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Editorial

Summary of a recent workshop/conference report on validation and implementation of bioanalytical methods: Implications on manuscript review in the Journal of Chromatography B

Validation of bioanalytical methods has rightfully become a primary consideration when manuscripts are reviewed for their suitability for publication in the Journal of Chromatography B. The Journal publishes many assays for specific compounds in various biological matrices, and in such publications, evidence should be provided that the method is suitable for its intended purpose. The validation experiments necessary to demonstrate the method's suitability will of course vary depending on that method's purpose, and Editors and Reviewers should be sensitive to such differences. For example, quantitative analysis of a component in a single cell may well be supported by no more than very limited data on reproducibility, whilst a method developed to support studies necessary for registration of a new pharmaceutical would have to be extensively validated according to internationally accepted guidelines.

Widespread consideration of validation issues is due in part to the attention this topic has received over the past several years by the pharmaceutical sciences community and related regulatory agencies, leading to the development of a formal guidance. A primary influence on validation policy has been the proceedings of a series of AAPS/FDA Bioanalytical Workshops, the first of which was held in Crystal City, VA, USA in December of 1990, and published in 1992 [1]. This original meeting was followed up by validation workshops held for small molecules (January 2000) and large molecules (March 2000), both also held in Crystal City, VA, USA. These consensus-building conferences resulted in establishment of a final FDA guidance in May of 2001 [2]. Although not stated specifically, it is commonly thought that this guidance only applies to small molecules. Separate recommendations were proposed for macromolecules in March 2003, which were further refined in another validation workshop for macromolecules in May 2003. The latest installment of validation consensus meetings was held in May of 2006 for the purposes of reviewing and evaluating the existing practices and to clarify the FDA guidance. This workshop addressed quantitative bioanalytical methods focusing on both chromatographic and ligand-binding assays, a departure from the previously used categorization of small versus large molecules. The workshop report was recently published in the AAPS Journal [3] and can be viewed at the following web link http://www.aapsj.org/view.asp?art=aapsj0901004.

The report discusses the differences between ligand-binding assays and chromatographically based methods for small molecules and offers several justifications why ligand-binding assays should be considered differently in terms of method validation. This proposed difference is based mainly on the premise that several additional sources of variability exist for ligand-binding assays. A validation acceptance criterion of 20% precision and accuracy extended to 25% at the lower limit of quantification (LLOQ) was proposed. Further, a "total error" criterion (inaccuracy plus imprecision) of ±30% would be applied to this data. This proposed criterion is a reasonable compromise given the inherent variability of ligand-binding assays since the total error would remain the same as that established in the FDA guidance, and pharmacokinetic judgments could be made with similar statistical confidence. The quality control accuracy criterion in which 4 out of 6 controls need to meet ±20% for ligand-binding assays is more liberal, however, than the current guidance for small molecules. This has again been justified through an acknowledgement of higher variability in these methods. Other recommendations for selectivity and stability are generally consistent with the current guidance. The report also focused on several important issues that would apply to both chromatographic and ligand-binding assays. Narrowing the standard curve range or revising quality control sample concentrations was recommended to avoid the problem of a mismatch between the concentration range of samples and the established range for calibrators and controls. A procedure for assessment of carryover was suggested but the report stopped short of recommending an acceptance criterion for blank samples in carryover experiments. The need to perform metabolite screening studies in early drug development was recognized and it was recommended that incurred (samples from subjects dosed with the drug being studied) be reanalyzed to show reproducibility and accuracy of the method. Several recommendations on how results and

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procedures should be documented were presented, which were specific to drug development studies. Procedures for stability studies were put forward with very specific recommendations for how these studies should be carried out but fell short of defining what constitutes a stable analyte. The lack of a criterion for stability acceptance is especially important because it is widely thought that nominal rather than assayed values should be used for "time zero" baseline comparisons. If nominal values are used, one must consider that any criterion applied be broad enough to account for both inaccuracy and imprecision of the method (total error).

