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A Global GLP Approach to Formulation Analysis Method Validation and Sample Analysis

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Abstract

Nonclinical pharmacokinetic (PK) and toxicokinetic (TK) toxicology safety studies are performed using good laboratory practice (GLP) regulations to ensure the availability of safe medicines. International GLP regulations uniformly require that dose concentration, homogeneity/uniformity and stability be known prior to administration. However, the United States Food and Drug Administration (US FDA) and the Organisation for Economic Co-operation and Development (OECD) both confirmed that GLPs do not apply to validation of analytical methods used to determine the concentration of GLP test article in drug dosage forms. It is our assertion that the outcome of nonclinical toxicology safety studies is inherently dependent upon accurate and precise dose formulations. In this paper, we attempt to provide supporting evidence as to why formulation method validation and sample analysis for supporting nonclinical toxicology studies should be consistently conducted under the framework of GLP principles across the globe. GLP studies are planned, performed, monitored, recorded, reported and archived according to a protocol, study plan or standard operating procedure (SOP) which is authorized prior to performing the experiments. All applicable experimental parameters and associated acceptance criteria are pre-defined. The FDA asked for responses to the Advance Notice of Proposed Rulemaking for 21 CFR Part 58 GLPs for Nonclinical Laboratory Studies [Docket No. FDA–2010–N–0548] on December 21, 2010. Several comments were received stating that guidance regarding the validation of formulation analysis methods and subsequent use for supporting GLP toxicology study sample analysis is warranted at this time and should be conducted consistently. Adherence to GLP principles for method validation and sample analysis would inherently improve the quality of nonclinical safety studies. Furthermore, the recently published White Paper titled, "Nonclinical dose formulation analysis method validation and sample analysis" should be the keystone of this effort.

Keywords: GLP; Regulatory; Nonclinical; Pharmacokinetic; Toxicokinetic; Formulation; Formulation Method Validation; Formulation Sample Analysis; Acceptance Criteria

Abbreviations: AAPS: American Association of Pharmaceutical Scientists; ADME: Adsorption Distribution Metabolism Excretion; API: Active Pharmaceutical Ingredient; AS: Autosampler (Post-Processed); BFG: Bioanalytical Focus Group; BT: Bench Top (Pre-Processed); cGMP: Current Good Manufacturing Practice; CMC: Chemical Manufacturing and Control; CoA: Certificate of Analysis; CVG: Canadian Calibration and Validation Group; EMEA: European Medicines Agency; EPA: Environmental Protection Agency; FDA: United States Food and Drug Administration; FIFRA: Federal Insecticide, Fungicide and Rodenticide Act; F/T: Freeze/Thaw; GBC: Global Bioanalysis Consortium; GCC: Global Contract Research Organization Council; GLP: Good Laboratory Practice; HPLC: High Pressure Liquid Chromatography; ICH: International Conference on Harmonisation; JMHW: Japanese Ministry of Health and Welfare; JMAFF: Japanese Ministry of Agriculture, Forestry, and Fisheries; k': Capacity Factor; LC-MS: Liquid Chromatography Mass Spectrometry; LLOQ: Lower Limit of Quantification; MHRA: Medicines and Healthcare Products Regulatory Agency; MSDS: Material Safety Data Sheet; N: Theoretical Plates; OECD: Organisation for Economic Co-operation and Development; PK: Pharmacokinetic; PR: Percent Recovery; QC: Quality Control; R^2: Coefficient of Determination; RSD: Relative Standard Deviation; SOP: Standard Operating Procedure; SST: System Suitability Test; STD: Standard; T: Tailing Factor; TK: Toxicokinetic; t_R: Retention Time; TSCA: Toxic Substance Control Act; UV: Ultraviolet

Introduction

New pharmaceutical products require significant resources from concept to final market introduction. Every step along the way is paved with trials and tribulations. A successful new drug application requires a thorough nonclinical (also known as "preclinical") toxicology safety package (e.g., adsorption, distribution, metabolism, and excretion (ADME), pharmacokinetic (PK) and toxicokinetic (TK)), a successful clinical program (Phases I, II, III and IV), and a comprehensive chemistry, manufacturing and controls (CMC) package. All toxicology safety packages are highly dependent upon accurate and precise analytical methods for quantifying the drug dosage formulations which are administered to the hosts. If the vehicle is free of interference, the dose formulations are carefully prepared according to the batch records, and the aliquots are stored appropriately, then the dose formulations should be at the correct concentrations when administered to the hosts. However, attention to detail alone is not enough to ensure a successful formulation or regulatory compliance.

