

**INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY,  
BIOLOGY AND CHEMISTRY****Review Article****Analytical Method Validation: An Updated  
Review**

**G. Geetha, Karanam Naga Ganika Raju, B. Vignesh Kumar  
and M. Gnana Raja\***

Sankaralingam Bhuvanewari College of Pharmacy, Annaikuttam,  
Virudhunagar District, Tamil Nadu, India.

**ABSTRACT**

The development of sound Analytical method(s) is of supreme importance during the process of drug discovery, release to market and development, culminating in a marketing approval. The objective of this paper is to review the method development, optimize and validation of the method for the drug product from the developmental stage of the formulation to commercial batch of the product. Method development for the interested component in finished product or in process tests and the sample preparation of drug product and to provide practical approaches for determining selectivity, specificity, limit of detection, limit of quantitation, linearity, range accuracy, precision, recovery solution stability, ruggedness, and robustness of liquid chromatographic methods to support the Routine, in process and stability analysis.

**Keywords:** Analytical method development, validation parameters, acceptance criteria.

**INTRODUCTION**

The reliability of an analytical finding is a matter of great importance to drive the formulation scientist in the developmental stage and impurity profile in stability study and dissolution data of the stability study as well as routine analysis. The importance of validation is producing reliable and repeatable results for routine analysis and stability analysis. This is especially true in the context of quality management and accreditation, which have become matters of increasing importance in analytical in dissolution and impurity profile in the recent years. Therefore, this topic should extensively be discussed on an international level to reach an accord on the extent of validation experiments and on acceptance criteria for validation parameters of analytical methods<sup>1</sup>.

**NEED OF ANALYTICAL METHOD  
VALIDATION**

It is essential to employ well-characterized and fully validated analytical methods to yield reliable results in the laboratories while analysing the registration batch and accelerated

stability testing samples. It is also important to emphasize that each analytical technique has its own characteristics, which will vary from analyte to analyte. In these instances, specific validation criteria may need to be developed for each analyte. Moreover, the appropriateness of the technique may also be influenced by the ultimate objective of the study. When sample analysis for a given study is conducted at more than one site and commercial batch for people consumption, it is necessary to validate the analytical method(s) as per ICH guidelines and to provide proper validation information for different sites and different parameter and to establish inter and intra laboratory reliability<sup>2</sup>.

**ANALYTICAL METHOD DEVELOPMENT  
AND VALIDATION**

Analytical method development is the process by which a specific analytical method is to be developed for drug products from the stage of in process to finished product and minivaldation to be done before starting the analyses of routine samples, investigation samples and stability samples. Analytical method development and

finalizing the method consists of

1. Standardizing the working standard from reference standard.
2. Optimizing the chromatographic condition, concentration of standard and sample solution and extraction procedure of the sample.
3. Analytical method verification or mini validation to be done before analysing (routine samples) tests like assay, dissolution and related substance in development laboratories etc.
4. Prior starting the validation the satisfactory result should be found in mini validation and formulation should be finalized<sup>3</sup>.

## TERMINOLOGY

### Validation

It is accepted that during the course of a typical drug product development program, a defined analytical method will undergo many modifications because composition changes, lower strength may be added or percentage of coating material may change on the formulation. Because of the changes the analytical method may be modified and if modified it should be verified so it requires different levels of validation. Two different levels/types of method validations, complete validation and partial validation or mini, validation, are defined and characterized as follows.

### Complete validation

Complete validation is necessary before executing clinical batch or registration batch of drug product. If any modification in the formulation or if any impurity found in the stability study the existing method to be modified and validated again, and the parameters are detailed below for the complete validation given table 1.

### Mini validation

Mini validations is required for all the test methods like Assay, Related substance, UOD and Blend Uniformity for analysing the routine samples prior starting the complete validation some parameters to be checked as per ICH Guidelines detailed below given table 2 and acceptance criteria discuss later<sup>4</sup>.

## VALIDATION PARAMETERS

### Linearity

A linear relationship should establish across the range as per table 3 given below. It may be demonstrated directly on the active substance by linear dilution and percentage level of each parameter given below table 3. The linear range of the method must be determined regardless of the all stage of the formulation during drug

development. ICH guidelines recommend evaluating a minimum of five concentrations to assess linearity and wider range of concentration and other approaches should be justified. The correlation coefficient, Y-intercept, Slope of the regression line and residual sum of square shall be submitted by appropriate statistical method<sup>5</sup>. The linearity solutions are prepared by performing serial dilutions of a single stock solution, for related substance, residual solvent impurity blend or solvent blend shall be used. The response of the interested peak is plotted against the, corresponding theoretical concentration.

