JOURNAL OF PHARMACEUTICAL SCIENCE AND BIOSCIENTIFIC RESEARCH (JPSBR)

(An International Peer Reviewed Pharmaceutical Journal that Encourages Innovation and Creativities)

A Comparative Review on Bioanalytical Method Validation as Per Various Regulatory Guidelines

Minakshi Dhoru*[1], Kajal Shah[2], Pinak Patel[3], Krunal Detholia[4]

1. Assistant Professor, Department of Quality Assurance, Smt. S. M. Shah Pharmacy College, Mahemdavad, Gujarat, India
2. Student of M.Pharm, Department of Quality Assurance, Smt. S. M. Shah Pharmacy College, Mahemdavad, Gujarat, India
3. Head of Department, Department of Quality Assurance, Smt. S. M. Shah Pharmacy College, Mahemdavad, Gujarat, India
4. Assistant Professor, Department of Pharmaceutics, Smt. S. M. Shah Pharmacy College, Mahemdavad, Gujarat, India

Article history:

Received 10 Jan 2019
Revised 26 Jan 2019
Accepted 28 Jan 2019
Available online 10 Feb 2019

Citation:
Dhoru M., Shah K., Patel P., Detholia K. A Comparative Review on Bioanalytical Method Validation as Per Various Regulatory Guidelines **J Pharm Sci Bioscientific Res**. 2019. 9(1):119-129

For Correspondence:
Minakshi Dhoru
Assistant Professor, Department of Quality Assurance, Smt. S. M. Shah Pharmacy College, Mahemdavad, Gujarat, India.

(www.jpsbr.org)

ABSTRACT:

Bio-analysis is a sub-discipline of analytical chemistry for quantitative measurement of drug and their metabolite in biological fluids. Before using any method in routine use validation should be carried out. There are various different regulatory guidelines on Bioanalytical method validation, US FDA guideline comes into effect in 2001 for Bioanalytical Method Validation that Provide Guidance for Industry and is accepted universally by Pharma- industries and research organisation. MHLW and EMA also come in account with US FDA guideline. Recently USFDA released a new draft on bioanalytical method validation in May 2018.The present review include a discussion on evaluation of several validation parameters such as sensitivity/selectivity, accuracy, precision, recovery specificity, calibration curve various stability analysis.

KEY WORDS: Bioanalysis, Validation, USFDA, MHLW, EMA, Regulatory guidance.

INTRODUCTION:

The term "Bio-analysis" refers to identification and quantification of an analyte or its metabolite in biological matrix (blood, plasma, urine, serum, saliva, cerebrospinal fluid). Bioanalysis in pharmaceutical industries supports in drug discovery and development. It includes various steps like sampling, sample preparation, analysis, calibration and data evaluation and reporting [1-3]. Bio-analysis study is very important for determining the drug efficacy, side effect and bioavailability of drug. Therefore studies data can be made more accurate by following an accurate and robust method development and validation [4-5]. A reliable and reproducible methods and techniques are always very demanding for the drugs and its metabolites studies for bioavailability (BA), bioequivalence (BE) and pharmacokinetic (PK) parameter for conducting the preclinical studies and therefore Bioanalytical Method Validation (BMV) plays a crucial role [6-7]. Bioanalytical is a vast field identified globally around 90's by United States Food and Drug Administration (USFDA) and American Association of Pharmaceutical Sciences (AAPS) with an aim to harmonize the principles of method validation. USFDA released the first guideline for the bioanalytical method validation in May 2001 and recent updated guidelines were also made available in May 2018. European Medicines Agency (EMA) and Ministry of Health, Labour and Welfare (MHLW), Japan in also issued guidelines for BMV. Even though there is a general understanding between regulatory authorities worldwide on the evaluation of validation parameters, there are still some differences in the methodology and acceptance criteria employed for validation. In this article, an attempt is made to combine the concepts of validation as per different regulatory guidelines.

