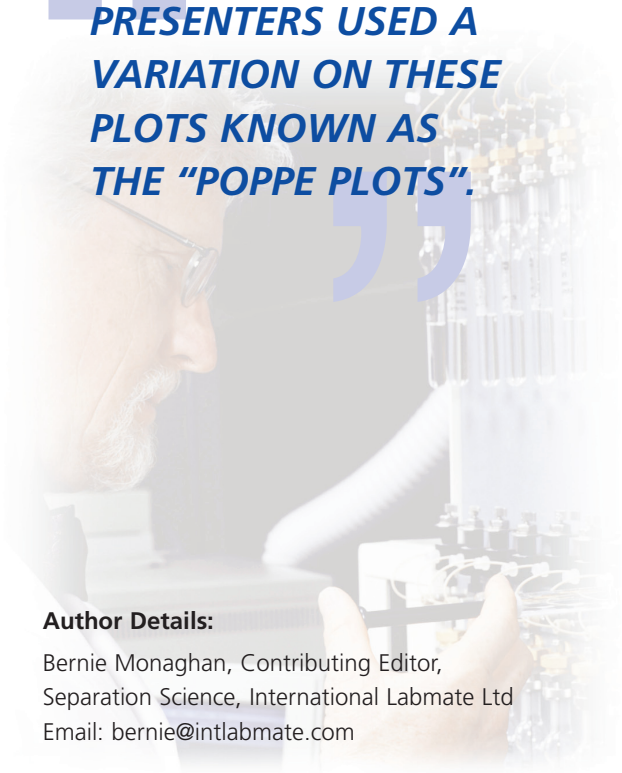
Chromatography Focus

SIZE NO LONGER MATTERS - IT'S ALL ABOUT SPEED

Bernie Monaghan

Although not 100% strictly true, attendees to the Chromatographic Society's spring meeting, incorporating the AGM of the Society, could come away with that impression after listening to presentations from leading Academics and senior scientists from the Pharmaceutical industry when addressing problems faced whilst developing strategies aimed at maximising both quality and productivity. The meeting title was 'Advances in Liquid Separations and Hyphenated Techniques' and was held at The Natural History Museum, London on May 16th. Almost 50 delegates were present and along with over 30 registrations from Exhibitors, committee members and speakers ensured that a good cross representation of the separation science community was represented. This resulted in stimulating and enlightening discussions following each of the presentations, many of which were of the 'work still in progress' topics at least ensuring that issues of real importance to practicing chromatographers were addressed.

> ***"SEVERAL OF THE PRESENTERS USED A VARIATION ON THESE PLOTS KNOWN AS THE "POPPE PLOTS".***



Author Details:

Bernie Monaghan, Contributing Editor,
Separation Science, International Labmate Ltd
Email: bernie@intlabmate.com

Dr Steve Rumbellow the Society Treasurer and principal organiser of the meeting welcomed delegates and speakers alike and passed the Chair for the morning session to Dr Derek Stevenson from the University of Surrey who in turn introduced the first speaker, Keith Brindred from GSK Stevenage who spoke on 'Quality and Speed – how do you get both? What makes a good chromatographic method and which tools are out there to help you?'

The current situation in many laboratories these days is more instrumentation, more samples to process and less people to do the work, in other words the system needs to operate at maximum efficiency to cope. The Departments that the presenter works with at GSK have changed markedly with much instrumentation located in individual chemistry labs these days as opposed to a central analytical laboratory enabling most scientists to view sample results remotely. Since the yearly throughput could be anywhere up to half a million samples then having systems in place that allow productivity to be maximised is essential. Ten individual variables were discussed relating to good chromatographic separations. One extremely interesting conclusion was that from an average cost of £2 per sample, the majority of that appears to be directly instrument related.

Dr Ying Wang made the second presentation from Pfizer Global R+D, Cambridge, UK who spoke on "New optimisation strategies and tools for HPLC method development". Dr Yang introduced the concept on modern day approaches to Methods Optimisation by discussing the needs for the activity and also the benefits that commercially available approaches deliver. Automation Software modules were outlined and defined as in *Figure 1*.