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For Good Laboratory Practice (GLP) regulated studies, the formulation doses must be verified for concentration, uniformity (i.e. homogeneity) and stability [1-13]. Explicit regulatory guidance does not currently exist for nonclinical dose formulation analysis method validation or sample analysis. In fact, there has been minimal global regulatory or industry emphasis regarding how to conduct GLP formulation dose analysis in support of nonclinical studies. The White Paper titled "Nonclinical dose formulation analysis method validation and sample analysis" [1] was intended to provide a consensus opinion regarding method validation and sample analysis for nonclinical GLP regulated studies. The only GLP regulatory guidances published are in regards to bioanalytical method validation [14-21]. There has been pronounced emphasis on global harmonization of bioanalytical studies through the efforts of international organizations such as the American Association of Pharmaceutical Scientists (AAPS) Bioanalytical Focus Group (BFG), the Canadian Calibration and Validation Group (CVG), the Global Bioanalysis Consortium (GBC) and the Global Contract Research Organization Council (GCC) [22-38]. Undeniably, regulatory guidance for the validation of analytical procedures has been developed for final active pharmaceutical ingredients (also known as API or drug substances) and final drug product formulations (also known as drug products) testing under current Good Manufacturing Practice (cGMP) regulations [39-44]. There has been progress made towards harmonizing cGMP API and drug product method validations [45,46]. There are also some GLP / cGMP regulation comparative documents available [47,48]. However, this lack of GLP regulatory guidance results in nonclinical GLP formulation analysis laboratories relying on regulations which are neither fit for purpose or phase appropriate for conducting formulation method validations and subsequent sample analysis (for example, Bioanalytical GLP or cGMP).

The FDA recently requested responses to the Advance Notice of Proposed Rulemaking for 21 CFR Part 58 GLPs for Nonclinical Laboratory Studies [5]. Over 160 comments were received from the pharmaceutical and bioanalytical industry, contract research organizations, and others. Many of the respondents stated that consistency regarding the validation of formulation analysis methods and subsequent use for supporting GLP toxicology study sample analysis is warranted at this time. The White Paper titled, "Nonclinical dose formulation analysis method validation and sample analysis" [1] has been reviewed by well over one thousand readers, and several global organizations have begun aligning their SOPs with the fundamental recommendations. We believe that the time is ripe for a global approach for conducting formulation method validation and sample analysis.

Nonclinical PK and TK formulation method validation studies typically include the parameters of dose concentration range, system suitability, method linearity, accuracy and precision, specificity / selectivity, carryover, sensitivity, pre-processed stability (bench top (BT) stability), post-processed stability (autosampler (AS) stability), short term stability and long term stability. The acceptance criteria for each parameter are defined in study protocols, study plans, or standard operating procedures (SOPs) in advance of study execution. The dose formulation samples are not true "unknowns", since nonclinical toxicology studies are performed at a target (nominal) dose concentration range. This paper attempts to provide recommendations of best practices on a global harmonized basis with proposed acceptance criteria for nonclinical dose formulation method validation and sample analysis. Like the White Paper [1], this paper will focus on small molecules and the use of high pressure liquid chromatography with ultraviolet or mass spectrometry detectors (HPLC-UV, LC-MS).

Present status of GLPs

All across the globe, industry in general is looking for a better solution to the work that needs done in today's global economy. Formulation analysis assessments supporting nonclinical toxicology studies must be efficient, effective and compliant. As each calendar year passes by, the world seems to get a little smaller and more of us find ourselves asking the same question; "I can't find any guidance on that, what do I do?" In the realm of GLP we are constantly reminded to refer to the basics, the predicate rules.

