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Dockets Management Branch (HFA-305)
Food and Drug Administration
5630 Fishers Lane, Rm. 1061
Rockville, MD 20852

7 February 2000

RE: Docket No. 98D-1195
Guidance for Industry Bioanalytical Methods Validation for Human Studies

Dear Sir/ Madam,

We appreciate Dr. Vinod Shah's invitation to comment upon the following document which he extended at the recent AAPS Workshop on Bioanalytical Methods Validation: *Guidance for Industry on Bioanalytical Methods Validation for Human Studies.*

Pharmacia & Upjohn, as a global research and development organization whose goal is to develop novel therapeutic entities to meet unmet medical needs, has a great interest in the guidelines proposed by the FDA on these issues. Our global scientific staff involved with Bioanalytical Methods Validation has carefully reviewed the draft guidance. The following individuals are providing the attached comments and suggestions for your consideration.

Pharmacokinetics & Bioanalytical Research
Timothy Heath
Paul Bombardt
(Kalamazoo, Michigan)

Clinical Pharmacology & Pharmacokinetics
Dean Knuth
(Kalamazoo, Michigan)

Drug Metabolism Research
Chris James
(Nerviano, Italy)

Drug Metabolism Research
Lena Palmer
Ewa Wanag
(Stockholm, Sweden)

Again, thank you for the opportunity to comment. Should any clarification of our input be required, please don't hesitate to contact Jenny Peters at (616)-833-8141 who will put you in contact with the above-mentioned scientists.

Sincerely,

PHARMACIA & UPJOHN COMPANY

Jenny L. Peters
Director Regulatory Intelligence

98D-1195

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I. INTRODUCTION

We would like to recommend that the scope of the guideline be carefully defined and, in particular, that it is clarified if the document applies to bioanalytical support for non-clinical studies.

In recognition that the proposed guideline is likely to be used throughout the world and in order to avoid adding more definitions for words commonly used in many areas of analysis, we suggest adopting ICH definitions (Validation of Analytical Procedures, ICH Q2A, March 1995) as far as possible. These seem to be generally applicable.

II. BACKGROUND

The guidance should instruct what needs to be done, not how; thus, in the third paragraph, the sentences beginning with "*For minor modifications, such as ...*" to the end of this paragraph should be deleted as these spell out specific "how to" details. Enumeration of examples leads to omissions or inclusion of conditions that in the context of a particular method fall outside the category in which they are contained. For instance, "*change of the detector*" is given as a major modification requiring full validation. In the case where two UV detectors have been operationally qualified with respect to wavelength calibration and the linearity of their range, their performance is thus identical, which should obviate the need for full validation.

Within the fourth paragraph, the first two sentences beginning with "*The analytical laboratory...*" and ending with "*...21 CFR 320.29*" should be deleted as GLPs do not cover human studies. The final guidance should reference, but should not overlap, existing guidelines/requirements stated in the FDA's GLPs or GCPs, many of which are mainly administrative. The guidance should remain focused on method validation procedures and data generation using appropriate bioanalytical methods in the analytical laboratory.

Again, in the fourth paragraph, the sentence beginning with "*The SOPs should cover all aspects...*" should be modified, replacing "*SOPs*" with "*written instructions*" or "*procedures*". Collection and shipping of samples for clinical studies, especially in multicenter trials, is always described in the protocol. Often, however, several CROs are involved for the in-life portion of a study, sample shipment and storage of the samples, sample analysis and data reporting, and finally the data analysis. To expect the analytical facility to write, distribute, and monitor SOPs in this type of situation is virtually impossible and not realistic.

The fifth sentence of the fourth paragraph states *"They also should include record keeping, security and chain of sample custody...."* We recommend removing the phrase "*chain of sample custody*" yielding *"They also should include record keeping, security and accountability systems that ensure integrity of test articles, sample preparation, and analytical tools, such as methods, reagents, ...*" The term "*chain of sample custody*" implies extensive legal documentation with hand-to-hand delivery; samples shipped via air, e.g. by Fed Ex, would not be in anyone's custody, and legally would not conform to "*sample custody*" rules. Keeping the phrase *"accountability systems that ensure integrity of test articles"* would meet the spirit of the guidance but not bind the bioanalytical community to the legalistic nightmare of *"chain of custody"*.

Finally, the last paragraph in this section addresses the process for which analytical validation may be divided into. We recommend labeling as:

> *"(2) Assay Validation*
> *(3) In-Study Assay Guidelines."*

These headings are more specific and use appropriate terminology in capturing the intent of the guidelines.

