

Intelligent method development: why it is important to the drug industry

Method validation is covered in scientific meetings and courses far more frequently than is method development, yet the development phase is important because if the method foundations are not strong, then validation can only confirm that fact; it cannot improve the method.

Joseph Chamberlain reports from a meeting that explored best practices for the intelligent development of informative, robust methods

Intelligent method development incorporates the philosophy that the method chosen is fit for its purpose, Ken Leiper, of Benson Associates, explained. Why has the topic of intelligent method development assumed such importance, he asked. The answer lies in the predicament that the pharmaceutical industry has found itself in recent years.

Compared with other major industries, such as food and automobiles, the pharmaceutical industry is woefully inefficient. It is now generally accepted that the sector's poor performance relative to other sectors is largely one of manufacturing performance, directly related to the conservatism of the industry and the regulatory authorities, which effectively block the innovative application of new technology. Analytical science should not be exempted from these criticisms. The traditional methods of quality control or quality assurance have tended to concentrate on the end product, such as testing the specifications of a tablet, leading to considerable wastage when specifications fail. Yet, as analytical scientists we have all been trained not only to ask the right questions but, more importantly, also to ask the relevant questions to ensure that our technologies are deployed efficiently and effectively to business advantage.

In a ranking of the stages of production of medicines, it is sampling and sample preparation that are most susceptible to variation, not the measurement or data reduction. Analysis was and still is largely product-focused, laboratory-based, invariably requires significant sample preparation, is about what can be measured not what needs to be measured, is carried out in solution in the chemical domain, and destroys all physical information. Invariably the information generated correlates poorly with drug product process performance. In contrast intelligent method development requires the adoption of new technologies which will improve overall quality. The challenge for the analytical community is how to make measurement more meaningful, said Mr Leiper.

Best columns and solvents for HPLC

There are two types of analyst, said Mel Euerby, of AstraZeneca R&D, Charnwood: the tinkerer and the thinker.

It is the thinker who is needed for intelligent method development in high-performance liquid chromatography, where there are so many potentially interacting parameters that

determine the best separations. The thinker will seek to understand the chemistry of the analyte, the stationary phase, and the mobile phase in designing the analytical method.

There are over 700 stationary phases available world-wide for HPLC. This dilemma of column choice is exacerbated by the fact that manufacturers' data are confusing and often contradictory. Pharmacopoeial classifications of stationary phases do not help in that, for example, the US Pharmacopeia has generic classes as well as highly specific ones. A greater knowledge of stationary phase chemistry permits a more rational selection of appropriate phases to be made based on matching the properties of analyte and stationary phase. The classification developed by Dr Euerby and colleagues considered such factors as the area of silica coverage, hydrophobicity, steric selectivity, hydrogen bonding capacity and ion exchange capacity at both basic and acid pH values. The chemometric tool of principal component analysis can then be used to identify similar columns for back-up phases and columns with orthogonal chromatographic properties for method development.

For selection of suitable mobile phases, retention modelling and resolution prediction software has been used to aid the rapid and systematic selection of optimal mobile phases and operating conditions. The orthogonal nature of differing organic modifiers in isocratic and gradient chromatography can be tailored using binary, ternary and quaternary mobile phase compositions. The use of mobile phases of pH greater than 8 is now possible following the development of new silica phases, extending the possibility of enhanced selectivity for basic analytes.

George Okafo, of GlaxoSmithKline, Stevenage, concentrated on the optimisation of separations in HPLC. In the drug development process, the type of analytical method that is required matches the phase of product development. In the early development phase, analytical methods are typically simple methods with generic elution conditions. In the mid to late development phase, activities are typically centred around developing a commercially viable synthetic route. It is around this point that intelligent method development and optimisation play significant roles in establishing methods that are fit for purpose. In the traditional one-factor-at-a-time approach, a critical parameter is varied across an

experimental range, while holding other conditions constant and repeating the process with a different parameter until an acceptable system is found. However the result is usually sub-optimal, unlikely to be robust, and may require redevelopment during the further development of the drug. Additionally interaction effects cannot be readily assessed using these methods. Over the years, modern approaches to method optimisation have been developed that allow multiple experimental parameters to be examined to reveal all potential interactions. Moreover, the design and separation space can be mapped out for the chromatography to provide not only an optimised method, but information about method robustness. Typically, the analyst will select key instrumental or method parameters and an appropriate experimental design before running real experiments under conditions defined by the design. Readily available software tools can be used to examine the interaction between different experimental parameters in a simple, visual and systematic manner. Examples discussed showed remarkably accurate predictions for excellent separations.