BIOANALYTICAL METHOD VALIATION

Method validation is a process that are used to demonstrate that a method will successfully meet or exceed the minimum standards as per given guidelines. The main purpose of validating bioanalytical procedure is to demonstrate that it is suitable for its intended purpose of use [8].

Need of validating a bioanalytical method:

1. To yield reliable result that can be satisfactorily interpreted [8].
2. It is recognized that bioanalytical methods and techniques are constantly undergoing changes and improvements and are at the cutting edge of the technology [8].
3. Specific validation criteria may need to be developed for each analyte as each bioanalytical technique has its own characteristics and that may vary from analyte to analyte [9].
4. When sample analysis for a given study is conducted at more than one site, it is necessary to validate the bioanalytical method(s) at each site and provide appropriate validation information for different sites to establish inter-laboratory reliability [9-10].

Parameters of validation and there comparison as per different guidelines:

1. Specificity/selectivity

Table1: Comparison of Validation parameter "Selectivity/Specificity" as per different guidelines [11-19].

Selectivity/ Specificity	USFDA Guidelines for BMV(2013)	USFDA Guidelines for BMV(2018)	MHLW Guidelines for BMV(2013)	EMA Guidelines for BMV(2013)
Definition	It is the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the sample.	It is the ability of the method to assess, the analyte in the presence of other components that are expected to be present (e.g., impurities, degradation products, matrix components, etc.).	It is the ability of an analytical method to measure and differentiate the analyte and the internal standard in the presence of other components in samples.	It is ability of analytical method to differentiate the analyte(s) of interest and IS from endogenous components in the matrix or other components in the sample
Method	Analyses of blank samples of the appropriate biological matrix (plasma, urine, or other matrix) should be obtained from at least six sources and tested for interference.	Analyses of blank samples of the appropriate biological matrix from at least six individual sources.	Evaluation is done by using blank samples obtained from at least 6 individual sources. The absence of interference with each analyte and internal standard should be confirmed and if matrix is of limited availability, less sources are used.	Analyses using at least 6 individual sources of the blank matrix, which are individually analysed and evaluated for interference. Use of fewer sources is acceptable in case of rare matrices.
Acceptance criteria	Not mentioned	• Blank and zero calibrators should be free of interference at the retention times of	Response should be free from interference, if occur should not be more than 20% of LLOQ for and	Response should be free from interference, if occur should not be more than 20% of LLOQ for

		the analyte(s) and the IS. • Spiked samples should be ± 20%LLOQ. • The IS response in the blank should not exceed 5% of the average IS responses of the calibrators and QCs.	analyte and 5% for internal standard.	and analyte and 5% for internal standard.

1. Accuracy

Table2: Comparison of Validation parameter "Accuracy" as per different guideline [11-19].

Accuracy	USFDA Guidelines for BMV(2013)	USFDA Guidelines for BMV(2018)	MHLW Guidelines for BMV(2013)	EMA Guidelines for BMV(2013)
Definitions	It is the closeness of mean test results obtained by the method to the actual value (concentration) of the analyte.	It is the degree of closeness of the determined value to the nominal or known true value under prescribed conditions.	It is the degree of closeness between analyte Concentration determined by the method and its theoretical concentration.	It is the degree of closeness of the determined value obtained by the method to the nominal concentration of the analyte (expressed in percentage).
Method	Analysed using minimum of 5 determinations per concentration and minimum of 3 concentrations in the range of expected study sample concentrations is recommended	Analysis should be done with at least 3 independent runs, 4 QC levels per run (LLOQ, L, M, H QC), and ≥ five replicates per QC level.	Within-run accuracy should be evaluated by replicate analysis of at least 5 concentration level in a single run. Between-run accuracy should be evaluated by the analysing 3 concentration level in a run.	Within-run accuracy should be evaluated by analysing 4 concentration level, 5 samples per level in a run. Between –run accuracy is analysed using 3 runs on two different days.
Acceptance criteria	The mean value should be within 15% of the nominal value except at LLOQ, where it should be less than 20%. The deviation of the mean from	• The run should meet the criteria of calibration curve. Within-run and between runs accuracy should be within ± 15% of nominal concentrations except	The mean value should be within ±15% of the theoretical concentration, except at the LLOQ, where it should be within ±20%.	The mean value should be within 15% of the nominal values for the QC samples, except for the LLOQ which should be

	the nominal value serves as the measure of accuracy	LLOQ, where it should be within ±20%.		within 20% of the nominal value.

