Calibration & Validation Group

WORKSHOP REPORT
The Canadian Workshop on the Crystal City AAPS/FDA White Paper
May 17th 2007 Montreal, Quebec, Canada

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(Workshop Chair)

Introduction

The Canadian Workshop on the Crystal City AAPS/FDA White Paper was held on May 17th in Montreal (QC), Canada. This event was organized by the Calibration & Validation Group (CVG is a scientific non-profit organization based in Toronto) as a 1-day full immersion workshop for contract research organizations and pharmaceutical companies involved in providing bioanalytical data for bioavailability, bioequivalence, pharmacokinetic, and comparability studies.

The following topics were lectured by recognized experts from regulatory and industry and enriched by interactive discussion from audience:

- "Calibration Curve and QC Ranges" (Eric Ormsby, A. Manager, Office of Science, Health Canada-Therapeutic Product Directorate, HC-TPD, and 2006 Crystal City AAPS/FDA White Paper Contributor)
- "Incurred Sample Re-analysis" (Dr. Saleh Hussain, Director Bioanalytical Operations, Anapharm, A PharmaNet Company)
- "Matrix Effect and Hemolysis Effect for MS Based Assays" (Dr. Nicola C. Hughes, Director Bioanalytical Laboratory Biovail Contract Research, A Division of Biovail Corporation)
- "Approaches to Assay Specificity in LC-MS/MS Methods" (Xia Yin, B.Sc, Scientific Leader, Bioanalytical Method Development, Bioanalytical Development, BCD, Apotex Inc.)
- "Confirmatory Re-analysis of Study Samples – An Interactive Session"(Dr. Mario L. Rocci Jr, CEO Prevalere Life Science Inc, AAPS Advisor, 2006 Crystal City AAPS/FDA White Paper Editor)
- "Metabolite Issues" (Dr. Fabio Garofolo, VP Bioanalytical Services, Algorithme Pharma Inc., CVG Regulatory Advisor, Chair of the LC-MS Discussion Group in Toronto, CVG LC-MS Instructor across Canada)

Attendance

106 professionals (experts, managers, directors, and executives from contract research organizations, pharmaceutical companies, and Canadian regulatory agency) mainly from Canada, but also from USA and Europe attended this workshop. The attending companies were: Aegera Therapeutics; Agilent Technologies; Algorithme Pharma; Anapharm; Apotex; Ba - Cetero Research; Barr Laboratories; Baxter Healthcare; Bio Pharma Services; BioDynamics Research Limited; Biovail Contract Research; Cangene Corporation; Cantest; CEDRA Corporation; Centre de Recherche du CHUL; Charles River

Laboratories; ConjuChem Biotechnologies; Custom Biologics; Diteba Research Laboratories; Eliapharma Services; Genpharm; GlaxoSmithKline; Health Canada – TPD; ITR laboratories Canada; Lek Pharmaceuticals d.d. (Sandoz); MDS Pharma Services; MDS Sciex; Merck Frosst Canada; MethylGene; Neurochem; NoAb BioDiscoveries; PainCeptor Pharma; Pharmalytica Services; Pharmascience; PRACS Institute - Cetero Research; Prevalere Life Science; QLT; Quest Pharmaceutical Services; Spark Holland; Theratechnologies; Thermo Fisher Scientific; Université de Montreal; University Health Network, Toronto General Research Institue; Varian Canada; ViroChem Pharma; Waters Limited; Watson Laboratories.

Goals and Objectives

This workshop focused on discussing, reviewing, sharing perspectives, providing potential solutions in order to agree upon consistent approaches on the main "hot topics" presented in the 2006 Crystal City White Paper published in the AAPS Journal, and available on line at: http://www.aapsj.org/view.asp?art=aapsj0901004

This white paper summarizes the conclusions of the 3rd FDA/AAPS Bioanalytical Workshop, held on May 1-3, 2006 in Arlington, VA, USA.

The following topics were suggested for discussion by the audience and CVG members both on-site and on-line:

- What is your approach for incurred samples reproducibility?
- Matrix Effects and Hemolysis Effect: How and why?
- Ion suppression and matrix effect: Do we need full or partial validation for the same compound in different species?
- Autosampler Stability and re-injection reproducibility: are you still using a fresh curve?
- Are standards being set by 483s and not by consensus, i.e. regulating by 483? Overreaction to avoid future 483s.
- Can storage at -70^{o}C and -80^{o}C be considered equivalent?
- Changing type of anticoagulants: what validation parameters should be evaluated?
- Contamination Criteria: What criteria are you using?
- Acceptance of nonlinear calibration models? How much "quadratic" is acceptable?

However, due to time constraints, only the first topic on incurred sample reproducibility was thoroughly discussed. In the next paragraph, the conclusions of the discussion on incurred sample reproducibility have been reported.

Discussion on Incurred Sample Re-analysis

Topic Introduction: Incurred samples have received great interest recently for assessing the reliability of bioanalytical methods used for bioavailability, bioequivalence, pharmacokinetic, and comparability studies. Reproducibility using incurred samples needs to be performed, but guidelines on how to perform it are not well defined yet.

The FDA message at the 3rd Crystal City Workshop on Quantitative Bioanalytical Method Validation (May 1-3, 2006) was clear and straightforward: 50% of the BE studies audited

in 2005 were reported to have significant bioanalytical deficiencies. Moreover, FDA investigators have seen examples where repeat analysis of incurred samples yielded a 30% to 80% difference in the analyte concentration results.

During the Canadian Workshop on the Crystal City AAPS/FDA White Paper, the most common approaches presently used in the industry to test incurred sample reproducibility were clearly identified and their advantages and disadvantages were thoroughly discussed.

