Manipal Journal of Pharmaceutical Sciences

Volume 6 | Issue 1 Article 10

3-1-2020

# Analytical Method Development and Validation: Requirements in Pharmaceutical Field

Hetal Bhejaliya
*Department of Pharmaceutics, Shree Dhanvantary Pharmacy College, Near Kim Railway Station (East), Kim, Surat-394110, Gujarat State, India*

Rohan Barse
*Department of Quality Assurance, Shree Dhanvantary Pharmacy College, Near Kim Railway Station (East), Kim, Surat-394110, Gujarat State, India*, barserohan@gmail.com

Review Article

# Analytical Method Development and Validation: Requirements in Pharmaceutical Field

Hetal Bhenjaliya, Rohan Barse*

Email: barserohan@gmail.com

## Abstract

Analytical method validation needed during the production and manufacture of drugs and such analytical procedure is sufficient for their intended purpose. The development of methods usually requires the collection of method specifications and the decision on the type of instrumentation. This needs a system of analyzing herbal products, new processes, and reactions, new compounds, active ingredients (macro analysis), residues (microanalysis), impurity profiling, etc.

**Key words:** Analyte, Qualitative, Quantitative, Standard, Validation

## Introduction

The creation of methods usually requires the collection of method specifications and the types of instrumentation to be determined[1]. It includes an analytical approach for herbal products, new processes and reactions, new ingredients, active substances (macro analysis), residues (microanalysis), impurity profiling, etc. This review article discusses the steps involved in developing and validating a drug molecule analytical approach in the pharmaceutical field[2,3]. Analytical measurements are linked to every aspect of society and there are countless explanations as to why these measurements are made. It is obviously important to determine the correct outcome and to be able to demonstrate that it is accurate.

Some of the prominent Quality Standards organizations are[5]:

**Hetal Bhenjaliya[1], Rohan Barse[2]**

1 *Department of Pharmaceutics, Shree Dhanvantary Pharmacy College, Near Kim Railway Station (East), Kim, Surat-394110, Gujarat State, India*

2 *Department of Quality Assurance, Shree Dhanvantary Pharmacy College, Near Kim Railway Station (East), Kim, Surat-394110, Gujarat State, India*

* Corresponding Author

Date of Submission: 25-07-2019, Date of Revision: 27-01-2020
Date of Acceptance: 29-01-2019

1. World Health Organization (WHO)
2. Pharmaceutical Inspection Cooperation Scheme (PIC/S)
3. United States Food and Drug Administration (US FDA)
4. The International Conference for Harmonization (ICH)
5. Current Good Manufacturing Practice (cGMP) regulations
6. Good Laboratory Practice (GLP) regulations.
7. Pharmaceutical Inspection Cooperation Scheme (PIC/S)

**Reasons valid for developing new analytical process**

1. Costly reagents and solvents required current analytical procedures. It also requires burdensome extraction procedures and separation.
2. Existing methods may be unreliable.
3. A similar sample matrix may not contain a suitable method for a specific analyte.
4. Existing technologies could be too complicated, cumbersome, not easily automated.
5. Current techniques may not have been appropriately resilient.
6. Cannot consider analytical methods for quantifying the analyte in biological fluids

**How to cite this article:** Bhenjaliya H, Barse R. Analytical Method Development and Validation: Requirements in Pharmaceutical Field. *MJPS* 2020; 6(1): 60-65.

**Goals for a new or better theoretical process**

1. Direct transmission of qualitative or quantitative data to laboratory computers for assessment, analysis, printing, and transmission via a network to other locations.
2. Sample preparation reducing the time, energy, materials and sample volume consumed by using simple quality assurance and quality control procedures reduced costs per analysis.
3. Qualitative description of special interest analytes, with some structural details.
4. Upon installation of the instrumentation and consideration of analyte parameters, the specifications should be used to further build, refine and test the system.

**Steps in the analytical method development[6]**

**1. Analyte standard characterization**

- While analyzing multiple components in the sample matrix, the number of components that pose the data is noted, and normal usability is calculated.
- Sample stability methods such as spectroscopic, high performance liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry (MS), etc.
- Consider the availability of standards for degradation products, possible impurities and synthetic precursors. The purity of all standards to be used in method development should be established and documented.

**2. Method requirements**

- Find the aims or parameters of the analytical methods to be set and describe the figures of the analytical merit.
- Additional criteria (time, energy, effort, time of analysis, available tools) tool limitations (pressure and solvents) and cost per analysis

**3. Literature search and prior methodology**

- Consider the objectives of the analytical methods to be established and all literature information relating to the drug is checked for relevant books, articles, United State Pharmacopoeia/ National Formulary (USP/NF), Association of analytical communities and American society for testing and materials publications for physicochemical properties, synthesis, solubility, and correct analytical methods.
- To decide if any analytical work on the analyte has ever been carried out within the company, and if so, to collect data, findings, records, memos, and publications.