Automation Software Modules

- **Computational and optimization module:** modeling chromatographic behaviors of analytes predicting optimal conditions
- **Automation and data exchange module:** controlling HPLC software and hardware system providing necessary data exchange facility
- **Artificial intelligence module (expert system):** simulating the actions of human chromatographer in a method development procedure

Figure 1. Automated options

Computational help is certainly required when consideration is given to the Independent parameters involved in a separation (solvent types and strength, pH, buffer type and concentration, temperature, type of column and flow rates) and the response factors such as Resolution, Retention time Selectivity and Robustness). Reviewed were the offerings from Dry Lab (LC Resources), OSIRIS (Datalys) and Turbo TMD (Perkin Elmer), all of which were based on algorithm modelling, ChromSword and ACD/LC Simulator (Advanced Chemistry Development) based on molecular structure considerations and MultiSimplex (Grabitech Solutions AB) which was based on a Chemometrics method. The strengths and weaknesses (non-suitability) of each was discussed along with other methods including those based around Factoral Design, Central Composite Design, and 5 other options.

Finally an Example was shown based around the use of Experimental Design and Response Surface Methodology developed in conjunction with the University of Bradford. The plots based upon the theoretical optimisation parameters were shown and compared against the data obtained in practice (*Table 1*) with excellent collaboration. The system claimed faster and more efficient method development, with optimum separation conditions delivering more robust separations and offering a better understanding of the retention mechanism. The programme allowed integration with other programs to allow development of automated systems.

Table 1. Correlation values

Retention Time	T1	T2	T3	T4	T5	T6	T7	T8
Experimental	0.78	1.00	1.19	1.67	1.93	11.34	21.13	36.69
Predictive	0.77	1.05	1.27	1.77	2.04	11.77	22.16	39.22
Resolution	R1	R2	R3	R4	R5	R6	R7	
Experimental	2.09	1.58	3.64	1.85	24.91	11.38	9.82	
Predictive	1.95	1.56	3.56	1.73	24.13	11.04	9.52	

Following the morning coffee break Prof Pat Sandra, taking time out from his organisational duties as co-chairman of HPLC 2007, to give a presentation entitled "Towards high efficiency in fluid based separation techniques". Although at first sight this topic may appear somewhat academic it actually encompasses the following range of possibilities, UPLC™, HT (High Temperature) LC, SFC, LCxLC, SFCxLC, CEC and potentially myriad combinations of the above. After observing and congratulating delegates to the fact that Industry was contributing to the development and advancement of theoretical Chromatography by practical means, Prof Sandra observed that more work needs to be done to bring efficiencies in LC based separations towards those routinely observed in capillary GC separations in terms of efficiency and plate numbers. He went on to describe how factors other than particle size could be used to obtain extra efficiency such as elevated temperatures and coupled columns with more modest particle diameters than UPLC types. The seemingly obsession with speed of analysis was qualified by the observation that speed should always be linked to the specific application. Attempts to maximise speed by increasing linear flow may cause loss of resolution or fail due to Instrument limitations. An example of a sample with over 200 components, one of which may be a potential biomarker illustrated the need to maximise the plate number for the separation. Never one to miss a trick Prof. Sandra commented that anyone interested is seeing the most up to date information on this separation and others should "come to HPLC 2007 in Ghent next month where more will be revealed"

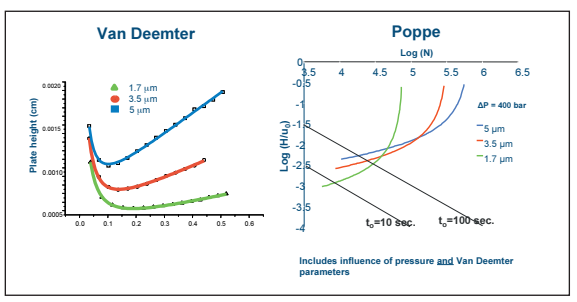


Figure 2. Complimentary data information

Many of the delegates, by virtue of their background in Pharmaceutical industry and the current myriad of products containing small particles (around 2um) to aid the quest for higher throughput, are familiar with Van Deemter and Knox plots showing the optimum flows for certain particle diameters and how they vary with efficiency (*Figure 2*).