All GLP texts, regardless of their origin, stress the same five important common themes: resources (organization, personnel, training, facilities and equipment), rules (protocols and written procedures), characterization (test items and test systems), contemporaneous documentation (raw data, final report and archives), and quality assurance. The information provided in Table 1 lists pertinent definitions for key GLP regulatory aspects [1-13]. All GLPs apply to the chemical procedures used to characterize the test and control substances/items/articles - the pertinent information must be documented; for example using a relevant certificate of analysis (CoA; identity, strength, purity, stability, composition and uniformity, as applicable) and material safety data sheet (MSDS; safe handling, storage and disposal) documents. All GLP texts contain similar language regarding the mixtures of test and control substances/items/articles with carriers/excipients/vehicles. No matter what semantic you prefer or require, the purpose is still the same; testing by an appropriate analytical method shall be conducted using a qualified, calibrated and maintained system, and conducted to determine the following properties of the mixture: uniformity (homogeneity), concentration and stability. Concentration assessment is required periodically, but not on every prepared formulation. Homogeneity assessment is required on suspensions, but is not required on true solutions. GLP is just a framework system to guide you through these five very important, yet common themes. Compliance with GLP principles is intended to assure the quality and integrity of the nonclinical toxicology safety data.

Discussion and Conclusions

Method validation

The White Paper titled "Nonclinical dose formulation analysis method validation and sample analysis" [1] presented a mechanism by which analytical methods used for dose formulation analysis could be validated in order to comply with multiple regulatory agencies. In order to accomplish this, the type of validation required must first be determined. The type of validation is based on the phase of the compound and status of any existing methods. Table 2 lists the experiments needed for each type of method validation.

An early phase compound is one being used in a study lasting ≤ 3 months and may be limited in availability. A full validation is performed when a method is being instituted for the first time for regulated analysis and is the most comprehensive validation type. A partial validation is performed when there is a change made to a validated method that may include changes to dose range or vehicle composition. A transfer validation is performed when a validated method is being performed by a second laboratory as written.

