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# FACILITATING INCURRED SAMPLE REANALYSIS BY EMPLOYING A LABORATORY INFORMATION MANAGEMENT SYSTEM

During the course of a drug development programme, bioanalytical assays will be validated and used to quantify drug and metabolites in samples from a variety of different biological matrices. To ensure that the assay is reproducible in each matrix, a subset of samples in each matrix needs to be reanalysed. Incurred Sample Reanalysis (ISR) has become an accepted way to assess the quality of all types of bioanalytical assays and has become widely used within the pharmaceutical industry and by regulatory agencies. ISR occurs when samples taken from an in vivo study are reassayed an additional time for quality assessment purposes. The main objective in performing confirmatory reanalysis of incurred samples is to demonstrate that the assay is reproducible.

The bioanalytical methods used to support the drug development process need to be validated within the study context to ensure that they are robust and capable of producing accurate, precise and reproducible results that are appropriate for a specific analytical application and to satisfy FDA requirements. Incurred sample reanalysis has become an accepted way to assess the quality of bioanalytical assays. Therefore, pharmaceutical organizations need to develop industry standard best practices and deploy high performance tools, like a LIMS, that deliver the functionality to meet the latest incurred sample reanalysis requirements.

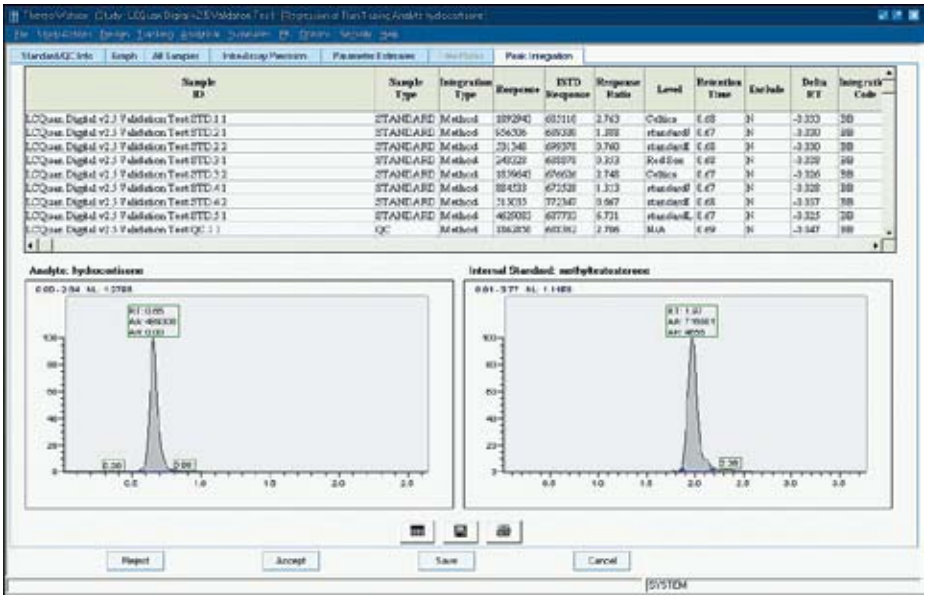

**Figure 1:** The advanced, bidirectional digital interface between Thermo Scientific Watson and LCQUAN, the data acquisition system for the Thermo Scientific TSQ Quantum mass spectrometer series, enables the secure transfer of worklist information and results data, together with integrated peak viewing in Watson.

## Background and History

In pharmacokinetic studies, bioanalytical method validation is crucial to minimizing random error and systematic bias, which ensures the quality of the analytical results. The validation of a bioanalytical assay, according to Good Laboratory Practice (GLP) requirements, requires the preparation of QC samples by spiking the biological matrix with reference material diluted previously in solution at set concentrations. Viswanathan, *et al.* (US Food and Drug Administration) has previously observed that ISR serves to further validate sample reproducibility and accuracy of the reported analytical results.[1] Thus it is very important that guiding principles for the validation of bioanalytical methods are established and circulated in the scientific community.

In 1999, the FDA issued a draft Guidance on Bioanalytical Methods Validation. This guidance was shared with the industry for comments and further discussion. In January 2000, more than 600 key scientists from the pharmaceutical industry, contract research organizations (CROs) and regulatory representatives participated in the Crystal City II conference on Bioanalytical Method Validation. Following this conference, the FDA circulated its first official guidance for bioanalytical methods, titled “Guidance for Industry: Bioanalytical Method Validation” in May 2001.[2]

Following this publication, many uncertainties still remained related to process guidelines for bioanalytical method validation owing to differing interpretations of the FDA guidance. Further clarification was needed in the industry, specifically regarding non-chromatographic, ligand-binding assays. To answer the growing questions related to bioanalytical methods validation, the Crystal City III conference was held in May 2006, during which the necessity of performing incurred sample reanalysis was discussed and process guidelines for bioanalytical methods validation further clarified. Viswanathan states that during the third AAPS/

