EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 3
Issue: 3
497-502
Year: 2016

REVIEW REPORT ON REGULATORY AGENCIES REQUIRMENT AND EXPERIMENTAL APPROCH FOR METHOD VALIDATION AND METHOD VERIFICATION

U. R. Maniyar*[1], Kishor Koshe[2], Dr. M. V. Katariya[3], Dr. G. S. Karva[4], S. H. Jaiswal[5], Dr. V. R. Katariya[6]

[1]Sr. Manager, Macleods Pharmaceuticals Ltd. Daman 396210 (U.T.)
[2]DGM, Macleods Pharmaceuticals Ltd. Daman 396210 (U.T.)
[3]Professor, Department of Chemistry, Deogiri College, Aurangabad-431005.
[4]Lecturer, Department of Pharmaceutics, Government college of Pharmacy, Aurangabad-431005.
[5]President, Macleods Pharmaceuticals Ltd. Mumbai 400059.
[6]Lecturer, Kamla Nehru Pharmacy College, Aurangabad.

***Author for Correspondence: U. R. Maniyar**
Sr. Manager, Macleods Pharmaceuticals Ltd. Daman 396210 (U.T.)

Article Received on 11/01/2016 **Article Revised on 02/02/2016** **Article Accepted on 24/02/2016**

ABSRACT
The objective of presented review article is to provide the detailed theoretical approach regarding the method development and validation as per various regulatory agencies guidelines required for the drug material and product thereof. The present review also deals with the most appropriate experimental approach for analytical method validation and method verification which is required for the routine analysis of pharmaceutical products for qualitative and quantitative estimation. In brief the objective of this review is to estimate the uncertainty of measurement of developed method using statistical application.

KEYWORDS: ICH Guidelines, method development and validation, regulatory agencies.

INTRODUCTION

The method of analysis used for the routine analysis of pharmaceutical drug substance and drug product in quality control should produce reproducible result; incense the method should be stability indicating. Hence it is necessary to evaluate the new developed method is most suitable and is giving the exact result. The target for analytical estimation for quantitative as well qualitative analysis is to obtain consistent, reliable and accurate reproducible result and hence validated analytical method help to achive the goal.

REGULATORY AGENCIES REQUIRMENT

The industrial guidelines provided by USFDA for method validation viz. one for analytical2 and other for bio-analytical methods validation3. The chapter 1225 of USP contained a specific guidelines regarding method validation. It focuses the validation of compendia procedures by providing definitions for validation of different analytical parameter. USP 2011 classifies the validation methods into four different categories depends on their use which covers the different validation parameter. The categories are as follows

Category I: Analytical procedures for quantitation of major components in bulk drug substances or active ingredients (including preservatives) in drug products.

Category II: Analytical procedures for determination of impurities in bulk drug substances or degradation compounds in drug products. These procedures include quantitative assays and limit tests.

Category III: Analytical procedures for determination of performance characteristics (e.g., dissolution, drug release, etc.).

Category IV: Identification tests.

Table 1: different category with validation element as per USP

Data Element Required for Validation					
Analytical Performance Characteristic	**Category I**	**Category II**		**Category III**	**Category IV**
		Quantitative Limit	**Limit Test**		
Accuracy	Y	Y	#	#	N
Precision	Y	Y	N	Y	N
Specificity	Y	Y	Y	#	Y

Detection Limit	N	N	Y	#	N
Quantitation Limit	N	Y	N	#	N
Linearity	Y	Y	N	#	N
Range	Y	Y	#	#	N
Note: Y- Yes, N-No and #-May be required, depending on the nature of specific test.					

International Conference on the Harmonization "ICH" is elaborate the differrent terminology and definitions and published guideline to determine the basic requirements of validation of Analytical Procedures in quality guideline Q2R(1) which is summarize as follow.