Nonclinical Laboratory Studies	**FDA (58.2 (d)):** Nonclinical laboratory studies are in vivo or in vitro experiments in which test articles are studied prospectively in test systems under laboratory conditions to determine their safety; are exclusive of studies which utilize human subjects or clinical field trials in animals; exclusive of exploratory studies for efficacy; and are exclusive of studies to determine physical or chemical characteristics of a test article. **EPA (792.3):** Study means any experiment at one or more test sites, in which a test substance is studied in a test system under laboratory conditions or in the environment to determine or help predict its effects, metabolism, product performance (efficacy studies only as required by 40 CFR 158.640), environmental and chemical fate, persistence and residue, or other characteristics in humans, other living organisms, or media. The term "study" does not include basic exploratory studies carried out to determine whether a test substance or a test method has any potential utility. **OECD (Section I, 2.3.1.):** Non-clinical health and environmental safety study, henceforth referred to simply as "study", means an experiment or set of experiments in which a test item is examined under laboratory conditions or in the environment to obtain data on its properties and/or its safety, intended for submission to appropriate regulatory authorities.
PK Studies	A study of how a medicine is handled by the body, usually involving measurement of blood concentrations of drug and its metabolite(s) (sometimes concentrations in urine or tissues) as a function of time. Pharmacokinetic (PK) studies are used to characterize absorption, distribution, metabolism and excretion of a drug, either in blood or in other pertinent locations. When combined with pharmacodynamic measures (a PK/PD study) it can characterize the relation of blood concentrations to the extent and timing of pharmacodynamic effects.
TK Studies	Drug safety evaluations consisting of standard animal toxicology studies for the assessment of drug exposure: hazard screens or systemic toxicology.
CHMP	The Committee for Medicinal Products is the EMEA's committee responsible for elaborating the agency's opinions on all issues regarding medicinal products for human use.
FDA	FDA regulation (21 CFR Part 58) for studies regulated under the Federal Food, Drug, and Cosmetic Act and the Public Health Service Act. FDA regulations apply to nonclinical laboratory studies conducted for test articles studies in test systems under laboratory conditions to determine their safety.
OECD	Organization for Economic Cooperation and Development (OECD) GLPs Section 8(8.1) regulation (ENV/MC/CHEM(98)17) the OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 1. OECD regulations apply to non-clinical safety studies to obtain data on the properties and/or safety with respect to human health and/or the environment of the pharmaceutical products, pesticide products, cosmetic products, veterinary drugs, food and feed additives, industrial chemicals, environmental safety studies conducted in the laboratory, greenhouses or in the field.
EPA	Environmental Protection Agency (EPA) GLPs 40CFR160, Subpart G, Section 160.120(2). Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and Part 792 Toxic Substances Control Act (TSCA).
MHRA	Medicines and Healthcare products Regulatory Agency, UK (Statutory Instrument 1999/3106) MHRA (UK GLPs) is an executive agency of the Department of Health in the UK that regulates medicines and medical devices as well as blood and therapeutic products/services derived from tissue engineering.
Test Article/Item/Substance	**FDA (58.3(b)):** Test article means any food additive, color additive, drug, biological product, electronic product, medical device for human use, or any other article subject to regulation under the act or under sections 351 and 354- 360F of the Public Health Service Act. **EPA (792.3):** Test substance means a substance or mixture administered or added to a test system in a study, which substance or mixture: 1) Is the subject of an application for a research or marketing permit supported by the study, or is the contemplated subject of such an application; or 2) Is an ingredient, impurity, degradation product, metabolite, or radioactive isotope of a substance described by paragraph (1) of this definition, or some other substance related to a substance described by that paragraph, which is used in the study to assist in characterizing the toxicity, metabolism, or other characteristics of a substance described by that paragraph. **OECD (Section I, 2.4.1.):** Test item means an article that is the subject of a study. **UK GLPs:** Test item means an article that is the subject of a regulatory study. **In this paper**: we will refer to the "analyte" (active pharmaceutical ingredient / drug substance / test article / control article / reference substance / test item).
Test System	**FDA (58.3, (i)):** Test system means any animal, plant, microorganism, or subparts thereof to which the test or control article is administered or added for study. Test system also includes appropriate groups or components of the system not treated with the test or control articles. **EPA (792.3):** Test system means any animal, plant, microorganism, chemical or physical matrix, including but not limited to soil or water, or subparts thereof, to which the test, control, or reference substance is administered or added for study. "Test system" also includes appropriate groups or components of the system not treated with the test, control, or reference substance. **OECD (Section I, 2.3.6.):** Test system means any biological, chemical or physical system or a combination thereof used in a study. **UK GLPs:** Test System means any biological, chemical or physical system or a combination thereof used in a regulatory study.
Carrier/Excipients/Vehicle	**OECD (2.4, 4.):** Vehicle means any agent which serves as a carrier used to mix, disperse, or solubilize the test item or reference item to facilitate the administration/application to the test system. **UK GLPs:** Vehicle means any agent which serves as a carrier used to mix, disperse, or solubilize the test or reference item to facilitate the administration or application to the test system. **EPA Parts 160 and 792:** Carrier means any material, including but limited to, feed, water, soil, and nutrient media, with which the test substance is combined for administration to a test system. **EPA Parts 160 and 792:** Vehicle means any agent which facilitates the mixture, dispersion, or solubilization of a test substance with a carrier.