FDA Bioanalytical workshop, it was suggested that the reproducibility in the analysis of incurred samples be evaluated in addition to the usual prestudy validation activities performed. The concept of incurred sample reanalysis was established in the conference report (published in 2006) in the American Association of Pharmaceutical Scientists (AAPS) journal, titled "Workshop/Conference Report — Quantitative Bioanalytical Methods Validation and Implementation: Best Practices for Chromatographic and Ligand Binding Assays."[1]

## Market-Driven Needs

Although the Workshop conference report indicates that incurred sample reanalysis is necessary, it does not prescribe in detail how the ISR should be performed. There are several aspects that need to be considered. Pharmaceutical companies and CROs need to consider these implications for their data processing systems. The advantages and disadvantages of each approach should be evaluated. Considerable scientific judgment is required both in the preparation of the process and in the interpretation of ISR results. First, the selection of samples for repeat analysis has to be considered. The selection of samples for ISR may be done randomly, quasi-randomly or by choosing pharmacokinetic concentration-time profiles. Whereas the random selection of samples yields an unbiased, objectively chosen set of samples, it has some disadvantages too. The random samples may not represent a proper cross-section of concentrations results: it may result in the selection of only a limited number of analytical runs and studies with many BLQ samples, which may result in a large number of non-quantifiable samples being selected for ISR. Quasi-random selection — random sample selection within low, medium and high concentration ranges — could be used to diversify the range of ISR samples to be selected, although the procedure is more complex than purely random selection. One advantage of selecting complete PK profiles is that they are easy to select for blocked sample designs, such as bioequivalence studies, and are less susceptible to selection errors, although this approach may not be appropriate for other types of studies such as Phase III clinical trials.

IN PHARMACOKINETIC STUDIES, BIOANALYTICAL METHOD VALIDATION IS CRUCIAL TO MINIMIZING RANDOM ERROR AND SYSTEMATIC BIAS, WHICH ENSURES THE QUALITY OF THE ANALYTICAL RESULTS.

Secondly, the criteria for confirmation of acceptable incurred sample repeat results needs to be considered. Typically, the degree of conformity of the original result with the ISR sample is calculated as a percentage such as

**%Difference = (ISR result – original result)/original result x 100%**

or a variant thereof. An assessment needs to be made whether the denominator comparator should be the original value obtained or the mean of the original and the ISR result. The former method assumes that the original value is correct but can yield conflicting conclusions with some pairs of numbers. For example, suppose that the original value is 100 ng/mL and the ISR result is 140 ng/mL and the limit for sample accuracy is ±30%. Then the deviation is +40%, which fails to stay within the prespecified limit. If the assayed results are swapped, such that the original value is 140 ng/mL and the ISR result is 100 ng/mL and the same ±30% limit is used, then the deviation is -28.6% which passes specification. Use of the mean value as the denominator overcomes this inconsistency. Additionally, the bioanalyst also needs to consider how to handle the calculations if the ISR sample is repeated more than once; should the individual ISR replicates each be assessed or should the mean or median of the ISR replicates be used? Another way to estimate the degree of conformity of the original result is to use a simple numerical difference expressed in the concentration units of measurement. It should be noted that the conference report does not propose a numerical limit for the percentage deviation.

Thirdly, there needs to be an evaluation of the overall study results for ISR samples. A prespecified proportion of ISR samples in a study must be within a specification limit. This limit may be defined as a ratio; for example, 67% or 2/3 of the study's ISR samples must pass. Companies also need to consider what actions to take if these limits are exceeded. Also, care should be taken to ensure that the study-level failures are not bunched at one end of the concentration range. The choice of a data processing system for bioanalytical support needs to take into account the factors and choices described above. And while there are many laboratory information management systems (LIMS) available for pharmaceutical work, a dedicated bioanalytical data system such as Thermo Scientific Watson LIMS may help to facilitate bioanalytical data processing and ISR sample selection and reporting.

## FDA Guidance

The FDA publication, "Guidance for Industry: Bioanalytical Method Validation," provides general recommendations for the validation of the bioanalytical methods used in human clinical pharmacology, bioavailability and bioequivalence studies requiring pharmacokinetic evaluation. The guidance also applies to bioanalytical methods used for non-human pharmacology/toxicology and preclinical studies. Recent FDA audits have shown that

