Bioanalytical method validation

Aiming for enhanced reproducibility

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In pharmacokinetic studies, bioanalytical method validation is crucial to minimizing random error and systematic bias, which ensures quality of analytical results. Bioanalytical method validation is a controlled procedure that comprises all the vital steps to establish that a certain method is capable of producing accurate, dependable and reproducible results that are appropriate for a specific analytical application. Currently, there is a strong emphasis on incurred sample reanalysis (ISR), which serves to further validate sample reproducibility and accuracy of the reported analytical results. Thus it is very important that guiding principles for the validation of bioanalytical methods are established and circulated in the scientific community. The US Food and Drug Administration's guidance document for bioanalytical method validation, "Guidance for Industry, Bioanalytical Method Validation," advocates extensive validation of the bioanalytical methods supporting pharmacokinetic studies.
This article highlights how highly reproducible validated bioanalytical methods for the quantitative determination of drugs and/or metabolites in biological matrices can be achieved by advancing analytical sensitivity.

The Importance of Higher Bioanalytical Reproducibility – Incurred Sample Reanalysis

A validated bioanalytical method must generate reproducible and accurate data to allow valid interpretation of the studies they support. In May 2001 the FDA's Center for Drug Evaluation and Research (CDER) circulated its first official guidance for bioanalytical methods, in cooperation with the FDA's Center for Veterinary Medicine (CVM) [1]. This document, titled "Guidance for Industry, Bioanalytical Method Validation," provides general recommendations for the validation of 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.

Ensuring precision and accuracy at the lower limit of quantitation (LLOQ) of a bioanalytical method after an instrument has been repeatedly exposed to biological matrices is a challenge faced by every bioanalytical laboratory. This is especially important if ISR is performed in between preventive maintenance schedules on an instrument that has analysed several batches of clinical study samples. Ensuring results accuracy on the repeat samples is critical because the validated method now has to withstand the impact of drug, metabolites, and any concomitant medications in the matrices.

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As these components rarely occur during the method development and validation process, it is vital for the validated method to perform within the established norms of reproducibility and accuracy during incurred sample analysis.

The reproducibility and accuracy of measurements that determine the concentration of various components in incurred samples was the subject of significant debate in a recent paper published 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" [2]. Suggestions to re-assay a subset of previously assayed incurred samples and compare them to the original value to ensure the veracity of the measurement were proposed. It was specified that re-evaluation of the bioanalytical method would be required if significant deviation of more than 15-30% from the original measurement was observed for any reason.

Analytical Solutions for Increased Sensitivity

Selective reaction monitoring (SRM) assay using a triple stage quadrupole mass spectrometer coupled to a high performance liquid chromatograph, or LC-MS/MS, is the most common method for bioanalysis. Minimal sample preparation techniques such as protein precipitation or simple solid phase extraction results in the continual build-up of proteins, salts, sugars, phospholipids

and other components in a biological matrix, particularly on the analytical column, and consequently in the ion source, pushing LC-MS/MS methods to the limit.

Bioanalytical methods require extensive validation prior to performing reproducible analyses at high levels of sensitivity after repeated exposure to samples in a biological matrix. For validated bioanalysis, ensuring high levels of sensitivity helps ensure trouble-free compliance with inter-day and intra-day assay requirements, allowing the user to process thousands of real samples before scheduled preventive maintenance is needed. Sensitivity is a measure of response; the better the response or the higher the number of ions detected, the better the analytical precision. Mathematically, precision is related to the square root of the number of ions detected. Therefore, to achieve better precision, more ions must be counted. Additionally, high sensitivity allows for lower per cent coefficients of variation (%CV) a measure of reproducibility, to be delivered where they are most crucial – between the LLOQ and the low-QC level.

The main challenge is detecting the ions of interest versus the ions that constitute the chemical noise. Most commonly a triple quadrupole system with higher selectivity (specificity) as well as higher sensitivity (response) will perform better in the presence of chemical background noise, provided ion suppression has been accounted for, usually through rigorous sample preparation protocols and the use of isotopically labelled internal standards. Despite these preparatory steps high chemical background noise levels associated with complex biological matrices can severely limit precision, specificity and reproducibility.

The key is to increase sensitivity without a commensurate increase in detectable noise; this would enhance the precision and accuracy of the measurement. Sensitivity is continually eroded by the build-up of biological matrix components in the ion source and in order to regain performance, cleaning is required and usually accomplished during the lab's preventive maintenance schedules. However, an instrument that can continue to perform effectively despite repeated exposure to biological matrices offers considerable advantages, not only in saving time and money, but in ensuring the assay is robustly reproducible. A robust ion source is a key component for ensuring accurate and precise measurements over time. This ultimately bears the brunt of non-volatile

components present in bioanalytical samples. (3)

New developments have allowed the creation of ion optics sources that run on a novel radio-frequency (RF-only) driven stacked lens (S-Lens) technology to capture almost every ion and effectively transfer it into a quadrupole mass analyser. The design of such instruments means that the S-Lens technology efficiently pumps out solvent-laden gas sampled from the atmospheric region (primary source of chemical noise), focuses the ions of interest into a tight beam and delivers it to an orifice that does not have direct line of sight with the in-coming shock-wave. This precisely calculated orifice serves as a baffle between the S-Lens and the high vacuum region ensuring most of the chemical noise is pumped away while the ions are transmitted. The lack of direct line of sight keeps the ion optical path cleaner longer, thus expanding the time between preventive maintenance schedules. Productivity is increased while maintaining sensitivity.

Solutions

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. Enhancements to laboratory information management systems (LIMS) have been 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 greatly enhances the productivity and reliability of the work that is done by scientists involved with ISR processes. The challenges related to 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. Developing software and instruments with increased levels of functionality and precision allows scientists facing these challenges to greatly improve their processes and the reliability of their submissions.

Control of data by a 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. Multi-level 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.

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Conclusion

The FDA's "Guidance for Industry, Bioanalytical Method Validation" and the critical

dialogue between the industry and the FDA, communicated through several peer reviewed white papers, provides the necessary frame-work required to develop a robust bioanalytical method. Such a process serves to reduce differences in approach within the industry concerning validation and implementation of bioanalytical methods. Ultimately, method validation is a process that demonstrates that a method will successfully meet or exceed the minimum standards set by the FDA guidance for accuracy, precision, selectivity, sensitivity, reproducibility, and stability of incurred samples.

References

[1] Guidance for Industry, Bioanalytical Method Validation. Center for Drug Evaluation and Research (CDER), Internet: http://www.fda.gov/downloads/Drugs/

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