For related substance method relative response factors and relative retention times for each impurity should establish with respect to the active compound. Response factors allow the end user to not using the impurity material for each analysis, and it is useful to correcting for response differences and final impurity calculation. To determine the relative response factors, linearity curve method or about 0.2 and 0.4 percent for each impurity and the active compound should be injected and find the relative response factor from the slope of the linearity curve or ratio between the response factor of impurity and response factor of active compound<sup>6</sup>.

### Selectivity (Specificity)

For clinical and before registration batch of the drug product, the analytical method must demonstrate specificity including degradation study. The method must have the ability to separate each known impurity and degradation product at the quantitation level and if any blank, placebo peaks are found it should be properly separated from impurity peak and interested peak.

For identification tests, discrimination of the method should be demonstrated by obtaining positive results for samples containing the analyte and negative results for samples not containing the analyte. The method must be able to differentiate between the analyte of interest and compounds with a similar chemical structure that may be present. For a high performance liquid chromatography (HPLC) identification test, peak purity evaluation should be used to assess the homogeneity of the peak corresponding to the analyte of interest<sup>7</sup>.

For assay/related substances methods, the active peak and each impurity should be adequately resolved from all impurity/degradant peaks, placebo peaks, and blank peaks. Resolution of each impurity peaks and impurity peak from interested peak should complice US Pharmacopeia. Blank, Placebo and sample

matrix components should be analyzed without the active present in order to identify possible interferences.

If filters are to be used to clarify sample solutions, an aliquot of filtered sample diluents should be analyzed for potential interferences and result should be compared with centrifuged sample. If the impurities/degradants are unknown or unavailable, forced degradation studies should be performed. Forced degradation studies of the active pharmaceutical ingredient (API), placebo and finished product, using either peak purity analysis or a mass spectral evaluation, should be performed to identify and separate the potential degradation products<sup>8</sup>.

The forced degradation studies should consist of exposing the API, placebo and finished product to acid, base, peroxide, heat, light conditions and moisture or water until adequate degradation of the active has been achieved. An acceptable range of degradation may be 10-30% for assay and about 10% for related substance but may vary based on the active being degraded. Over degradation of the active or known impurity should be avoided to prevent the formation of secondary degradants. If placebo material is available, it should be stressed under the same conditions and for the same duration and as the API and finished product. The degraded placebo samples should be evaluated to ensure that any generated degradants are resolved from the analyte and impurity peak of interest.

Forced degraded sample should pass the peak purity and does not show any purity flag by using a photodiode array detector for chromophoric compound. Non chromophoric compound or GC sample should confirm mass spectral evaluation of degradation product peak and each known impurity peak should not show any significant changes in the fragmentation pattern from the parent compound<sup>9</sup>.

#### **Precision**

Precision reflects the closeness of agreement of a series of measurements between the series measurement obtained from multiple sampling from the same sample under the same condition at the same time. Precision may be considered in three levels repeatability, intermediate precision and reproducibility<sup>10</sup>.

#### **Repeatability**

Repeatability expresses the precision under the same operating condition over a short interval of time. It is also termed intra-assay precision. A minimum of six replicate sample preparation of a same sample or homogenous sample prepared at the 100% test concentration.

#### **Intermediate precision**

Intermediate precision reflects within-laboratory variations such as different days, different analysts, and different equipments. Intermediate precision testing can consist of two different analysts, each preparing a six sample preparations, as per specified analytical method. The analysts execute their testing on different days using separate instruments and analytical columns.

#### **Reproducibility**

Reproducibility expresses the precision of a method with in the laboratory variation like different days, different analyst and different equipments etc. Each testing site can prepare a total of six sample preparations, as per the analytical method. Results are evaluated to ensure statistical equivalence among various testing sites. Acceptance criteria similar to those applied to intermediate precision also apply to reproducibility.

#### **Accuracy**

Accuracy should be performed at a minimum of five concentration levels, for LOQ<sup>11</sup> and maximum concentration will be six replicate preparation and median concentration must be three replicate as per specified test method or it can be spiked synthetic mixture of product component and the acceptance criteria and number sample preparation detail given in table 6.

