

# Why robust analytical methods have to exist at every stage of a medicine's life

While ICH validation guidelines provide a framework for the core activity of measurement validity, actual requirements are heavily influenced by the purpose and uncertainty of the measurement. This meeting, reported by **Joe Chamberlain**, examined key strategies for the successful validation of analytical methods at all stages of a medicine's life, from clinical trials through to post-marketing

The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) consists of the regulatory authorities of Europe, Japan and the US and experts from the pharmaceutical industry in these three regions. The World Health Organization (WHO), the European Free Trade Area (EFTA), Switzerland and Canada are also included as observers. The ICH guidelines are invariably referred to by the principal regulatory authorities for analytical validation, said Mark Broughton, of Aventis Pharma, Holmes Chapel. Analysts can thus agree on the core definitions for accuracy (the closeness of agreement between the true value and that found), precision (the closeness of agreement between a series of measurements), specificity (verification that the analytical method responds only to the substance of interest) and range (the range of sample concentrations over which the analytical procedure has been shown to have suitable performance). Other useful agreed attributes include limit of detection, limit of quantitation, linearity and robustness.

The guidelines describe circumstances where revalidation is required and explain that the degree of revalidation required depends on the nature of the change. Method maintenance is not covered by ICH but is an expectation of current good manufacturing practice and should be defined locally along with a process for carrying out validation and the standards expected.

## A regulator's view

Geoff Houghton, of the Medicines and Healthcare products Regulatory Agency, described the requirements of the EU regulators in new drug applications or variations, concentrating on the reasons for analysis rather than the technical aspect. Since July 2003, the product licence (or, in European terms, the marketing authorisation) has benefited from a new common technical document format, with a section devoted to quality data. Although the bulk of a submission on the analytical method may describe its technical aspects, it is the validation which is most critical. Importantly, validation may need to take into account changes unrelated to the analytical method which may have an unexpected impact on the validity of an existing method. For example, a change in formulation from film-coating to enteric-

coating may require identification tests for new excipients, revalidation of existing identity tests and even revalidation of pharmacokinetic assays used in bioequivalence studies. Internal procedures should exist so that analytical development and quality control departments are alerted to such changes.

Paradoxically, the EU licensing authorities require more validation for a change of method than for the methods designed to support a new chemical entity, as the change in method will require cross-validation with the original method. Dr Houghton stressed that requirements of the regulatory authorities often coincide closely with the minimum that competent analysts would choose to do to ensure that their methods are valid and robust. Since the analyst knows more about a particular technique than any regulator, the analyst should think about why something is being done rather than how, and then is more likely to be doing the right thing for satisfying the authorities, concluded Dr Houghton.

## Non-chromatographic test methods

Validation of analytical methods is essentially about accuracy and precision, said Arjen Tinke, of Johnson & Johnson, Beerse, Belgium. For chromatographic methods, the use of "spiked" samples is often used to evaluate accuracy but for non-chromatographic methods, orthogonal comparisons (that is, the application of an alternative assay) are more useful. For example, loss on drying can be checked by a gas chromatographic method and laser diffraction for particle size analysis can be checked by image analysis.

The ultimate test method is one that needs to be applied only once, and to achieve this with confidence the best precision is required. To determine the nature and source of any deviation it is important to develop powerful analytical test methods. From a speed and cost point of view, it is a challenge to develop such precise test methods. However, the test methods that are used in R&D may differ from those that are used in the final quality control procedure, since in R&D the manufacturing process and the product may not yet be fully defined.

The use of additional test methods will lead to a more complete mapping of the product characteristics, or to a better correlation between the various physical or physiological parameters. Some typical R&D methods can be used in a later stage, as an or-

thogonal method in the validation of the accuracy of a QC method.

Apart from high accuracy and precision, QC test methods should meet additional requirements with regard to compliance, speed and cost. For the analytical support of R&D and QC processes, science and compliance should be considered as equally important. However, for R&D test methods the cost for analysis may be higher, and the compliance level may be limited.

## Statistical case studies

Phil Woodward, of Pfizer Global R&D, Sandwich, Kent, presented case studies where collaborations between analytical chemists and statisticians added value in pharmaceutical R&D. A typical series of chromatographic analyses would include interpolated standards which are used to quantify the analytical samples so bracketed. The required frequency of interpolation will depend on the amount of drift in the analytical response. Simple statistical procedures can be used to determine the lowest frequency consistent with reasonable analyses per day for given degrees of drift, injection repeatability, and the accuracy required. Thus productivity can be increased while maintaining data quality.

A high degree of robustness is required in chromatographic methods such as HPLC which may need to be applied in different laboratories using different equipment and subject to different degrees of variation. In the search for the most appropriate operating conditions (column material, solvents, flow rate, temperature, pH, detector settings) the experimental design is important. Experiments can be monitored by recording retention times, resolution, peak responses and tailing factors, after statistical methods have been applied to give the best coverage of the design space, detect interactions and ensure increased precision. Overall there is an increased efficiency in the use of resources, even if a single optimum is rarely achieved.

Frequent failure of tests for linearity in gas chromatographic assays, despite a belief that

**Details** The one-day symposium on method validation organised by the **Joint Pharmaceutical Analysis Group** took place at the Royal Pharmaceutical Society's London headquarters on 20 May. Dr Chamberlain is a former editor of *The Journal of Pharmacy and Pharmacology*

the method was fundamentally sound has led to the application of statistical methods to resolve this problem.

Mr Woodward noted that simple regression analysis is not useful in testing for linearity, particularly if the samples are inappropriately spaced over the test range; naturally tight values close to the origin will cause a non-zero intercept test to fail rarely, whereas values with a wide spread at the high end will often trigger a curvature alert. However, by plotting residuals it can be clearly seen that variation increases with concentration and hence, with magnitude of response. In this case, an appropriate modification of the statistical model is to use weighted least squares.