2. Precision

Table: 3 Comparison of Validation parameter "Precision" as per different guidelines [11-19].

Precision	USFDA Guidelines for BMV(2013)	USFDA Guidelines for BMV(2018)	MHLW Guidelines for BMV(2013)	EMA Guidelines for BMV(2013)
Definition	The closeness of individual measures of an analyte when the procedure is applied repeatedly to multiple aliquots of a single homogeneous volume of biological matrix.	The closeness of agreement (i.e., degree of scatter) among a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions.	Describes variation between individual concentrations determined in repeated measurements	The closeness of repeated individual measures of analyte.
Method	Measured using a minimum of 5 determinations per concentration where minimum of 3 concentrations in the range of expected study sample concentrations is recommended.	Analysis should be done with at least 3 independent runs, 4 QC levels per run (LLOQ, L, M, H QC), and ≥ five replicates per QC level	Within-run precision should be evaluated by replicate analysis of at least 5 concentration level in a single run. Between-run precision should be evaluated by the analysing 3 concentration level in a run.	Within-run precision evaluate using minimum of 5 samples per concentration level in a run. Between –run precision is analysed using 3 runs on two different days
Acceptance criteria	The within-run and between run precision CV value should not exceed 15% for the QC samples, except for the LLOQ which should not exceed 20%	The within-run and between run precision CV value should not exceed 15% for the QC samples, except for the LLOQ which should not exceed 20%	Measured concentrations determined at each level should not exceed 15%, except at the LLOQ, where it should not exceed 20%.	The within-run and between run precision CV value should not exceed 15% for the QC samples, except for the LLOQ which should not exceed 20%.

3. Recovery

Table 4: Comparison of Validation parameter "Recovery" as per different guidelines [11-19].

Recovery	USFDA Guidelines for BMV(2013)	USFDA Guidelines for BMV(2018)	MHLW Guidelines for BMV(2013)	EMA Guidelines for BMV(2013)
Definition	It is the detector response obtained from an amount of the analyte added to and extracted from the biological matrix, compared to the detector response obtained for the true concentration of the analyte in solvent.	Refers to the extraction efficiency of an analytical process, reported as a percentage of the known amount of an analyte carried through the sample extraction and processing steps of the method	It is measure of the efficiency at which an analytical method recovers the analyte through the sample-processing step.	Not mentioned
Method	Determined by comparing the analytical results for extracted samples with unextracted standards at three concentrations (low, medium, and high)	Determined by comparing result of extracted samples versus extracts of blanks spiked with the analyte post extraction (at L, M, and H level)	Determined by comparing the analyte response in a biological sample spiked with the analyte and processed, with the response in a biological blank sample that is processed and then spiked with the analyte	Not mentioned
Acceptance criteria	Recovery of the analyte need not be 100%, but the extent of recovery of an analyte and of the internal standard should be consistent, precise, and reproducible.	Not mentioned	Not mentioned	Not mentioned

4. Sensitivity/Lower Limit of Quantification

Table5: Comparison of Validation parameter "Sensitivity/Lower Limit of Quantification" as per different guidelines [11-19].