III. REFERENCE STANDARD

The first paragraph under *"III. Reference Standard"* states: *"A master standard (a synthetic batch for which identity and purity are clearly established and acceptable) should be maintained for each reference standard. All subsequently synthesized batches are to be compared chromatographically with that master standard."* It is our opinion that the following recommendations be considered:

- The reference to a Master Standard should be deleted from the guidance document. A Master Standard is more applicable to the GMP environment where compound supply is not an issue. In addition, the guidance does not address what tests are needed to qualify a master standard.
- In the clinical setting, reference standards, particularly with respect to metabolites, are often in short supply (10 to 100 mg). The implication from the guidance is that assays for metabolites should not be performed if a master standard cannot be produced. There are also cases where a slightly dissimilar standard, e.g. a different salt or crystalline form of a compound, may be transparent with respect to the bioanalytical method and therefore can be used. Additionally, applying the master standard criteria to internal standards is not generally warranted.

We propose the following to be inserted into the guidance:

> *"Well characterized reference standards should be used. The source and lot number, certificates of analyses (when available) and/or internally or externally generated evidence of identity and purity should be furnished for each reference standard."*

This paragraph goes on to state that *"All reference materials should be checked prior to use to determine if there are significant interfering chromatographic peaks at the retention time of the analyte and/or internal standard, using the analytical procedure to be used in the study."* We feel that the guidance should clarify what reference materials are meant by this statement, i.e. concomitant medications, reference standards of analyte(s) and internal standard, and/or reference standards of known degradation products or impurities.

Finally, it is unclear from the guidance as to what frequency the reference materials have to be checked prior to use and what actions are implied? Are corrections allowed? The use of the terminology "interfering peaks" is not relevant for immunoassays.

IV. PRE-STUDY VALIDATION

The title of *"IV. Pre-Study Validation"* does not capture the perspective that validation may be a continuing process. Given that issues concerning stability and specificity (selectivity), for example, are sub-headings, a more appropriate title for this section would be *"IV. Assay Validation"*. Validation can and should be viewed as an on-going process, as in the evaluation of incurred samples. *"Pre-Study Validation"* gives the incorrect and unachievable notion that all validation must be complete prior to the onset of sample analysis.

The paragraph also states that *"Pre-study validation should include analytical method development and documentation."* We recommend eliminating *"analytical method development"* from this sentence. Method development efforts are research-oriented and should not be included as a component of validation. In addition, the phrases *"(4) quality control samples,"* and *"(6) acceptance criteria"* should be eliminated from the last sentence, as these are not performance parameters.

A. Specificity

Our recommendation is that the term *"specificity"* be replaced with *"selectivity"*, not only here but also throughout the entire document (Persson 1998).

A sentence at the end of the first paragraph in this section stating *"The potential impact of ionization suppression effects in mass spectrometry based assays should be considered."* should be included (Fu 1998).

The second paragraph in the section currently entitled "*Specificity*" addresses the need to evaluate the presence of interference in blank samples. We agree that validation of methodology requires consideration of interference; however, it is difficult and not appropriate to define how to conduct such evaluation. The presence of an interference and the ultimate impact it has on the ability to generate valid data is dependent on the 'consistency' of the interference. The precision and accuracy of the method at the LLOQ which is determined in the validation addresses the impact of the interference.

B. Calibration Curve

In the first paragraph, "*same biological matrix*" should be defined – does that imply that it should be taken from patients (e.g. hepatic or renal failure, diabetics, etc.)?

The first paragraph also states that "*Concentrations of standards should be chosen on the basis of the concentration range expected in a particular study.*" While it is recognized that ideally the curve range selected should be close to the range in concentrations anticipated for a clinical study, this is not always practical. For first-time-in-man studies, concentrations can only be roughly approximated. For drug interaction studies, concentrations may be significantly decreased or increased. Concentrations also vary between a normal patient population, and in diseased, hepatically or renally impaired patients. Thus, we recommend that as long as a method demonstrates acceptable performance over the curve range validated, the method may be used in a fashion that is reasonable for a given study.

The number of calibration standards in the draft guidance is listed as *"five to eight"*. This should be stated as a minimum. Also, these numbers conflict with the requirement stated under the linearity section for "*four out of six*" calibration standards. The number of acceptable standards should be stated as a minimum percentage as this is more easily implemented when the numbers of calibration standards are not in multiples of three.