Characterisation of glycoproteins

The "Measurements for biotechnology" programme is part of the UK's national measurement system supported by the Department of Trade and Industry. It aims to generate new approaches to measurement in biotechnology, which will lead to standardisation and thus comparability, both nationally and internationally. Paula Vickers, of LGC Ltd, reported one aspect of this programme which brings together the key technical resources of protein and glycoprotein analysis. Partners in the project include academic institutes, instrument manufacturers and biopharmaceutical manufacturers.

Glycans are important biotechnology products and the heterogeneity of N-linked glycosylation is a key method of characterisation. In the LGC project, the range of methods available was evaluated via a questionnaire completed by members of the partnership, and specific techniques were selected for evaluation in depth.

The "intelligent method development" symposium, organised by The Joint Pharmaceutical Analysis Group, took place at the Royal Pharmaceutical Society's London headquarters on 8 December 2005

The Joint Pharmaceutical Analysis Group is a focus for the presentation and discussion of matters of importance to those interested in pharmaceutical analysis. The remit of the group is "to encourage, assist and extend the knowledge and study of pharmaceutical analysis and quality control by the holding of scientific meetings, by the promotion of lectures, practical demonstrations and discussion, or by any means consistent with the aims and objects of the sponsoring bodies and the with the rules of the group".

The group's sponsoring bodies are the Royal Pharmaceutical Society and the Royal Society of Chemistry. Membership of the group is open to member of either society and is free to members of the Royal Pharmaceutical Society.

Pharmacists wishing to join the group should apply in writing, giving their registration number, to the Secretariat, Joint Pharmaceutical Analysis Group, Room 403, Royal Pharmaceutical Society, 1 Lambeth High Street, London SE1 7JN.

For quantification of the degree and sites of glycosylation of intact glycoprotein or glycopeptide, a colorimetric anthrone method that distinguishes between glycosylated and non-glycosylated intact proteins was considered to be underused compared with a variety of capillary electrophoresis methods. For characterisation of different glycoforms present in cleaved glycans, capillary gel electrophoresis-laser induced fluorescence was evaluated, particularly with a view to determining the goodness of resolution, quantitation and repeatability. Fourier-transform ion cyclotron resonance mass spectrometry was also evaluated for showing that high accuracy mass spectral data can be used to ascertain structural information of glycoforms present.

The project is now developing a good practice guide to assist in the choice of analytical methods for glycoprotein heterogeneity analysis, which will be presented at a practical workshop at the end of the project, said Dr Vickers.

Differential scanning calorimetry

Calorimetry is a widely used analytical tool, primarily because heat is a universal indicator of chemical and physical change, said Simon Gaisford, of the School of Pharmacy, University of London. In addition, the physical form of any sample is irrelevant, which means its range of applications is near limitless. The most commonly used form of calorimetry in pharmaceuticals is differential scanning calorimetry (DSC). Here, the heat flow to or from a sample and inert reference is measured as a function of temperature. DSC's many applications include polymorph detection and the measurement of glass transitions and amorphicity. However, the relatively slow scan rates (typically 5–20°C/min) mean that considerable information on the

sample is lost because the time required for inter-molecular rearrangements is shorter than the experimental run time.