#### **Solution Stability**

The solution stability is stability of standard and extracted sample solution (ready to inject) from the sample or matrix and analyzed as per specified method, and it should be stored properly in room temperature and refrigerated condition depending upon the stability of the sample and standard solution.

The stability of standard and sample solution should be established in room temperature and refrigerated, if refrigerated before analysing it should be thawing to room temperature.

A minimum two preparation of standard and sample solution should be prepared and analysed as per specified method. The analysed solutions stored in necessary condition and the stability can be established for two days or solution stability can be established by an hour basis depending upon the nature of the product<sup>12</sup>.

#### **Ruggedness (Robustness)**

Robustness<sup>12, 13</sup> of an analytical procedure 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. Robustness must not necessarily include in minivaldation or in preclinical stage validation, but in complete validation and before transfer the analytical method to another laboratories it should be established, the procedure and acceptance criteria detail in table 8.

#### Limit of quantification and detection (LOD and LOQ)

The LOQ is the lowest amount of an analyte in a sample that can be quantitatively determined with suitable precision and accuracy. There are different approaches to the determination of LOQ. LOQ is a parameter to the quantitation of the sample at low level in the compound and is used particular for the impurities, degradation products and/or residual solvents. LOD and LOQ can be established from signal to noise ratio method and linearity curve method.

LOQ based on signal to noise ratio (SIN): This approach can only be applied if there is no much baseline noise, LOQ can be calculated as per US Pharmacopoeia or it can be derived from the computer aided soft ware's. For related substance and residual solvents a least concentration of impurities can be spiked in to the test solution (if no impurities present in the test solution) or synthetic mixture of product component of placebo preparation. The low concentration solution gives signal to noise ratio is about 10, that could be the LOQ and the low concentration solution gives signal to noise ratio is about 3, that could be the LOD of the particular impurity and it should be established for each impurities and interested peak<sup>14</sup>.

#### DOCUMENTATION

The validity of an analytical method should be established and verified by laboratory studies and documentation of successful completion of such studies should be provided in the validation

report. General and specific SOPs (standard operating procedure) and good record keeping are an essential part of a validated analytical method.

1. Summary information,
2. Method development, degradation study data and establishment of Relative Retention Factor, Relative Retention Time and LOQ etc.

#### Summary information

Summary information should contain detail of certificate of analysis of reference standard and/or working standard, validation protocols and summary reports, including analytical method development report and if any minivaldation reports.

- ✚ Summary table with a list contains validity period of the certificate of analysis reference standard and/or working standard, protocol number should be allotted for each and every method and the summary report of the all the validation should be addressed in a proper way.
- ✚ An operational description of the analytical method.
- ✚ A forced degradation stability studies and supporting data.
- ✚ A description of experiments conducted and if any error occurs during a validation, the incident report, original and repeated data detail should be covered.
- ✚ Documentation of intra and inter-assay precision and accuracy.
- ✚ Legible annotated chromatograms or mass spectrograms, if applicable
- ✚ Any deviations from SOPs, protocols, or (Good Laboratory Practice) GLPs (if applicable), and justifications for deviations and the incident report to be captured.

Table 1

Type of analytical procedure	Identification	Test for Impurities		Assay Dissolution Content/potency
		Quantitation limit		
Characteristics				
Accuracy	-	+	-	+
Precision	-	+	-	+
Repeatability	-	+	-	+
I. Precision <sup>1</sup>	-	+(1)	-	+(1)
Specificity (2)	+	+	+	+
Detection Limit	-	-(3)	+	-
Quantitation limit	-	+	-	-
Linearity	-	+	-	+
Range	-	+	-	+

- signifies that this characteristics is not normally evaluated.  
+ signifies that this characteristics is normally evaluated.

- (1) in case where reproducibility has been performed, intermediate precision is not needed.  
 (2) Lack of specificity of one analytical procedure could be compensated by other supporting analytical procedure(s).  
 (3) May be needed in some cases.

**Table 2**

Analytical methods	Parameters
Assay Content Uniformity or Blend Uniformity Uniformity of Dosage Units Dissolution	Precision, Accuracy, specificity only blank and placebo interference, Linearity and solution stability.
Related substance	Precision, Accuracy, specificity includes forced degradation study, LOD and LOQ, Linearity and solution stability.
Residual solvents	Precision, Accuracy, specificity only blank and placebo interference, LOD and LOQ, and Linearity.