1. Limit of Quantitation (LOQ)

The following sentence is included in this section: " *No interference present in blanks at the retention time of the analyte at this concentration (LLOQ), or typical response at this concentration at least five times greater than any interference in blanks at the retention time of the analyte.*" The requirement that typical response of the LLOQ should be five times greater than any interference present in blank matrix should be removed from the guidance. Our proposal is that the guidance should be amended to read: "*Any interference of the analyte should not preclude accurate and precise determination of the analyte at the LLOQ.*" If the LLOQ is validated with an accuracy and

precision of ≤20% and performs acceptably in the presence of an interfering response, the draft guidance's requirement may not be relevant.

The guidance does not address the assay of endogenous components where a background level of 'interference' is expected. The expected physiological concentration ranges are usually well established for endogenous compounds.

A calibration standard below the LLOQ could be considered to better define the true LLOQ when required by the analytical technology (i.e. immunoassays).

The statement "*Analyte peak (response) identifiable, discrete, and reproducible...*" should be stated as the mean response, and not as individual results.

2. Linearity

We recommend that wording in the first paragraph be as in the first conference report (Shah 1992). The requirement of "*minimal or no weighting*" should be eliminated.

Correlation coefficient is an insensitive measure and should be dropped as an acceptance criterion. The correlation coefficient measures the degree of correlation, not the linearity of two variables, and usually not in the case where one variable is fixed (Bolton 1984).

C. Precision, Accuracy, and Recovery

We propose the addition of a statement which reflects the provision of accepting methods with precision and/or accuracy >15%. One of the requirements of being able to generate valid data is validating the methodology. However, in rare instances as dictated by the scope of the study, a method with performance characteristics outside of the 15% norm may still be acceptable. With this rationale, we propose the addition of the following sentence after the paragraph regarding the accuracy of a method.

"In exceptional circumstances, methods with CVs>15% (20%) and/or an accuracy deviating by more than 15% (20%) may be scientifically justified and in accordance with the scope of the study."

We suggest eliminating the paragraph on recovery. While it may be important to evaluate the recovery/extraction efficiency of an analytical method, this exercise should be examined during method development. Establishing criteria for

recovery/extraction efficiency is not relevant to validation and therefore should not be included in the guidelines.

D. Quality Control Samples

We recommend changing the title of this section to "*Validation Control Samples*". The first sentence which reads "*Pre-study validation of analytical method should be carried out using at least three batches of biological matrix, where each batch is collected from a different source.*" should be modified. The necessity of examining different batches of biological matrix is method-dependent. Consequently, this sentence should be modified to read "*The evaluation of multiple batches of biological matrix during assay validation of the analytical method should be considered, to address the impact of such issues as ionization suppression when using LC-MS.*"

The requirement that a reference standard be included in each batch should be eliminated.

Guidelines should be provided with respect to ensuring that no error occurred in the preparation of stock solutions. However, there are different ways to evaluate. It is not necessary to require that, "*Quality control samples at concentrations noted below should be made from a stock solution separate from that use to prepare standards*", as this may introduce an avoidable bias in the data. We recommend that this statement be changed to:

> *"Replicate stock solutions should be prepared with subsequent evaluation for assessing their congruence."*

E. Stability

We acknowledge that stability evaluation is a critical step with respect to method validation and that each of the 5 sections highlighted under "*Stability*" requires attention to ascertain analyte stability. However, much of the descriptive text in this section specifies how to conduct evaluation of stability. Given that there are multiple valid approaches to assess stability, the guidelines for stability should tell us what to address (Freeze/Thaw, Short-Term Room Temperature, Long-Term Stability, Stock Solution Stability and Autosampler Stability), but not how we should conduct the evaluation (Dadgar 1995; Timm 1985; Causon 1997). Additionally, the requirement of measuring stability relative to various container systems and matrices where the expected difference is unlikely to be meaningful, e.g. polypropylene versus polystyrene or sodium heparin versus lithium heparin, should be removed from the guidance. We also recommend that sections 1, 2, 3, and 5 be modified as follows:

1. Freeze and Thaw Stability

The second sentence beginning with *"At least three aliquots..."* should be modified to indicate that samples should be frozen and thawed in the same manner as the conduct of a particular method and anticipated study conditions. Thus, stability samples should be stored at the intended storage temperature and thawed in accordance with how study samples will be treated. This cycle should be repeated two more times and then analyzed on the third cycle (Shah 1992, Buick 1990).

2. Short Term Room Temperature Stability

The title should be modified to *"Short-Term Stability"* since, for compounds with known instability problems at room temperature, samples would always be kept at lower temperatures for processing. The new title allows flexibility in the study conditions to more closely describe the intended processing conditions.