Sensitivity	USFDA Guidelines for BMV(2013)	USFDA Guidelines for BMV(2018)	MHLW Guidelines for BMV(2013)	EMA Guidelines for BMV(2013)

Definition	It is defined as the lowest analyte concentration that can be measured with acceptable accuracy and precision (i.e., LLOQ).	It is defined as the lowest analyte concentration in the matrix that can be measured with acceptable accuracy and precision (i.e., LLOQ).	It is the lowest concentration of an analyte at which the analyte can be quantified with reliable accuracy and precision.	It is the lowest concentration of analyte in a sample which can be quantified reliably, with an acceptable accuracy and precision.
Method	Establish LLOQ using at least five samples independent of standards and determine%CV	The lowest non zero standard on the calibration curve defines the sensitivity (LLOQ).	LLOQ should be adapted to expected concentration in the study	LLOQ should be adapted to expected concentration in the study and LLOQ should be established using a minimum of five determinations
Acceptance criteria	Should be at least 5 times the response compared to blank response. LLOQ analyte peak (response) should be identifiable, discrete and reproducible with a precision of 20% and accuracy of 80–120%	• The analyte response at the LLOQ should be ≥ five times the analyte response of the zero calibrator. • The accuracy and precision should be ± 20% of nominal concentration (from ≥ five replicates in at least three runs).	The response of analyte at the LLOQ should be at least 5 times the response of a blank sample. Mean accuracy and precision at the LLOQ should be within ±20% of the nominal (theoretical) concentration and not more than 20%, respectively.	Analyte signal should be at least 5 times the signal of a blank sample and the accuracy at LLOQ should be within 80–120% with precision ≤20%

5. Calibration curve/Standard curve

Table6: Comparison of Validation parameter "Calibration curve/Standard curve" as per different guidelines [11-19].

Calibration curve	USFDA Guidelines for BMV(2013)	USFDA Guidelines for BMV(2018)	MHLW Guidelines for BMV(2013)	EMA Guidelines for BMV(2013)
Definition	It is the relationship between instrument response and known concentrations of the analyte.	It is the relationship between the instrument response and the calibration standards within the intended quantitation range.	Relationship between the theoretical concentration and the response of the analyte.	The response of the instrument with regard to the concentration of analyte
Method	Analysed using minimum of six	Consist of a blank(no analyte, no IS), a zero	Analysed using blank sample, a zero sample	A minimum of six calibration

	runs conducted over several days, with at least four concentrations (including LLOQ, low, medium, and high) analyzed in duplicate in each run.	calibrator (blank plus IS), and at least six, non-zero calibrator levels covering the quantitation range, including LLOQ in every run.	(blank sample spiked with internal standard)), and at least 6 concentration levels of calibration standards, including an LLOQ sample.	concentration levels should be used, in addition to the blank sample (processed matrix sample without analyte and without IS) and a zero sample (processed matrix with IS).
Acceptance criteria	LLOQ response should be ≥5 times the response to blank response and precision should be below 20% of the CV and accuracy within ±20%, ULOQ should have precision below 15% of CV and accuracy within ±15% of the nominal concentration. For calibration curve, the standard should be below 15% of nominal concentration, except for LLOQ where calibrator should not deviate by 20% and 75% of non-zero including LLOQ should be within limit.	• Non-zero calibrators should be ± 15% of nominal (theoretical) concentrations, except at LLOQ where the calibrator should be ± 20% of the nominal concentrations in each validation run. • 75% and a minimum of six non-zero calibrator levels should meet the above criteria in each validation run.	The accuracy of back calculated concentrations of each calibration standard should be within ±20% of the theoretical concentration at the LLOQ, 109 or ±15% at all other levels and at least 75% of the calibration standards, with a minimum of 6 levels, including the LLOQ and the highest levels, should meet the above criteria.	The back calculated concentrations of the calibration standards should be within ±15% of the nominal value, except for the LLOQ for which it should be within ±20%. At least 75% of the calibration Guideline on bioanalytical method validation

6. Stability

Table7: Comparison of Validation parameter "Stability" as per different guidelines [11-19].