**Table 3: US FDA guidelines for analytical method validation**

S.No	Test Methods	Parameter : Linearity <sup>ii</sup>
1	Assay	50 to 150 % of the test concentration and correlation coefficient should obtain $\geq 0.999$
2	Content Uniformity or Blend Uniformity	50% to 150 % of the test concentration from the lower strength and correlation coefficient should obtain $\geq 0.999$
3	Uniformity of Dosage Units	50% form the lower strength and to 150 % of the test concentration from the higher strength (if different strengths are available) and correlation coefficient should obtain $\geq 0.999$
4	Dissolution	50 to 150 % of the test concentration. If sustained product 10% to 150 % of the test concentration and correlation coefficient should obtain $\geq 0.999$
5	Related Substance and residual solvents	LOQ to 300% of each known impurity and correlation coefficient should obtain $\geq 0.95$

**Table 4**

Test Methods	Parameter : Specificity
Assay	No interference from blank <sup>iii</sup> , Placebo <sup>iv</sup> and degraded impurity
Content Uniformity or Blend Uniformity	No interference from blank and Placebo.
Uniformity of Dosage Units	No interference from blank and Placebo.
Dissolution	No interference from blank and Placebo.
Related Substance	No interference from blank, Placebo and any degraded or any impurity.
Residual solvents	Percentage interference can be considered for the final calculation if not more than 2.0% for blank as well as placebo <sup>v</sup> .

<sup>i</sup> Above table, wider range has been selected because we could avoid revalidation due to strength change or while adding any lower strength.

<sup>ii</sup> Diluents used for standard and test preparation.

<sup>iii</sup> Synthetic mixture of product component except active ingredient.

<sup>iv</sup> Synthetic mixture of product component including active ingredient.

Table 5

Test Methods	Parameter : Precision <sup>i</sup>
Assay	Six sample preparation to prepare as per specified method. The mean assay value and individual assay value should obtained 95.0% to 105.0 % and percent RSD should obtain 2.0% of six assay value and confidence intervals also can be calculated.
Content Uniformity or Blend Uniformity	Ten sample preparations to prepare as per specified method and sample collected in triplicate, three different places and one pooled sample. The mean assay value and individual assay value should obtain 90.0% to 110.0 % and percent RSD should obtained 2.0% for ten assay values.
Uniformity of Dosage Units	Ten dosage units should prepare as per specified method. The mean assay value and individual assay value should obtain 90.0% to 110.0 % and percent RSD should obtained 2.0% for ten assay values.
Dissolution	Six dosage units should prepare as per specified method. The mean assay value and individual assay value should obtain 95.0% to 105.0 <sup>vi</sup> % and percent RSD should obtained 5.0%.
Related Substance	Six sample preparation to prepare as per specified method or known impurity can be spiked. The mean and individual percentage impurity spiked may be 85.0% to 115.0 % and the percent RSD should obtain 15.0% of each individual impurity.
Residual solvents	Six sample preparation to prepare as per specified method or known solvent can be spiked. The mean and individual percentage solvent spiked may be 85.0% to 115.0 % and the percent RSD should obtain 15.0% of individual solvents.

Table 6

Test Methods	Parameter : Accuracy
Assay Content Uniformity or Blend Uniformity Uniformity of Dosage Units Dissolution	A minimum six sample preparation to prepare at lower and higher concentration, three preparations in the middle concentration as per specified method or active can be spiked with synthetic mixture of product component. The mean recovery value and individual recovery value should obtain 97.0% to 103.0 % and percent RSD should obtain 2.0%.
Related Substance	A minimum six sample preparation to prepare at LOQ level and 200% from of it and three preparations in the middle concentration as per specified method or impurity blend can be spiked. The mean recovery value and individual recovery value should obtain 85.0% to 115.0 % and percent RSD should obtain 15.0%.
Residual solvents	A minimum six sample preparation to prepare at LOQ level and 300% from of it and three preparations in the middle concentration as per specified method or solvent can be spiked. The mean recovery value and individual recovery value should obtain 85.0% to 115.0 % and percent RSD should obtain 15.0%.