The sentence beginning *"Three aliquots of each of the low and high concentrations..."* should be modified to indicate that samples should be thawed in the same manner as study samples and maintained under ambient conditions for the expected duration in the intended study (Buick 1990).

3. Long-Term Stability

The last sentence which states, *"The concentrations of all the stability samples should be compared to the mean of back calculated values...."* should be deleted. Numerous techniques for evaluating stability are valid and documented in the literature. Alternate methods may be applicable in any given instance. For example, the method of Timm (Timm, 1985) employs a statistical procedure which compares fresh and stored response confidence intervals. With respect to comparison of study condition storage versus ultra cold storage as the reference, Dadgar et al (Dadgar 1995) have shown that this also is a valid approach.

5. Autosampler Stability

The last two sentences in the first paragraph should be combined to read:

"The stability of both the drug and the internal standard should be assessed over the anticipated run time for the batch size to be used in studies."

F. Acceptance Criteria

We propose that with respect to acceptance criteria, a method is considered fully valid when it meets the precision and accuracy requirements. The criteria established under "S*ensitivity*" should be captured under *"Precision and Accuracy*" and not as a separate sub-heading. While "*Specificity*" and *"Stability"* are critical issues, these are adequately addressed elsewhere in the guidelines and need not be evaluated with respect to Acceptance Criteria. Acceptance Criteria should be viewed solely based on accuracy and precision of applying the analytical methodology for analysis of samples.

We also propose that the following statement be added:

"In exceptional circumstances, methods with CV's >15% (20%) and/or an accuracy deviating by more than 15% (20%) may be scientifically justified and in accordance with the scope of the study."

V. IN-STUDY VALIDATION

We would like to see the section title changed to **"*In-Study Assay Guidelines*".** Reporting of bioanalytical data to regulatory agencies assumes that a method has undergone validation and that written procedures for the conduct of analysis and reporting of data exists.

The second paragraph, fourth sentence, should state:

"The calibration curve should cover the same concentrations, including the calibrators at the LOQ (upper and lower), as defined in the "Assay Validation."

The requirement that all study samples from a subject should be analyzed in a single run should be stricken. If the method has documented acceptable inter-assay variability, this should not be a requirement. This is not feasible for some study designs (e.g. population PK, therapeutic drug monitoring).

The third paragraph should address acceptance criteria for calibration standards. Our recommendation for acceptance criteria: that back-calculated values must be within ±15% of the nominal value, except at the LLOQ, which may be within ±20%. At least 75% of calibration standards must be acceptable, or the curve is rejected. Again, the number of acceptable standards should be stated as a minimum percentage, as this is more easily implemented when the numbers of calibration standards are not in multiples of three. Also, if the rejected calibration standard(s) are at the low or high end of the curve, the curve can be truncated in accordance with the laboratory's SOP.

It should be stated that at least 66% of all QCs must be within 20% of nominal and a minimum of half the QCs at each level meet this criteria. A minimum of duplicate QC's at three levels (low, mid and high) is required.

VI. DOCUMENTATION

We agree that as stated in the second paragraph, an SOP should state situations under which re-assay of samples is permitted. However, requiring that that re-assays be done in triplicate is not practical. In some instances ethical considerations prevent the collection of larger volume of biological matrix, such as the case in pediatric studies. Re-assays of samples from such studies may not be possible. The widely accepted method of Lang (Lang, 1991) is recommend for reassay decision making.

The inclusion of serial chromatograms of 20% of subjects is unnecessary, as this will add little useful information beyond what would already be expected in the report and imposes a significant cost and regulatory burden of paperwork on each study. We suggest that only representative chromatograms of subjects, calibration standards, and QCs should be provided. All other chromatograms, raw data and SOPs are always available for audit purposes.

ADDITIONAL REFERENCES

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Dadgar D, and Burnett PE. "Issues in evaluation of bioanalytical method selectivity and drug stability," *Journal of Pharmaceutical and Biomedical Analysis* 1995; 14: 23-31.

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Lang JR, Bolton S. "A comprehensive method validation strategy for bioanalytical applications in the pharmaceutical industry-1. Experimental considerations", *Journal of Pharmaceutical and Biomedical Analysis*, 1991; 9(5): 357-61.

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Shah VP, Midha KK, Dighe S et al. "Analytical methods validation: bioavailability, bioequivalence and pharmacokinetic Studies" Pharm Res 1992; 9:588-592.

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