### Clinical trials and comparators

An important series of tests often described as pre-formulation is used to identify a drug candidate's physical and chemical properties, said Mark Benger, of Quintiles. These tests are essential in assisting formulators to make dosage design choices and can help chemists in development of analytical methods in the early phases of drug development. The presentation focused on validation of dissolution and stability-indicating assays, and the implications of using comparators in clinical trials.

Once a set of conditions has been established the analytical method for dissolution testing should be validated to enable the procedure to be deemed fit for purpose. In the early phases it is recognised that a full validation package is not required to satisfy regulators in the US or Europe. Clearly, accuracy at the range of expected concentrations should be validated. "Spiking" experiments, such as those used for assessing precision and method repeatability, should be included. At this stage the dissolution procedure would not be fully tested for robustness but assurances should be made that the drug is stable in the medium of choice.

With regards to the stability assay, this, too, should be developed and validated with a view to further modifications to formulations which are to be expected as the clinical process evolves. The method should be specific with regards to the main component. The generation of degradants through limited stress conditions such as heat and light should also be undertaken.

It is also appropriate that mass balance is evaluated, whereby the sum of all detected components should approach 100 per cent of the initial assay value. Often during early development it is not possible to achieve full mass balance as unknown degradants may have significantly different ultraviolet absorption compared with that of the parent compound. Other tests to perform are accuracy, linearity, precision and limits of detection and quantitation. The early development stability-indicating assay is, therefore, an important tool in assisting in the understanding of degradation pathways that may govern the progression of the compound into late-stage clinical trials.

Another area of interest in the interim stage of a compound's development is the use of comparator studies to enable companies to test their candidate product against the market leader in blind clinical trials. Once the marketed product is blinded, for example by over-encapsulation, it is considered to be a new product which requires its own analytical validation. There are requirements to check that the use of a particular blinding process has not altered the release rate of the product or indeed the chemical stability.

### Late stage pharmaceutical development

Kevin Ryan, of Pfizer Analytical, Sandwich, Kent, provided a useful definition of validation. It is the process by which assurance is provided that we have developed a method that is fit for its intended purpose, ie, we understand clearly what the performance requirements are and we are confident that the method can meet these routinely.

Validation data are used to judge the capability of a procedure to generate analytical results of the expected quality, reliability and consistency. It reflects the method development process and consequently poor development inevitably means a poor validation data set. There is considerable guidance for method validation for the industrial analyst. Vital documents are ICH Q2a and Q2b, although these should not be applied blindly but on a case-by-case basis. Important documents include the US Food and Drug Administration "Draft guidance for industry — analytical procedures and method validation, and the "FDA reviewer guidance — validation of chromatographic methods".

### Measurement uncertainty

Analysts generally express the quality of their measurements in terms of a measurement error and are frequently over-optimistic in their estimations, often ignoring significant sources of error. However, measurement error is not a useful parameter for estimating and expressing measurement quality, said Steve Wood, from the Laboratory of the Government Chemist. Measurement uncertainty, which is estimated from what is known about a measurement rather than by speculating about unknown residual errors, is a more robust methodology and should be used instead. Measurement uncertainty is an effective way of expressing the errors that are inherent in any measurement method. It is a concept used increasingly by analytical scientists and is being adopted in international standards for certification and accreditation of measurement laboratories.

Evaluation of the uncertainty of any measurement method should be an integral part of method validation and knowledge of the uncertainty of a result is essential for comparison against specification or regulatory limits. The concept of uncertainty itself is straightforward but implementing it often causes confusion.

There are four key steps involved in quantifying uncertainty: what needs to be meas-

ured should be specified in detail; for each stage of the measurement procedure sources of uncertainty should be listed, identifying what causes the result to change; the uncertainty components then must be quantified; and, finally, the values are combined to give a quantitative measure of uncertainty as a range.

### New technologies

No clear guidelines exist for the validation criteria required for a new technology and consequently the process for the introduction of something novel can be somewhat complex, said Manuel Sanchez-Felix, of Lilly, US. Indeed, although the FDA states its support for the development and use of the most appropriate instrumentation, it has also recognised that the use of rare or exotic systems places a burden on the regulatory laboratory, and may delay registration. Until recently this potential to delay registration may have deterred the industry from introducing new technology but there has been a welcome change in approach that encourages the use of new technologies.

Vehicles for the introduction of new technologies include user groups (for example, the Pharmaceutical Analytical Science Group, the American Association of Pharmaceutical Sciences, and the Joint Pharmaceutical Analysis Group), working groups (particularly the New Technology Forum, a UK partnership established by the Royal Pharmaceutical Society, the Medicines and Healthcare products Regulatory Agency and the pharmaceutical industry), and the regulatory authorities.

Now that the industry has incentives and rapidly evolving processes, the regulatory environment is changing to one of process understanding and the guidelines now being developed by the regulatory authorities act as catalysts for the introduction of new technology, said Dr Sanchez-Felix.

### Society members' groups

The Royal Pharmaceutical Society has established special interest groups for community pharmacists, for veterinary pharmacists, for industrial, regulatory and technical pharmacists, for hospital pharmacists and for pharmacy academic staff. The groups hold meetings to consider topics of interest within their own fields of practice and they provide a source of advice to the Society's Council on specialist matters. Details of the groups can be obtained from the Society. Contacts are as follows: Community Pharmacists Group and Industrial Pharmacists Group, Ann Harrington, practice division (tel 020 7572 2411); Hospital Pharmacists Group and Veterinary Pharmacists Group, Liz Griffiths, practice division (tel 020 7572 2408); Academic Pharmacy Group, Rachel Ollerearnshaw, education division (tel 020 7572 2375).