Table 2: Basic requirements of Analytical Procedures validation in Q2R(1) guideline

Type of analytical procedure characteristic	Identification	Testing For Impurity		Assay/ Dissolution content / potency (measurement only)
		quantitation	Limit	
Accuracy	-	+	-	+
Precision				
Repeatability	-	+	-	+
Interm.Precision	-	+#	-	+#
Specificity$	+	+	+	+
Detection Limit	-	-@	+	-
Quantitation Limit	-	+	-	-
Linearity	-	+	-	+
Range	-	+	-	+

+ : Signifies that characteristic is normally evaluate
- : Signifies that characteristic is not normally evaluate
: In case where reproducibility (see glossary) has been performed, intermediate precision is not needed.
$: Lack of specificity of one analytical procedure could be compensated by other supporting analytical procedure (s)
@ : May be needed in some cases.

The terms validation and verification of analytical methods are interchangeably used. The difference is best explicated by USP Chapters <1225> and <1226>. Chapter <1225>[4] is titled: "Validation of Compendial Methods" which describes the validation of analytical methods with all validation parameters from introduction. The result is a validated method for a specific sample. This procedure is recommended for the validation of methods developed internally. Chapter <1226>[5] is titled "Verification of Compendial Methods." It provides recommendations of compendial methods that demonstrate a laboratory's ability to successfully run the method. Methods are also verified during method transfer by the receiving laboratory.

STRATEGY FOR ANALYTICAL METHOD VALIDATION AND VERIFICATION OF DRUG SUBSTANCES AND DRUG PRODUCT

Based on different validation guidelines the common approach for method validation and/or verification of drug substances and drug product is summarized in table 3 and table 4, respectively.

Table 3 : Validation/verification characteristics for Drug Substances

Type of analytical procedure	Identificafication		Related Substances		Assay (measurment only) content / potency	
characteristic	Valida.	Verifi.	Valida.	Verifi.	Valida.	Verifi.
Accuracy	No	No	Yes	No	Yes	No
Repeatability	No	No	Yes	Yes	Yes	Yes
Interm.Precision	No	No	Yes	No	Yes	No
Specificity$	Yes	Yes	Yes	Yes	Yes	May be
Detection Limit	No	No	Yes	No	No	No
Quantitation Limit	No	No	Yes	Yes	No	No
Linearity	No	No	Yes	No	Yes	No
Range	No	No	Yes	No	Yes	No

Table 4 : Validation/verification characteristics for Drug Product

Type of analytical procedure	Identification		Specific Test Uniformity Content Dissolution		Related Substances		Assay (measurement only) content / potency	
characteristic	**Valida.**	**Verifi.**	**Valida.**	**Verifi.**	**Valida.**	**Verifi.**	**Valida.**	**Verifi.**
Accuracy	No	No	May be	May be	Yes	May be	Yes	May be
Repeatability	No	No	Yes	Yes	Yes	Yes	Yes	Yes
Interm.Precision	No	No	Yes	No	Yes	No	Yes	No
Specificity	Yes	Yes	May be	Yes	Yes	No	Yes	Yes
Detection Limit	No	No	No	No	Yes	Yes	No	No
Quantitation Limit	No	No	No	No	Yes	Yes	No	No
Linearity	No	No	May be	No	Yes	No	Yes	No
Range	No	No	May be	No	Yes	No	Yes	No

EXPERIMENTAL PROCEDURE AND ACCEPTANCE CRITERIA:

When the relevant validation characteristics have been identified, the experimental procedure which will be used to investigate those characteristics needs to be defined.

Specificity

Specificity demonstrates that the response due to analyte of interesting the sample is not affected by potential interferences which may also be present in sample. Specificity should be conducted during the validation of identification test, impurities determination and assay. The general requirement that the sample and standard chromatograms should corresponds in retention time. The easiest way to perform the specificity for HPLC method is to perform this test in conjugation with forced degradation study. The utilization of mass spectrometry (MS) detector in series after Photo Diode Array (PDA) detector to obtain more information is encouraged in terms of mass-to charge ratio of parent ions, initial fragmentation pattern, and peak purity. Specificity is confirmed when API and impurities peak(s) is pure and there is no interference from placebo and blank solution at the retention time of peak.

Forced Degradation

Forced degradation (also called as stress testing) studies may provide the information to the degradation pathway and degradation product that could from during storage drug substance or drug product. The Overstressing can destroy the relevant compound or generate the irrelevant compounds. The extent of targeted degradation should be approximately anywhere 5% to 10%. Forced degradation studies carried out either in the solution state and/or in the solid state. Usually the on one batch of drug substance and/or one formulation blend (capsules and tablets)

Table 5: Solid state forced degradation studies.