Fast-scan differential scanning calorimetry (FS-DSC) has been developed to ameliorate these issues, using power-compensation DSC rather than heat-flux DSC, allowing heating rates up to 500°C/min. Through the use of such heating rates, FS-DSC experiments can be conducted on a time-scale that does not allow inter-molecular rearrangement. The data are therefore representative of the sample in its initial state, which often allows a much greater understanding of the material under investigation. Examples that illustrate the usefulness of the technique include the resolution of problems of decomposition, polymorph detection and quantification, amorphous content detection and quantification, and the determination of the glass transition temperatures in complex mixtures.

Automated methods

Andrew Walsh, of AstraZeneca, said that automated methods were little used in the development phase of a drug's life cycle. In the discovery phase there are many campaigns and automation is feasible for large numbers of analyses using generic methods; in the production phase there are large numbers of batches which make automation of specific methods worthwhile. Nevertheless, experience of dosage form development suggests that automation in this phase can also be worthwhile although each dosage form has its own problems.

In the development of pressurised metered-dose inhalers, for example, the variables in this complex device include the propellant, the lubricant, the surfactant, the retention valve, and the number of actuators. The challenges include wall, valve or stem deposition, cooling effects, electrostatics, sedimentation and creaming. Manual method parameters often bear little resemblance to their automated counterparts and it is therefore critical that the dosing process is well understood. Significant differences exist between manual and automated shaking, and the analyst-to-analyst variation is also surprisingly high. Conversely, although the automated system offers a constant shake, it has a non-sinusoidal profile because of robotic deceleration before reaching its maximum amplitude. It is important that the consequence of these differences is quantified and understood. There are also several methods of automated shaking which can determine the quality of data in terms of variability and active drug concentration.

Dissolution methods

Before a method can be assessed we need to understand what we are dealing with, said Steve Westcott, of Melbourn Scientific, and this is the essence of intelligent method development. One particular problem has been in the development of dissolution methods for lipophilic, or poorly water soluble, drugs. For a dissolution method the purpose may be to discriminate between variations in the physi-

cal form of the product or to assess the likelihood of biological absorption as attempted by the biological classification system. These different purposes will require a different type of analysis. For poorly soluble drugs the physical form and properties of the active pharmaceutical ingredient are the key factors for maximising the solubility of the drug and making it bioavailable. The crystal form and size are important, with smaller particle sizes being favoured. This has led to size reduction to nano-particles, which presents difficulties for the analysis because most filtration systems are ineffective for material of this size.

For evaluating dissolution of the dosage form the amount of drug to be administered is important, as are the type and amounts of the excipients. Liquid formulations or semi-solids, with the drug either as an emulsion or dissolved in the excipient, are easy ways of administering a partially dissolved drug. Inclusion complexes and lipid emulsions are put forward as alternatives but such strategies can present the analyst with significant challenges. Issues such as the manufacturing process and stability storage will need to be addressed by the method, sometimes after product development has been completed. Dissolution procedures can have a role to play in product development. It is not just a technique for quality control, although this will be its final goal, concluded Mr Westcott.

Near infrared spectroscopy

The rapidly emerging technique of near infrared spectroscopy (NIRS) and the recognition of process analytical technology combine to provide a prime example of intelligent method development, said Alan Rhoden, of Pfizer. NIRS can be used on-line or at-line to gain timely information in real-time feedback, and to enhance process knowledge and control. Continuous quality verification is important. The aim is to be able to monitor and reprogramme all processes as they happen, hence reducing wastage and cycle times. As a means of increasing the level of process information NIRS technologies have proven themselves to be excellent tools (see *PJ*, 29 October 2005, pp552–3).

The mixing (or blending) of the active pharmaceutical ingredient and excipients is one of the most common processing steps in the industry today and, therefore, one which repays increases in efficiency. In blending operations the use of real time analysis, such as is possible with NIRS allows blending to be terminated when the blend is ready and over-long blending times become unnecessary.

The use of fluidised beds for drying is also well established. It is highly efficient and produces good batch sizes. However reproducibility is based on good control of drying conditions, usually done by off-line moisture determination; NIRS can do this in real-time. Intelligent design extends to the NIRS instruments themselves: a simpler instrument in which wavelength range is restricted can improve signal to noise ratio, for example, said Dr Rhoden.