Mixtures/Formulations	**FDA (58.113):** For each test or control article that is mixed with a carrier, tests by appropriate analytical methods shall be conducted: To determine the uniformity of the mixture and to determine, periodically, the concentration of the test or control article in the mixture. To determine the stability of the test and control articles in the mixture as required by the conditions of the study either before study initiation, or concomitantly according to written standard operating procedures which provide for periodic analysis of the test and control articles in the mixture. Where any of the components of the test or control article carrier mixture has an expiration date, that date shall be clearly shown on the container. If more than one component has an expiration date, the earliest date shall be shown. **EPA (792.113)** For each test, control, or reference substance that is mixed with a carrier, tests by appropriate analytical methods shall be conducted: To determine the uniformity of the mixture and to determine, periodically, the concentration of the test, control, or reference substance in the mixture. To determine the stability of the test, control or reference substance in the mixture before the experimental start date or concomitantly according to written standard operating procedures, which provide for periodic analysis of each batch. Solubility and Interference of vehicle with article is also required. Where any of the components of the test, control, or reference substance carrier mixture has an expiration date, that date shall be clearly shown on the container. I f more than one component has an expiration date; the earliest date shall be shown. **OECD (Section II): 6.2.5.** If the test item is administered or applied in a vehicle, the homogeneity, concentration and stability of the test item in that vehicle should be determined. For test items used in field studies (e.g., tank mixes), these may be determined through separate laboratory experiments.
Reference Item / Control Substance	**FDA (58.3, (c)):** Control Article means any food additive, color additive, drug, biological product, electronic product, medical device for human use, or any article other than a test article, feed, or water that is administered to the test system in the course of a nonclinical laboratory study for the purpose of establishing a basis for comparison with the test article. **OECD (2.4, 2.):** Reference item ("control item") means any article used to provide a basis for comparison with the test item. **UK GLPs:** Reference item means any article used to provide a basis for comparison with a test item. **EPA Parts 160 and 792:** Control Substance means any chemical substance or mixture, or any other material other than a test substance, feed, or water, that is administered to the test system in the course of a study for the purpose of establishing a basis for comparison with the test substance for known chemical or biological measurements. **EPA Parts 160 and 792:** Reference Substance means any chemical substance or mixture, or analytical standard, or any material other than a test substance, feed, or water, that is administered to or used in analyzing the test system in the course of a study for the purpose of establishing a basis for comparison with the test substance for known chemical or biological measurements.

Note: The pertinent references used to prepare this table are numbers 1-13.

Table 1: Definitions.

Parameter	Early Phase	Full	Partial	Transfer
When is it conducted?	≤3 months Limited API	>3 months	Significant change	Between labs
SSTs (retention time, area, N, T, k', R_s)	X	X		
System Performance Check Standards		X		
Linearity & Range (R^2 >0.99)	X	X		
Number of QC Batch Runs	1	≥2	1	1
Carryover (1% of std. or 20% LLOQ)	X	X		
LLOQ (sensitivity, S/N ≥10)		X		
Specificity / Selectivity	X	X		
Accuracy (% recovery) and Precision (% RSD)	X	Intra-run and Inter-run	X	X
Stability (pre & post, storage, F/T, stock)		X		

Table 2: Four Types of Validations.

Quality control (QC) samples are prepared at three levels (low, mid and high) spanning the anticipated dosing levels of the in-life nonclinical toxicology safety study. The QC samples are prepared by adding a known amount of compound to the vehicle that is intended to be used in the study. Preparation of the QC samples are typically performed in class A volumetric flasks or by weight for solid vehicles. A QC batch run will typically contain triplicate preparations of each of the three QC levels. The stability of these solutions should be assessed at the anticipated storage condition for the in-life study. This is usually refrigerated or frozen. Stability should be assessed for the anticipated storage time from preparation to analysis, usually 2 weeks to 1 month. Recommended acceptance criteria are presented in Table 3.

The resulting analytical method document typically contains the following sections: method limitations (range of method, vehicle used in validation), reagents, preparation of solutions, preparation of standards, sample preparation / dilution, system suitability requirements, stability of solutions, instrument conditions, calculations, and safety indications. The method should be drafted prior to validation and finalized following successful validation. The validated method is then used for sample analysis.