Stress Test	Condition	Duration
Thermal (close container)	50°C/80°C (ambient RH)	1 wk and 2wks
Thermal / oxidative (open container)	50°C/80°C (ambient RH)	1 wk and 2wks
Thermal / humidity (open container)	40°C/75%RH	1 wk and 2wks
Light (closed container)	Ambient	Maximum 1.2 million lux hours and 200 watt hours / squares meter
Light / oxidative (open container)	Ambient	Maximum 1.2 million lux hours and 200 watt hours / squares meter

Table 6: Solution state forced degradation studies

Test factor	Condition	Duration
PH	10 mg in 2mL water	1 day and 3 days
	10 mg in 2mL water of 0.1M to 5M HCl	
	10 mg in 2mL water of 0.1M to 5M NaOH	
	All in amber volumetric flask and at room	
	temperature 10mg / 2mL 3% H_2O_2	
Oxidation (H_2O_2)	At 5°C and room temperature in amber volumetric flask. If DS is not soluble , then pH modifications may be necessary	1, 2, and 3 days
Light	50mg / 10mL water ambient	Maximum 1.2 million lux hour

		and 200 watt hours / squares meter, 6hrs, 1 day and 2 day
Heat	10mg in 2 mL water at 80°C	6 hrs, 1 day and 2 day

LINEARITY

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. It may be directly demonstrated on the analyte, or on spiked samples using at least five concentrations over the whole working range.

Appropriate statistical calculations are to be done, such as a linear regression cofficent, slope and intercept, residual sum of squares with a graphical presentation of the data.

RANGE

The range of an analytical method is the interval between the upper and lower levels (Including these levels) that have been demonstrated to be determined with precision, accuracy, and linearity using the method as written. For assay test, it requires a minimum of specified range to be 80 to 120 percent of the test concentration, and for the determination of an impurity, the range to extend from the limit of quantitation, to, 150 percent of the specification.

LIMIT OF DETECTION

It is the lowest concentration of analyte in a sample that can be detected, but not necessarily quantified. In chromatography, the detection limit is the injected amount that results in a peak with a height at least three times as high as the baseline noise level. As per ICH this signal/noise method describes three more methods.

- *Visual inspection:* The detection limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.
- *Standard deviation of the response based on the standard deviation of the blank:* Measurement of the magnitude of analytical background response is performed by analyzing an appropriate number of blank samples and calculating the standard deviation of these responses.
- *Standard deviation of the response based on the slope of the calibration curve:* A specific calibration curve is studied using samples containing an analyte in the range of the limit of detection. The residual standard deviation of a regression line, or the standard deviation of y-intercepts of regression lines, may be used as the standard deviation.

LIMIT OF QUANTITATION

The limit of quantitation is the minimum injected amount that produces quantitative measurements in the target matrix with acceptable precision in chromatography, typically requiring peak heights 10 to 20 times higher than the baseline noise.

For the limit of quantitation, the ICH recommends, in addition to the procedures as described above, the visual inspection and the standard deviation of the response and the slope of the calibration curve. Any results of limits of detection and quantitation measurements must be verified by experimental tests with samples containing the analytes at levels across the two regions. It is equally important to assess other method validation parameters, such as precision, reproducibility and accuracy, close to the limits of detection and quantitation. Figure 1 illustrates both the limit of detection and the limit of quantitation.

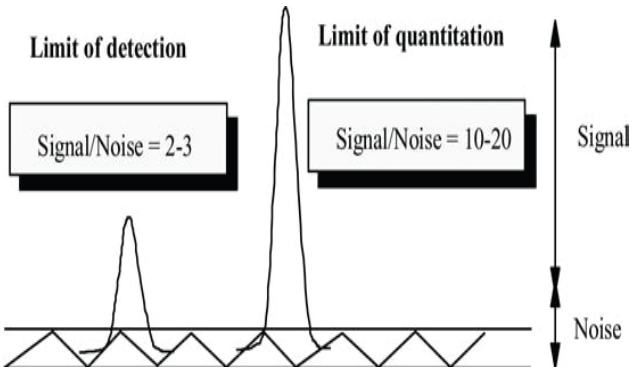

Fig. 1. Limit of detection and limit of quantitation via signal to noise

ACCURACY

Accuracy described as the closeness of agreement between the value that is adopted, either as a conventional, true, or accepted reference value, and the value found. It is the measure of the exactness of the analytical method developed. The accuracy of an analytical method may be determined by any of the following ways:

- Analysing a sample of known concentration and comparing the measured value to the 'true' value. However, a well characterized sample (e.g., reference standard) must be used.
- Spiked – placebo (product matrix) recovery method. In this method, a known amount of pure active constituent is added to formulation blank [sample that contains all other ingredients except the active(s)], the resulting mixture is assayed, and the results obtained are compared with the expected result.
- Standard addition method. In this method, a sample is assayed, a known amount of pure active constituent is added, and the sample is again assayed. The difference between the results of the two assays is compared with the expected anser.

In both methods (spiked – placebo recovery and standard addition method), recovery is defined as the ratio of the

observed result to the expected result expressed as percentage.
The accuracy should cover at least 3 concentrations (80, 100 and 120%) in the expected range.

PRECISION
Precision of analytical method expresses the closeness of agreement (degree of scatter) between the series of measurements obtained from multiple samplings of the same homogenous sample under the prescribed condition Precision can be establish through Repeatability and Intermediate Precision.

Repeatability: Repeatability represents the simplest situation and involves analysis of replicates by the same analyst, generally one injection after the other. Repeatability tests are mandatory for all tests delivering numerical data. Repeatability is divided into two parts: injection repeatability and analysis repeatability (multiple preparations). Results obtained by six different sample solution over the short period of time by the same analyst , on the same column, same equipment, on the same day . Determine the mean, standard deviation, %RSD and 95% confidence interval of the result obtained from six preparation. Repeatability can be calculated using Eq.(1) and Eq. (2) from a larger number of repeatedly prepared samples (at least six).

$$S = \sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \bar{x})^2}, \quad --(1)$$

% RSD = S/Mean*100 - - (2)

4.6.2 Intermediate Precision
Prepare the six different sample solutions from the same homogenous sample and analyse it typical variation include the analyst, instrument, day and column (if available). Calculate the result and determine the mean, standard deviation, relative standard deviation and 95%confedance interval of the result obtained from six preparations.
Calculate the absolute difference in the result obtained in Repeatability (mean value of six results) and intermediate precision ((mean value of six result)

4.7 ROBUSTNESS AND RUGGEDNESS
4.7.1 Robustness
The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variation in method parameters and provides an indication of its reliability during normal usage. The robustness of a method is evaluated by varying method parameters such as percent organic solvent, pH, ionic strength, temperature and determine the effect (if any) on the results of the method. The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study.

If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure.

4.7.2 Ruggedness
The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different laboratories, different analysts, using operational and environmental conditions that may differ but are still within the specified parameters of the assay. The testing of ruggedness is normally suggested when the method is to be used in more than one laboratory.

4.8 Stability and system suitability tests
It is most important parameter to establish method is stability indicating method. Stability of the sample, standard and reagents is required for a reasonable time to generate reproducible and reliable results.
System suitability test provide the assurance that on a specific occasion the method is giving, accurate and precise results. System suitability test are run every time a method is used either before or during analysis. The results of each system suitability test are compared with defined acceptance criteria and if they pass, the method is deemed satisfactory on that occasion.

5. CONCLUSION
Validation is a constant, evolving process that starts before an instrument is placed on-line and continues long after method development and transfer. In this review article we discussed about the importance and types of validation of analytical methods. From the above discussed matter we concluded that the validation of developed analytical methods is critical elements in the development of pharmaceuticals. Success in these areas can be attributed to several important factors, which in turn will contribute to regulatory compliance.