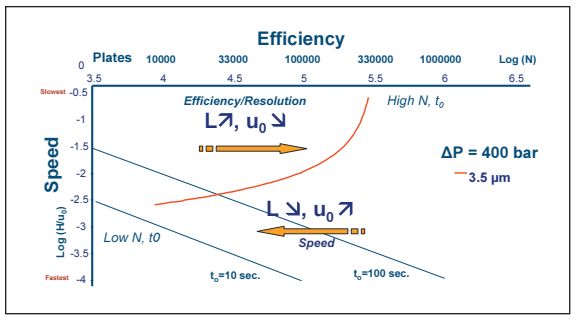


Figure 3. Example of Poppe plot and variables

Several of the presenters used a variation on these plots known as the "Poppe Plots". In the example shown here, for each particle size, the lines represent the maximum speed obtainable, when working at the maximum pressure of the system. Very high efficiencies are obtained using larger particles, at the cost of analysis time (large t^0). For lower plate counts, use of small particles provides the fastest analysis. A "Poppe Plot" is further illustrated for 3.5um material in *Figure 3*.

The problems associated with attempts within the Global Pharmaceutical Industry to increase productivity were discussed by Paul Ferguson from Pfizer Global R+D and are summarised in *Figure 4* which show the key challenges is to maintain high standards of drug quality and information whilst increasing productivity. From those particularly relevant to the meeting, enhancements specific to 4 main areas were discussed and examples presented of how each was evaluated. Chromatography was examined with a view to enhancing the resolution by increasing the speed of analysis.

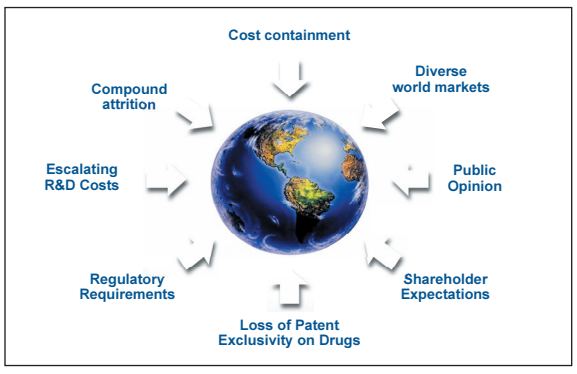


Figure 4. Issues facing multi-national Pharma

Figure 5. COMET system in place

Of particular use was a concept named COMET (*Figure 5*) (Comprehensive Orthogonal Method Evaluation Technology) that basically comprised of a 4 way parallel LC/MS with MUX™ ESI interface running orthogonal methodology. It was used to investigate the advantages of parallel analysis against serial systems. A typical advantage cited was the optimisation of a chiral separation, which reduced from 36 hours on serial system to 4 hours with a parallel system. In general parallelism supplies the required information in a 1/n (where n is the number of systems used) time frame. Other Chromatographic options examined were UHPLC, HT-LC and monoliths, the first two providing same or enhanced information in similar timeframes but monoliths are progressing to enhanced information in faster timeframes. Process improvements were also examined leading to interest in generic methodologies specifically UHPLC alternatives to HPLC screening techniques. Simulation by virtue of use of searchable databases and Optimisation and prediction techniques to minimise experimental time were also looking promising. The overall conclusion the speaker concluded was that combinations of the variables currently under examination are likely to provide the highest productivity increases.

The current situation in many laboratories these days is more Instrumentation, more samples to process and less people to do the work, in other words the system needs to work at maximum efficiency to cope. Keith Brindred from GSK spoke on this topic in his presentation entitled "Quality and Speed – how do you get both?