Table 7

Test Methods	Parameter : Solution Stability <sup>vii</sup>
Assay Content Uniformity or Blend Uniformity Uniformity of Dosage Units Dissolution	The percent assay value should obtain with in $\pm 2.0\%$ from the initial assay value for sample preparation and $\pm 2.0\%$ difference from the response from the initial standard preparation.
Related Substance	The percent total impurity value should obtain with in $\pm 0.04\%$ and individual impurity within $\pm 0.02\%$ from the initial assay value for sample preparation and $\pm 2.0\%$ difference from the response from the initial standard preparation.
Residual Solvents	Usually organic solvents are stable there will be no significant change in stability study.



Table 8

Test Methods	Parameter : Robustness <sup>iii</sup>
Assay Content Uniformity or Blend Uniformity Uniformity of Dosage Units Dissolution Related substance Residual solvents <sup>ix</sup>	System suitability with in the acceptance criteria.

Table 9

Test Methods	Parameter : LOD and LOQ <sup>x</sup>
Related substance	A minimum six sample preparation to prepare at Quantitation level and Detection level shall be just established. The impurity blend solution can be spiked as per specified test method. The mean recovery value should obtain 85.0% to 115.0 % and percent RSD should obtain 15.0%.
Residual solvents	A minimum six sample preparation to prepare at Quantitation level and Detection level shall be just established. The solvent blend solution can be spiked as per specified test method. The mean recovery value recovery value should obtain 85.0% to 115.0 % and percent RSD should obtain 15.0%.

## CONCLUSION

Analytical method validation and method transfer data playing a fundamental role in pharmaceutical industry for releasing the commercial batch and long term stability data therefore, the data must be produced to acceptable scientific standards. For this reason and the need to satisfy regulatory authority requirements, all analytical methods should be properly validated and documented. The aim of this article is to provide simple to use approaches with a correct scientific background to improve the quality of the analytical method development and validation process. This article gives an idea about number of sample preparation, procedure and acceptance criteria for all analytical method validation parameters in wider range. Applications of analytical method and method transfer are also taken into consideration in this article. These various essential development and validation characteristics for analytical methodology have been discussed with a view to improving the standard and acceptance in this area of research<sup>13-16</sup>

## REFERENCES

1. Thompson M, Ellison SLR and Wood R. Harmonised Guidelines for single Laboratory Validation of Method of Analysis. *Pure Appl Chem*. 2008;74:835-55.
2. Wood R. How to Validate Analytical Methods. *Trends Analyt Chem*. 2005;54:149-58.
3. McDowall RD. The Role of Laboratory information Management systems LIMS in Analytical Method Validation. *Anal Chim Acta*. 2077; 54:149-58.
4. Puluído A, Ruusanches I, Boque R and Rius FX. Uncertainty of results ID routine Qualitative Analysis in Analytical Chemistry. *J Pharm Biomed Anal*. 2005;22:647-54.
5. Kallner A. Quality specification based on the uncertainty of measurement. *Se and J Lab Invest*. 2005;59:513-6.
6. Trullols E, Ruisanchez I, Rius FX. Trends in Analytical Chemistry. *J Lab Invest*. 2003;23:137-45.
7. Valcarcel M, Cardenas S Gallego M. Sample Screening system in analytical chemistry. *Trends Analyt Chem*. 1999;23:137-45.
8. Ye C, Liu J, Ren F, Okafo N. Design of Experimental Date Analysis By JMP (SAS Institute) in Analytical Method Validation. *J Pharm Biomed Anal*. 2000;23:581-9.
9. Bressolle F, Bromet PM, Audran M. Validation of liquid chromatographic and gas chromatographic method Applications to pharmacokinetics. *J Chromatogr*. 2000;686: 3-10.
10. Lindner W, Wainer IW. Requirements for initial assay validation and publication in *J Chromatography B*. *J Chromatogr*. 2006;707:1.2.

11. Rodbord D, Feldrnan Y Jaffe M. Kinetics of Two-Site Immuno radiometric (Sandwich) Assay-II. *Immunochem.* 1995;15: 77-82.
  12. Vander HY, Nijhuis A, Verbeke JS, Vandegtnste BG, Massart DL. Guidance for roubustness/ruggedness test in method validation, *J Pharm Biomed Anal.* 2009;24:723-53.
  13. Validation of analytical procedure: methodology Q2B, ICH Harmonized Tripartite Guidelines, 1996:1-8.
  14. *US Pharmacopeia.* 2007; 1:582-583.
  15. *US Pharmacopeia.* 2007; 1:680-681.
  16. Gaurav Tiwari, Ruchi Tiwari. Bio analytical method an updated review. *Pharmaceutical Methods.* 2010;1:25-38.
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