Sample analysis

Following a successful method validation, dose formulation samples used in an in-life study may be analyzed using the method as written. Triplicate (≥ n=3) samples are analyzed in order to confirm the concentration of compound in the vehicle which was used to dose the subjects in an in-life study. Furthermore, dose formulations are assessed for homogeneity by analyzing triplicate samples from the top, middle and bottom of the bulk formulation. The homogeneity data is analyzed per strata (≥ n=3) as well as across the batch (≥ n=9). Stability of the actual dose formulations used in an in-life study may also be assessed by storing samples at the storage conditions used in the

Intra- and Inter-run Accuracy	Solutions:	100 ±10% recovery
	Suspensions:	100 ±15% recovery
	Solids:	100 ±20% recovery
Intra- and Inter-run Precision	Solutions:	≤5% RSD
	Suspensions:	≤10% RSD
	Solids:	≤20% RSD
Stability	Solutions:	100 ±10% recovery, ≤10% RSD
	Suspensions:	100 ±15% recovery, ≤10% RSD
	Solids:	100 ±20% recovery, ≤15% RSD

Table 3: Method Validation Parameters and Acceptance Criteria.

Injection Type	# of injections	Parameter(s)	Acceptance Criteria
Diluent Blanks	≥ 1 injected at the beginning of the sequence	Concentration	≤ LLOQ
System Suitability Test (SST) Injections	≥ 3 points injected at the beginning of the chromatographic sequence following the diluent blank	Retention Time (t_R)	As per SOP
		Peak Response	
		Tailing Factor (T)	
		Theoretical Plates (N)	
		Capacity Factor (k')	
		Resolution (R_s)	
Multi-Point Calibration Curve	≥ 5 points injected immediately following the SST injections	Coefficient of Determination (R^2)	≥ 0.99
		y-intercept	Not significantly different than 0
Single-Point Calibration Curve	≥ 5 points injected immediately following the SST injections	Coefficient of Determination (R^2)	≥ 0.99
		y-intercept	Not significantly different than 0
Stock Standard Comparison	≥ 5 injections of the stock standard diluted to mid range of a multipoint curve concentration or to the single point curve concentration	Concentration (PR)	100 ±5% difference
Performance Checks	Same solution as SSTs; bracketing 10 or less samples	Concentration (PR)	100 ±5% nominal
Post-Processed Samples	≥ 3 injections of each sample preparation	Concentration (PR) Solutions	100 ±10% (PR) ≤ 10 (%RSD)
		Concentration (PR) Suspensions	100 ±15% (PR) ≤ 10 (%RSD)
		Concentration (PR) Feeds / Solid Matrices	100 ±20% (PR) ≤ 15 (%RSD)

Table 4. Sample Analysis Parameters and Acceptance Criteria

study and analyzing them for content. Recommended sample analysis parameters and acceptance criteria are presented in Table 4.

Conclusion

The White Paper is widely accepted as an industry standard representation of formulation validation and sample analysis parameters / activities. The mechanism of method validation and sample analysis presented there complies with all regulatory agencies' expectations. There is no reason that the White Paper cannot be applied globally as a mechanism to validate analytical methods for formulations analysis and perform formulation sample analysis.

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References

1. Whitmire ML, Bryan P, Henry TR, Holbrook J, Lehmann P, et al. (2010) NonClinical dose formulation analysis method validation and sample analysis. The AAPS Journal 12: 628-634.
2. Food and Drug Administration (1978) 21CFR58 - Good Laboratory Practice for Nonclinical Laboratory Studies. Rockville, Maryland: US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research.
3. Food and Drug Administration (2004) Comparison Chart of FDA and EPA Good Laboratory Practice (GLP) Regulations and the OECD Principles of GLP. US Department of Health and Human Services, Food and Drug Administration, Office of Regulatory Affairs.
4. Food and Drug Administration (2007) Guidance for Industry, Good Laboratory Practices Questions and Answers. US Department of Health and Human Services, Food and Drug Administration, Office of Regulatory Affairs.
5. Food and Drug Administration (2010) 21CFRPart 58 – [Docket No. FDA-2010-N-0548] Good Laboratory Practice for Nonclinical Laboratory Studies. Federal Register 75: 80011-8013.