One factor overlooked by many laboratories who tend to view storage space (and retrieval from) of electronic data as 'taken for granted' without regard for the ever increasing numbers of samples that pass through any lab involved in Drug Discovery process at the moment. John Hollerton of GSK gave a talk entitled "So you can run faster . Now what do you do with all that data?" in which the issues related to data storage and retrieval were discussed. By using some rather amusing (and alarming) data the presenter illustrated the point that the numbers of samples processed these days in a major Pharmaceutical company requires a massive amount of storage space for the files. Looking at pricing for such space in various storage formats makes it an expensive operation that cannot be ignored when it comes to costing sample analysis.

Ease of retrieval of information is also an important factor to be considered when making strategic decisions on data storage. Improvements in the separation science field are not solely restricted to making life easier for scientists working in the Pharmaceutical Industry as Benedikt Kessler from Oxford University explained when he spoke on "How Proteomics benefits from advances in LC and MS technology"

Simon Perry from Syngenta spoke on the "Developments and Applications of UPLC-MS in Agricultural Research." The focus was very much on the gains that UPLC-MS brought to the desk of the scientists in a complimentary field to Pharmaceuticals and life sciences. Increasing numbers of samples are being analysed in attempts to trace potentially environmentally hazardous substances so speed and increased method sensitivity are important in making life easier for the scientist and safer for the rest of us. A small trade exhibition was also held for delegates with representation from the following companies; - Waters (main sponsors), Grace Davison, Hichrom, Shimadzu, Crawford Scientific, Iris Technologies, Vaportech with Andy Craze from Waters giving a presentation on the available Waters column chemistries to help in increasing productivity.

SEPARATION SCIENCE / SPECTROSCOPY MEETINGS CALENDER 2007/08

DATES	VENUE	MEETING	CONTACT
2007			
26th-29th August	Kaunas, Lithuania	**4th Nordic Separation Science Conference**	www.conference.vdu.lt/nosss4
5th-7th September	Siofok, Hungary	**7th Balaton Symposium on High Performance Separation Methods. In Memoriam Szaboics Nyiredy**	www.mett.hu
9th-12th September	Herriott Watt, Scotland	**BMSS Annual Meeting**	www.bmss.org.uk
9th-14th September	Antwerp, Belgium	**Euroanalysis XIV**	www.euroanalysisxiv.ua.ac.be
27th September	Runcorn, England	**Advances in GC Technology**	www.chromsoc.com
9th-11th October	Hannover, Germany	**Biotechnica**	www.biotechnica.de
23rd-24th October	Loughborough, England	**Big Prep 4**	www.chromsoc.com
21st-24th October	Orlando, USA	**ISPPP 2007 (International Symposium on the Separation of Peptides, Proteins and Polypeptides)**	www.isppp.org
21st-26th October	Cairns, Australia	**4th International Peptide Symposium "From Discovery to Therapeutics"**	www.peptideoz.org
15th November	Sunderland, England	**Impact of Separation Science in Pharmaceutical R+D**	www.chromsoc.com
9th-14th December	Cambridge, England	**LC-MS Short Course and Symposium**	www.bmss.org.uk
2008			
30th -Jan - 1st Feb	Bruges, Belgium	**HTC-10/ExTech 10 (Hyphenated Techniques in Chromatography and Advances in Extraction Techniques)**	www.ordibo.be/htc
10th-13th February	Dubai, UAE	**Arab Lab**	www.arablab.com
2nd-7th March	New Orleans, USA	**Pittsburgh Conference**	www.pittcon.org
9th-13th March	Berlin, Germany	**22nd International Symposium on MicroScale Bioseparations and Methods for Systems Biology**	www.msb2008.org
10th-11th March	Winchester, England	**Informatics 2**	www.chromsoc.com
1st-4th April	Munich,Germany	**Analytica**	www.analytica.de