Stability	USFDA Guidelines for BMV(2013)	USFDA Guidelines for BMV(2018)	MHLW Guidelines for BMV(2013)	EMA Guidelines for BMV(2013)
Definition	The chemical stability of an analyte in a given matrix under	Measure of the intactness an analyte in a given matrix under specific storage and	The chemical or biological stability of an analyte in a given solvent or matrix	The chemical stability of an analyte in a given matrix under specific

	specific conditions for given time intervals.	use conditions relative to the starting material for given time intervals.	under specific conditions over given time intervals.	conditions for given time intervals.
Freeze and Thaw Stability				
Method	Stability should be determined for a minimum of 3 freeze-thaw cycles.	Stability is determined after three freeze-thaw cycles. QC samples should be thawed and analyzed according to the same procedures as the study samples. QC samples should be frozen for at least 12 hours between cycles.	Stability is determined using 3 replicates per concentration i.e. HQC and LQC in the same condition as used for the study sample after freeze-thaw cycles.	Performed after freeze-thaw cycles which should be equal to or greater than the freeze/thaw cycles intended for the study samples
Bench-Top Stability				
Method	It should be designed and conducted to cover the laboratory handling conditions that are expected for study samples.	It should determine the stability of samples under the laboratory handling conditions that are expected for the study samples.	Stability is evaluated using 3 replicates per concentration of HQC and LQC samples with QC samples before and after storage	Evaluate the stability using at least triplicates of LQC and HQC
Long-Term Stability				
Method	The storage time in a long-term stability evaluation should equal or exceed the time between the date of first sample collection and the date of last sample analysis.	The storage time in a long-term stability evaluation should equal or exceed the time between the date of first sample collection and the date of last sample analysis. Long-term stability QCs should be compared to freshly prepared calibration curves and QCs. Determination of stability at minus 20ºC would cover stability at colder temperatures.	Performed on the samples that have been stored for a time that is longer than the actual storage period.	QC samples should be stored under the same conditions as the study samples and analyzed
Stock Solution Stability				

Method	The stability of stock solutions of drug should be evaluated when the stock solution exists in a different state or in a different buffer composition from the certified reference standard, the stability data on this stock solution should be generated to justify the duration of stock solution storage stability.	The stability of stock solutions of drug should be evaluated when the stock solution exists in a different state or in a different buffer composition from the certified reference standard, the stability data on this stock solution should be generated to justify the duration of stock solution storage stability.	Performed by using at least 3 replicates at each concentration levels of HQC and LQC	Bracketing approach can be used for the study of stability of stock and working solution
Processed /Extract Sample Stability				
Method	The stability of processed samples, including the time until completion of analysis, should be determined	Stability of processed samples, including the residence time in the autosampler against freshly prepared calibrators.	Not mentioned	Stability of the processed sample at room temperature or under the storage conditions to be used during the study (dry extract or in the injection phase),
Auto-sampler stability				
Method	Not mentioned	The stability of extracts in the auto- sampler is evaluated only if the auto-sampler storage conditions are different or not covered by extract (processed sample) stability.	Not mentioned	On-instrument/ autosampler stability of the processed sample at injector or autosampler temperature.
Acceptance criteria	Stability sample results should be within 15% of nominal concentrations.	The accuracy (% nominal) at each level should be ± 15%.	The mean accuracy in the measurements at each level should be within ±15% of the theoretical concentration.	The mean accuracy in the measurement at each level should be within ±15% deviation of the

				theoretical concentration

CONCLUSION

USFDA, EMA and MHLW guidelines are generally referred for the bioanalytical method validation. Recently USFDA have launched the new guidelines for the bioanalytical method validation. Even though the scientific basis for evaluation of parameters is same across these guidelines, but still there are differences in the acceptance criteria and methodology for few parameters. Till date USFDA and EMA are the most widely referred guidelines referred guidelines for the bioanalytical method validation. USFDA guideline provides guidance on additional issues such as endogenous compounds, biomarkers, diagnostic kits and also encourages the development and use of newer bioanalytical technologies. EMA lacks some important parameters such as recovery in its guidelines. As none of the guidelines appear to be restricting to their particular parameters which make it open that additional parameters which are not present in specific guidelines can be performed. The regulatory agencies should consider for implementing a common guidance for the bio-analytical method validation which would be acceptable worldwide.