Workshop conclusions:

1. **1992 HPB Canadian Guidance.** According to this guidance, 15% incurred samples should have been randomly selected and re-analyzed, though the re-assay values were not used in statistical analysis (Drugs Directorate Guidelines, “Conduct and Analysis of Bioavailability and Bioequivalence Studies"; Health Protection Branch, Health and Welfare Canada, 1992). However, the Therapeutic Products Directorate of Health Canada (TPD-HC) revoked the incurred sample re-analysis in 2003. The workshop audience agreed in considering the enormous amount of data accumulated by TPD-HC from 1992 to 2003, which is a very important source of information to evaluate the validity and efficacy of the incurred sample re-analysis approach retrospectively. However, later during the workshop report preparation, it was stated that TPD-HC, as a regulatory body, cannot use data from submissions for other purposes. Hence, it was suggested that the sponsors of the submission or the CROs, with the sponsor’s permission, recapitulate the results of these 15% repeats and send their comments in a non-confidential, scientific format directly to TPD-HC and/or to CVG. Then, TPD-HC and/or CVG could meaningfully summarize all these results to verify if they might be helpful. For instance, these results could be useful to evaluate if it is necessary to reconfirm incurred sample repeatability when moving from phase 1 single dose pharmacokinetic studies to multiple dose, patient studies, drug interaction studies or combination formulation studies.
2. **Incurred Single Sample Re-analysis.** This approach may indicate stability or specificity problems not apparent with normal QC samples. It is a real life situation, which tests the full profile and samples for many individuals. However, performing repeat measurements of the samples is costly and time consuming. Moreover, appropriate statistical methods should be defined to determine the level of reproducibility of incurred samples (e.g., QC Acceptance Criteria, Linear Regression and Confidence Interval). Incurred sample selection and percentage of samples re-assayed should be defined and be based on analyte concentration, patients population, special population, metabolism and clearance. Sample re-assay should be performed during and after sample analysis to better evaluate the stability of the study samples. Specific actions, if any, should be taken, once the re-analysis of incurred samples is completed and the results have been evaluated. If the results are contradictory, perhaps a confirmatory third analysis could be required.
3. **Pooled Sample Approach.** This approach is used to obtain low and high concentrations sets and measure them in replicates over several different batches. It can evaluate stability and measure both intra- and inter- batch precision. It also

provides sufficient material to test stability over time by pooling samples from day 1, re-assaying them after 2 weeks, and re-testing again after a longer storage time. However, pooling the study samples may decrease the non reproducibility of the method because it may reduce the anomalies and variability coming from individual study animals, subjects and patients. Hence, on the pooled sample approach, the overall workshop attendees' opinion was negative. Moreover, one scientist in the audience clearly stated that he did a study and confirmed, at least in his experiment, that pooling can indeed dilute potentially interfering substances in the matrix and give erroneous results.

4. **Pre-Study Approach.** This approach is based on the importance of performing a rigorous method development by using incurred samples obtained from a pre-study (3-5 subjects pilot study) to evaluate all the parameters which may influence the reproducibility such as:
    - Metabolites converting to parent
    - Endogenous compounds
    - Formulation
    - Special populations
    - Special studies
    - Protein binding differences in patient samples
    - Sample inhomogeneity
    - Matrix effects from high concentrations of metabolites
    - Concomitant medication interfering with the analytes
    - Variable recovery between analyte and internal standard in incurred samples
    - Decomposition products

    The major advantage of this approach is that it does not put at risk the study results and allows testing the incurred sample reproducibility before starting the sample analysis. Furthermore, this approach produce an in-depth understanding of the bioanalytical method before using it by thoroughly studying the behavior of drug and metabolite(s), various matrix effects and concomitant medications in incurred samples. Indeed, the 1992 Canadian guidance has already been suggesting this approach (pre-study verification with incurred samples) for all the studies in which the samples of blood were insufficient for duplicate analysis. However, during the workshop, it was pointed out that the IRBs might not give approval to dose subjects for collecting pre-study samples and that though this might work for healthy volunteers, there will still be the same reproducibility questions raised once patient studies begin, since the matrix of patients may be substantially different from normal subjects. Indeed, it will be very difficult to use a pre-study approach in patients.

In conclusion, even if during the workshop the discussions on the approaches taken to evaluate incurred sample re-analysis were considerable, the majority of the attendees agreed that the approach that had been adopted most often, since the Crystal City III meeting, was the repeat of individual samples from a given study, to cover both high and

low analyte concentrations. The acceptance criteria of assay reproducibility using this approach were based on 2/3 of the reportable values falling within 20% of the initial values (often in addition with other pre-defined statistical criteria). The audience in general understood the strong and clear rationale behind the FDA's wish to carrying out this type of re-analysis, but also agreed that the number of samples should not be as high as the 15% as previously recommended by the TPD-HC. The number of samples suggested during the Canadian Workshop for this type of re-assay approach was as low and 10-15 (randomly selected) samples up to 1 -2 complete batches of samples (50 to 100 incurred samples). At the workshop and during the preparation of workshop report, many laboratories stated that they have been carrying this approach since May 2006.

Follow up

The audience of this workshop has unanimously requested a follow up meeting to discuss the topics that were not evaluated during this workshop and to have an update on how industry and regulatory agencies worldwide are handling the incurred samples reproducibility. Hence, CVG is organizing **The 2nd Canadian Workshop on the Implications of Crystal City AAPS/FDA White Paper** that will be held on **April 2008** in **Montreal** *(Detailed registration information coming soon!).*

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