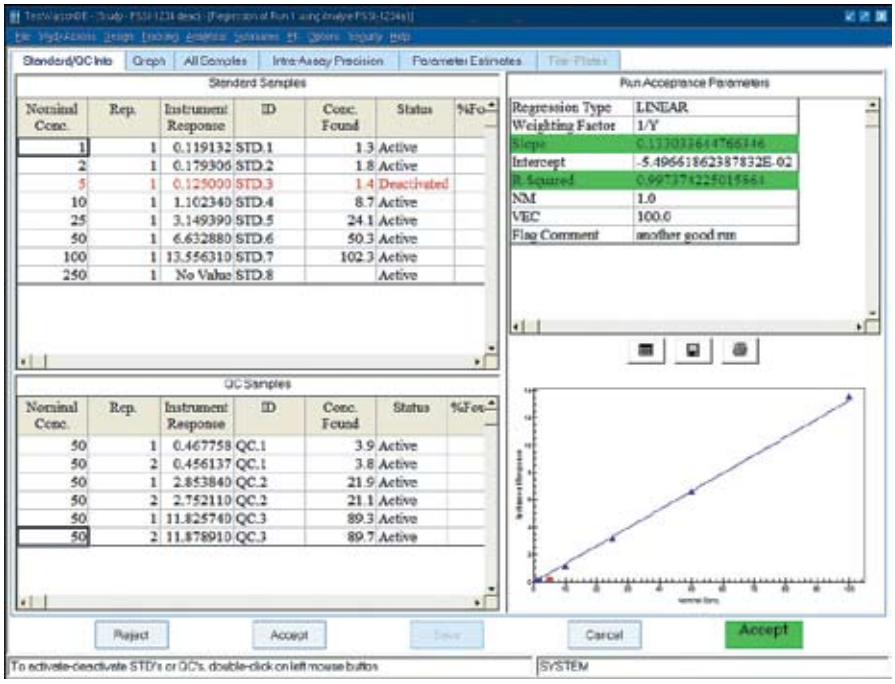


**Figure 2:** Thermo Scientific Watson LIMS analyses calibration curves from standards and back-calculates concentrations for QCs and unknowns. Configurable parameter flags alert the user to acceptability criteria. Colour-coding enhances the visual inspection of results.

ISR sometimes yielded dramatically different results, even when using a validated assay. To identify these cases, it has been determined that the reanalysis of a limited number of incurred samples should be systematically verified and should be part of assay validation.

Viswanathan explains that as bioanalytical tools and techniques have continued to evolve, and significant scientific and regulatory experience has been gained, the bioanalytical community has continued its critical review of the scope, applicability and success of the presently employed bioanalytical guiding principles. To perform accurate ISR, scientists need a solution that ensures data consistency; specifically, they require a methodology that considers both hardware and software functionality for a completely integrated process. For chromatographic assays to enhance the reliability of the ISR process, it is necessary that LC-MS instruments provide high sensitivity, precision and an increase in signal without a commensurate increase in noise. The combination will ensure consistent confirmation of the quality of the assay. To answer the software requirements, the automation of many of the manual analyses will ensure greater accuracy of ISR results and more easily satisfy FDA guidelines for the validation of bioanalytical methods.

## Harware and Software Solutions

The challenges associated with ISR apply to instruments as well as software and are related to the sensitivity and precision of the instrumentation. Instrument vendors are being asked to increase the level of precision and reliability of their LC-MS offerings to meet the evolving demands of scientists working on validating bioanalytical methods and engaged in ISR studies. Selective Reaction Monitoring (SRM) using a triple stage quadrupole mass spectrometer coupled to a high performance liquid chromatograph, or LC-MS/MS, is the most common chromatographic method for bioanalysis. Developing software and instruments with increased levels of functionality and precision will allow scientists facing these challenges to greatly improve their processes and the reliability of their submissions.

Enhancements to Laboratory Information Management Systems are currently being developed with the goal of meeting the challenges of the bioanalytical laboratory and addressing the ISR challenges by automating many of the current manual analyses. This will greatly enhance the productivity and reliability of the work that is done by scientists involved with ISR. Control of data by a laboratory information management system such as Thermo Scientific Watson LIMS provides users with the necessary workflow for the generation of analytical runs, and the importing, analysis, review and reporting of data and subsequent export of results to external systems. Multilevel security access capability, achieved by combining the mass spectrometer with a bioanalytical LIMS, offers system administrators the choice to modify user privileges from full system access to data review only. This ensures system security and audit traceability while maintaining data integrity with utmost flexibility and configurability.

## Conclusion

Incurred sample reanalysis has become an accepted way to assess the quality of bioanalytical assays. Therefore, pharmaceutical organizations and CROs need to develop and deploy industry standard best practices, SOPs to manage equipment and processes, and LIMS that deliver the functionality to meet the latest incurred sample reanalysis requirements. Scientists are looking to instrument and software providers to better streamline data processing and reporting. With improvements in both the sensitivity and precision of the instrumentation, and improved integration with the laboratory information management systems in place, scientists will experience a greatly simplified workflow, improved accuracy of results and reporting, which will result in significant time and cost savings, enabling pharmaceutical companies to bring their drugs to market faster. Pharma

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