6. REFERENCES
1. International Conference on the Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). Validation of Analytical Procedures:Text and Methodology Q2 (R1). (2005). Available from http://www.ich.org
2. International Conference on Harmonization (ICH) Q8(R2): Pharmaceutical Development (August 2009).
3. International Conference on Harmonization (ICH) Q11: Development and Manufacture of Drug Substances (Chemical Entities and Biotechnological/Biological Entities) (May 2011).
4. Australian Government Department of Health Ageing: Therapeutic Goods Administration "Finished product (medicine) analytical procedure validations for Complementary Medicines" March 2006.
5. WHO-Stability Testing of Active Substances and Pharmaceutical Products.Geneva; April 2006. (QAS/06.179)

6. United StatesPharmacopeia/National Formulary
General Chapter <621> Chromatography
General Chapter <1010> Analytical Data
– Interpretation and Treatment
General Chapter <1224> Transfer of Analytical Procedures
General Chapter <1225> Validation of Compendial Procedures
General Chapter <1226> Verification of Compendial Procedures
7. ASTM Standard, E29 - 2008 Standard Practice for Using Significant Digits in Test Data to Determine Conformance with Specifications, ASTM International, West Conshohocken, PA, (www.astm.org).
8. Hibbert DB, Quality Assurance For the Analytical Chemistry Laboratory, New York, Oxford University Press, 2007.
9. Potdar MA, Pharmaceutical Quality Assurance, 2nd edn. Pune, Nirali Prakashan, 2007.
10. Connors KA, A Textbook of Pharmaceutical Analysis, 3rd edn, New Delhi, John Wiley and Sons Inc., 2007.
11. Bailey LC,Chromatography, Remington: The Science and Practice of Pharmacy.: Lippincott William andWilkins
12. Willard HH, Merritt LL, Dean JA, Settle FF, Instrumental Methods of Chemical Analysis, Delhi, CBS Publishers and Distributors;
International Journal of Pharma Research and Development – Online
www.ijprd.com
13. www.wikipedia.com
14. Analytical Method Validation by Pedro Lopez Garcia
Dong MW, Modern HPLC for Practicing Scientists, New Jersey, A John Wiley and Sons Inc.,2006.
15. Snyder LR, Kirkland JJ, Glajch JL. Practical HPLC Method Development, A Wiley- Interscience Publication,2nd edn, 440-442.
16. Nageswara Rao R., Nagaraju V. An overview of the recent trends in development of HPLC methods for determination of impurities in drugs. J. Pharma. Biomed. Ana., 2003; 33: 335-377.
17. Saranjit singh., Tarun Handa., Mallikarjun Narayanam., Archana Sahu et al .A critical review on the use of modern sophisticated hyphenated tools in the characterization of impurities and degradation products. J. Pharma. Biomed. Ana. 2012.
18. Schweitzer, M.; Pohl, M.; Hanna-Brown, M.; Nethercote, P.; Borman, P.; Hansen, G.; Smith, K.; Larew J. Implications and Opportunities of Applying QbD Principles to Analytical Measurements. Pharm. Tech., 2010; 34(2): 52-59.
19. Bhatt, D.A.; Rane, S.I. QbD Approach to Analytical RP-HPLC Method Development and its Validation. Int. J. Pharm. and Pharm. Sci., 2011; 3(1): 179-187.
20. Monks, K.; Molnar, I.; Rieger, H.-J.; Bogati, B.; Szabo, E. Quality by Design: Multidimensional exploration of the design space in high performance liquid chromatography method development for better robustness before validation. J. Chromatogr. A 2012: 1232: 218-230.
21. Skoog, D.A., Holler, F.J, Crouch, S.R. Principles of Instrumental Analysis, Thomson Learning, 2006.
22. Ahuja, S.; Dong, M. Handbook of Pharmaceutical Analysis by HPLC, Elsevier, 2005.
23. Ahuja, S. and Jespersen, N. Modern Instrumental Analysis, Elsevier, Amsterdam, 2006.
24. Ahuja, S.; Scypinski, S. Handbook of Modern Pharmaceutical Analysis, Second Edition, Academic Press, 2011.
25. Nickerson, B. Sample Preparation of Pharmaceutical Dosage Forms, Springer, 2011.
26. Miller, J.C., J.N. Miller, and E. Horwood, Statistics for Analytical Chemistry , 3rd edition, Prentice Hall, 1993.
27. WHO/FIP Training Workshop Hyatt Regency Hotel, Sahar Airport Road Andheri East, Mumbai, India 28 April 2008 – 2 May 2008.