6. Organisation for Economic Co-operation and Development (1998) OECD Principles of Good Laboratory Practice (ENV/MC/CHEM(98)17).

7. Huntsinger DW (2008) OECD and USA GLP applications. Ann 1st Super Sanita 2008 12: 403-406.

8. Environmental Protection Agency (2007) 40CFR160 – Good Laboratory Practice Standards.

9. Environmental Protection Agency (2007) 40CFR792 – Good Laboratory Practice Standards.

10. Health and Safety – The Good Laboratory Practice Regulations 1999. 1999 No. 3106 Schedule 1.

11. Ministry of Health, Labor and Welfare (1997) No. 21 – Good Laboratory Practice Standards for Non-Clinical Safety Studies on Drugs (GLP).

12. MHRA (2011) Good Laboratory Practice: Frequently asked questions.

13. World Health Organization (2009) Handbook: Good Laboratory Practice (GLP): Quality practices for regulated non-clinical research and development. (2ndedn).

14. Shah VP, Midha KK, Dighe S, McGilveray IJ, Skelly JP, et al. (1991) Analytical method validation: bioavailability, bioequivalence and pharmacokinetic studies. Conference report. European Journal of Drug Metabolism and Pharmacokinetics. 16: 249-255.

15. Shah VP, Midah KK, Findlay JW, Hill HM, Hulse JD et al. (2000) Bioanalytical method validation - a revisit with a decade of progress. Pharmaceutical Research 17:1551-1557.

16. Food and Drug Administration (2001) Guidance for Industry: Bioanalytical Method Validation. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research.

17. Viswanathan CT, Bansal S, Booth B, DeStefano AJ, Rose MJ, et al. (2007) Quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. Pharmaceutical Research 24: 1962-1973.

18. Shah VP (2007) The History of Bioanalytical Method Validation and Regulation: Evolution of a Guidance Document on Bioanalytical Methods Validation. The AAPS Journal 9: E43-E47.

19. European Medicines Agency (2008) EMEA/ChmP/EWP/531305/2008 – Concept Paper/Recommendations on the Need for a (CHMP) Guideline on the Validation of Bioanalytical Methods. European Medicines Agency, Committee for Medicinal Products for Human Use.

20. Fast DM, Kelley M, Viswanathan CT, O'Shaughnessy J, King SP, et al. (2009) Workshop report and follow-up—AAPS workshop on current topics in GLP bioanalysis: Assay reproducibility for incurred samples—implications of Crystal City recommendations. The AAPS Journal 11: 238-241. DOI: 10.1208/s12248-009-9100-9.

21. European Medicines Agency (2009) EMEA/CHMP/EWP/192217/2009 - Draft Guidance on Validation of Bioanalytical Methods. European Medicines Agency, Committee for Medicinal Products for Human Use.

22. Garofolo F (2007) Workshop Report – The Canadian Workshop on the Crystal City AAPS/FDA White Paper.

23. Savoie N, Booth BP, Bradley T, Garofolo F, Hughes N et. al. (2009) The 2nd Calibration and Validation Group Workshop on recent issues in Good Laboratory Practice bioanalysis. Bioanalysis 1: 19-30.

24. Savoie N, Garofolo F, van Amsterdam P,Booth BP, Fast D, et al. (2010) 2009 White Paper on Recent Issues in Regulated Bioanalysis from The 3rd Calibration and Validation Group Workshop. Bioanalysis 2: 53-68.

25. Savoie N, Garofolo F, van Amsterdam P, Bansal S, Beaver C, et al. (2010) 2010 White Paper on Recent Issues in Regulated Bioanalysis & Global Harmonization of Bioanalytical Guidance. Bioanalysis 2: 1945-1960.

26. Lausecker B, van Amsterdam P, Brudny-Kloeppel M, Luedtke S, Timmerman P (2009) European Bioanalysis Forum and the way forward towards harmonized regulations. Bioanalysis 1: 873-875.