Liquid chromatography/tandem mass spectrometry (LC/MS/MS) has become the analytical method of choice for most analytical measurements in biological matrices for pharmaceutical applications. LC/MS/MS methods may demonstrate unique problems typical of a chemical reaction detector such as matrix effects from undetected components. The need for matrix effect evaluation was recognized in the FDA guidance but no specific recommendation about how this should be carried out was suggested. The current workshop report does a good job of defining the matrix effects, suggesting relevant and practical procedures to evaluate the problem and even recommends an acceptance criterion for matrix effect reproducibility. A very important topic specific to LC/MS/MS bioanalysis that is not dealt with in the workshop report is ion crosstalk from metabolites. This may be due to the difficulty of conducting crosstalk experiments. Another problem commonly associated with LC/MS/MS is its susceptibility to long term signal drift and the possibility that quantification limits may change on a run-to run basis. The establishment of an acceptance criterion for internal standard responses can address this problem, however, the workshop failed to reach any agreement on a procedure or criterion for this. Other topics that were discussed however for which the workshop participants could not reach agreement included cross-validation procedures and acceptance criteria along with some aspects of stability testing.

The proper validation of assays was described as a key issue for the assessment of manuscripts in previous editorials written by the Editors of the Journal of Chromatography B [4,5]. In these editorials, draft validation criteria were proposed for review of manuscripts submitted to the Journal. It should be noted however that these editorials, as well as the consensus reports and resulting guidelines, apply to "validated assays" in biologic matrices related to drug approval studies. In other fields, different criteria may be applied and for analytical purposes outside of a legally regulated environment, the researcher needs to decide on appropriate experiments to demonstrate fitness of the method, for the intended application. For example, Peters and Maurer published a paper in 2002 describing how requirements for validation are somewhat different in the field of forensic toxicology [6]. Further, the FDA bioanalytical guidance states that its purpose is to provide general recommendations and that the recommendations can be adjusted or modified depending on the type of analytical method used. Thus when assessing method manuscripts submitted to the Journal of Chromatography B, Editors and Reviewers should carefully consider the stated purpose of the method and the amount of validation performed. Even for manuscripts in which it is claimed the method is validated according to the FDA guidance, it is not appropriate for Reviewers to serve as an FDA auditor would, employing a validation checklist. Tables including supporting data such as sample analyte stability are not necessary to characterize the analytical method, unless stability is a significant issue in the conduct of the method.

Application of the FDA guidance may not be appropriate in many situations. For example, the FDA guidance for quality control acceptance in which 4 out of 6 quality control samples must be within 15% of their nominal concentrations, is based on a fixed range deemed to be appropriate for regulated bioanalytical studies. There are many benefits associated with a statistically derived confidence interval approach for quality control evaluation. Confidence intervals have been shown to be more efficient for error detection and more flexible for false rejection of data than the fixed interval approach described in the FDA guidance. Depending on how bioanalytical data in the broad sense will be used, a broader or narrower data rejection range may be more appropriate than that published in the FDA guidance.

Bioanalysis is not completely homogenous and reviewers must take care when assessing papers that are not involved in FDA regulated bioanalytical drug development. The impact of the guidance has been significant and a large number of submissions to the Journal of Chromatography B do involve bioanalytical drug development. At the risk of being over emphasized and applied, it is important to inform the readership of the Journal of Chromatography B of significant developments in the evolution of validation consensus. The editors of the Journal of Chromatography B will continue to be open to research papers as well as to papers describing analytical methods destined for use in regulated environments. Acceptance of research papers however is facilitated by considering the intent of the method when judging whether it is fit for its intended purpose and for publication.

References

[1] V.P. Shah, et al., J. Pharm. Sci. 81 (1992) 309.
[2] Guidance for Industry, Bioanalytical Method Validation, US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CEDER) May 2001.
[3] C.T. Viswanathan, et al., AAPS J. 9 (2007) E30.
[4] W. Lindner, I.W. Wainer, J. Chromatogr. B 683 (1996) 133.
[5] W. Lindner, I.W. Wainer, J. Chromatogr. B 707 (1998) 1.
[6] F.T. Peters, H.H. Maurer, Accred. Qual. Assur. 7 (2002) 441.

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