REFERENCES

1. Tijare, L., R. Nt, and M. Un. "A Review on Bioanalytical Method Development and Validation". Asian Journal of Pharmaceutical and Clinical Research, Vol. 9, no. 9, Dec. 2016, pp. 6-10.
2. Kirthi A, Shanmugam R, Prathyusha SM, Basha J. A review on bioanalytical method development and validation by RP-HPLC. Journal of Global Trends in Pharmaceutical Sciences. 2014;5(4):2265-71.
3. Moein MM, El Beqqali A, Abdel-Rehim M. Bioanalytical method development and validation: Critical concepts and strategies. Journal of Chromatography B. 2017 Feb 1;1043:3-11.
4. Causon R. Validation of chromatographic methods in biomedical analysis. Viewpoint and discussion. Journal of Chromatography B: Biomedical Sciences and Applications,1997, 689:175- 180.
5. Hartmann C, Smeyers-Verbeke J, Massart DL and McDowall RD. Validation of bioanalytical chromatographic methods, Journal of Pharmaceutical and Biomedical Analysis, 1998,17: 193-218.
6. Taleuzzaman M, Gilani SJ, Ali S, Hafeez A, Nagarajan K. Bio-Analytical Method Validation-A Review. Austin J Anal Pharm Chem. 2015;2(5):1053.
7. Kalakuntla RR, Kumar KS. Bioanalytical method validation: A quality assurance auditor view point. Journal of Pharmaceutical Sciences and Research. 2009 Sep 1;1(3):1.
8. Sonawane LV, Poul BN, Usnale SV, Waghmare PV and Surwase LH . Bioanalytical Method Validation and Its Pharmaceutical Application- A Review, Pharmaceutica Analytica Acta, 2014; 5: 288.
9. Bhusnure O.G.et al. Review on Bioanalytical Method Development and Validation by Chromatographic Techniques, International Journal Of Pharmacy And Pharmaceutical Research 2017;10(1): 76-97
10. Tiwari G and Tiwari R .Bioanalytical method validation: An updated review, Pharmaceutical Methods, 2010; 1: 25-38
11. Guidance for Industry Bioanalytical Method Validation (USFDA guidelines),2013,Web (http://academy.gmp-compliance.org/guidemgr/files/UCM368107.PDF)
12. Guidance for Industry Bioanalytical Method Validation (USFDA guidelines),2018, Web (https://www.fda.gov/downloads/drugs/guidances/ucm070107.pdf)
13. Guideline on Bioanalytcal Method Validation in Pharmaceutical Development (MHLW) ,2013,Web (http://www.nihs.go.jp/drug/BMV/BMV_draft_130415_E.pdf)
14. Guideline on Bioanalytical method validation (EMEA guidelines) ,2013,Web (https://www.ema.europa.eu/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf)
15. Sivacharan K, Girish B, Nitin A, Brijesh V and Jyoti P. International Guidelines for Bioanalytical Method Validation, A Comparison and Discussion on Current Scenario, Chromatographia, 2011;73: 201-217.
16. Graeme S. Bioanalytical Method Validation: Notable points in 2009 draft EMA guideline and differences with 2001 FDA Guidance, Bioanalysis, 2010; 2(5): 929-935.
17. Natasha S, Fabio G, Peter V and Surendra B. White paper on Recent Issues in Regulated Bioanalysis and

Global Harmonization of Bioanalytical Guidance, Bioanalysis, 2010; 2(12): 1945-1960.

18. S. Kollipara, G. Bende, N. Agarwal, B. Varshney and J. Paliwal. International guidelines for bioanalytical method validation: a comparison and discussion on current scenario, Chromatographia ,2011;73:201–217.
19. Kalakuntla R and Santosh Kumar K. Bioanalytical Method Validation: A Quality Assurance Auditor View Point, Journal of Pharmaceutical Sciences and Research, 2009; 1: 1-10.