27. Timmerman P, Lowes S, Fast DM, Garofolo F (2010) Request for global harmonization of the guidance for bioanalytical method validation and sample analysis. Bioanalysis 2: 683.

28. Bansal S, Arnold M, Garofolo F (2010) International harmonization of bioanalytical guidance. Bioanalysis 2: 685-687.

29. van Amsterdam P, Lausecker B, Luedtke S, Timmerman P, Brudny-Kloeppel M (2010) Towards harmonized regulations for Bioanalysis: Moving forward! Bioanalysis 2: 689-691.

30. Abbott R (2010) The Broadening scope of validation: Towards best practices in the world of bioanalysis. Bioanalysis 2: 703-708.

31. Premkumar N, Lowes S, Jersey J, Garofolo F, Dumont I, et al. (2010) Formation of a Global Contract Research Organization Council for Bioanalysis. Bioanalysis 2: 1797-1800. DOI: 10.4155/BIO.10.165.

32. van Amsterdam P, Arnold M, Bansal S, Fast D, Garofolo F, et al. (2010) Building the Global Bioanalysis Consortium: Working towards a functional globally acceptable and harmonized guideline on bioanalytical method validation. Bioanalysis 2: 1801-1803.

33. Tang D, Zhong D (2010) Regulated bioanalysis and the desire for harmonized regulations in China. Bioanalysis 2: 1913-1919.

34. Savoie N, Garofolo F, van Amsterdam P, Bansal S, Beaver C, et al. (2010) 2010 white paper on recent issues in regulated bioanalysis & global harmonization of bioanalytical guidance. Bioanalysis 2: 1945-1960. DOI: 10.4155/BIO.10.164.

35. Abbott RW, Gordon B, van Amsterdam P, Lausecker B, Brudny-Kloeppel M, et al. (2011) From challenges to solutions. Bioanalysis 3: 833-838.

36. Dumont I, Garofolo F (2011) The 1st conference in Asia-Pacific on recent issues in bioanalysis. Bioanalysis 3: 723-731. http://www.future-science.com/doi/pdfplus/10.4155/bio.11.47

37. American Association of Pharmaceutical Scientists, Bioanalytical Focus Group (2010) Submission of comments on 'Guideline on Validation of Bioanalytical Methods' (EMEA/CHMP/EWP/192217/2009). American Association of Pharmaceutical Scientists.

38. European Union GLP Working Group (2004) Guidance document for GLP inspectors and GLP test facilities: Cross-contamination of control samples with test item in animal studies.

39. Food and Drug Administration (1994) Reviewer Guidance – Validation of chromatographic methods. Rockville, Maryland: US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research.

40. International Conference on Harmonisation (1995) ICH Guidance Q2A – Text on validation of analytical procedures.

41. International Conference on Harmonisation (1995) ICH Topic Q 2 (R1) – Validation of Analytical Procedures: Text and Methodology. European Medicines Agency.

42. Food and Drug Administration (1996) Guidance for Industry: Q2B – Validation of Analytical Procedures: Methodology. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research, ICH.

43. Food and Drug Administration (2000) Guidance for Industry: Analytical Procedures and Methods Validation: Chemistry, Manufacturing and Controls Documentation. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research.

44. Food and Drug Administration (2010) Guidance for Industry: Validation of Analytical Procedures: Methodology. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Veterinary Medicine.

45. .Food and Drug Administration (2000) Q6A Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and Products: Chemical Substances. Federal Register 65: 83041-53063.

46. Thompson M, Ellison SLR, Wood R (2002) Harmonized guidelines for single-laboratory validation of methods of analysis (IUPAC Technical Report). Pure Applied Chemistry 74:835-855.

47. Huber L (2002) A Primer: Good Laboratory Practice and Current good Manufacturing Practice. Agilent Technologies.

48. Horvath, C (2008) Comparison of preclinical development programs for small molecules (drugs/pharmaceuticals) and large molecules (biologics/biopharmaceuticals): studies, timing, materials, and costs. John Wiley & Sons, Inc.