**4. Choosing a method**

- If no methods are available to investigate the analyte to be analyzed in the past.
- Adopt sample preparation and instrument conditions (e.g. HPLC) wherever possible to take advantage of the latest methods and technologies.

**5. Instrument setup and initial studies**

- Installation, function and performance evaluation of the instrumentation in respect of laboratory standard operating procedures shall be verified by defining the appropriate instrumentation
- Starting with an accurate, proven norm is essential, rather than a complex matrix of samples.

**6. Optimization**

- If initial analytical results are less than optimal, start the optimization cycle, keeping the method's goal in mind. If necessary, using computer-based optimization tools.
- Pay special attention to experimental design during optimization.

**7. Demonstration of investigative data of value with standards**

- First, give customized empirical merit figures for the rule, before dealing with the actual research. The norm can not meet the required figures of empirical validity, and the study of the sample is futile.
- If the analytical merit figures are standardized and recorded, including standardization of items such as integration parameters and any statistical data treatment (when necessary), then sample analysis can start.

8. **Evaluation of proven methods with actual samples: Derivation of figures of merit**
   - Working with actual samples, conduct sample preparation steps to ensure analyte peak detection capability apart from any other possible interferences and contaminants.
   - Ideally, a "dilute and shoot" sample preparation will minimize the time and cost of the overall analysis. At the same time, one must remember to provide an injection solution that is compatible with the HPLC/MS system.

9. **Validation of figures of merit**
   - Validate the method once it has been developed and optimized. Regulatory laboratories (FDA) perform method validation by evaluating and documenting the USP.
   - The eight parameters of method validation namely accuracy, linearity, range, limit of detection (LOD), limit of quantification (LOQ), specificity, ruggedness and sturdiness.

10. **Determination of percentage of sample recovery and quantitative sample analysis**
    - An average percentage of the recovery in a sample matrix of spiked, genuine standard drug that contains no analyte. Recovery optimization has to be shown from sample to sample for reproducibility (average ± standard deviation)

11. **Method validation**

- Perform zero blind studies to demonstrate that known levels can be accurately and precisely determined in a real sample.
- Perform double-blind studies to further demonstrate the quantitative accuracy and precision of the overall method.
- Demonstrate repeatability of analytical results, within a single laboratory.
- Demonstrate analytical figures of merit reproducibility (ruggedness), from lab to lab, analyst to analyst, instrument to instrument, and so on, as required.
- Carry out additional analysis using the analyte's credible sample matrix selected reaction monitoring (SRM)of major interest. If there is no SRM available, have one synthesized outside, contract laboratory that will guarantee composition authenticity and identity.

12. **Preparation of written protocols and procedures**
    - Ensure that all necessary and sufficient details of the method are stated so that other labs can reproduce, as closely as possible, the experimental conditions.
    - Provide specific suppliers, addresses, catalog numbers, batch numbers, purity levels, and any other unique identifying features that will ensure that other analysts obtain the exact items to duplicate the method.

13. **Transfer of method technology to outside laboratories: Interlaboratory collaborative studies**
    - Continue method validation (ruggedness) outside the original laboratory by performing interlaboratory collaborative studies. interlaboratory studies can be accomplished by splitting known, authenticated samples and dispensing them to other laboratories while providing them with a complete procedure of the overall, final method.

14. **Comparison of interlaboratory studies**
    - Summarize and statistically compare validation results from interlaboratory collaborative studies to demonstrate whether the method can be transferred to other facilities and provide similar accuracy and precision of the quantitative results.

15. **Preparation of summary report on overall method validation results**
    - Prepare a summary report that includes results from all laboratories where the method was employed, with qualitative and quantitative results statistically treated.

16. **Summary report of final method procedures and results, and preparation of journal article for submission.**

***Sampling collection for the development of an analytical tool***

Make the quantification of impurities accurate at low levels which is essential in defining the quality of pharmaceutical products. To that end, a great deal of time is devoted to developing methods to meet these needs. The first step of this development project must be to define and gather a set of samples containing any potential and actual impurities that need to be assessed by the purity method. With this set in hand, subsequent development experiments can assure that a method or methods can accurately and completely determine the purity of a pharmaceutical product.

This paper provides detailed guidance in selecting the set of samples that contain the compounds of interest that must be quantified at low levels for a pharmaceutical product. A list of potential components and their sources is provided. Guidance is given on sample-screening techniques and when to eliminate samples that are redundant or unnecessary. Finally, techniques are outlined to enrich and combine samples in order to minimize the sample set.

**Method Development in Chromatography** [7]

***Problems in method development***

1. Stored samples are initially accurate but slowly become inaccurate with low bias
2. Absorption issue: A serially diluted curve is concave. The response factors drop with decreasing concentration. An increased exposure due to the number of dilutions, surface area contact, and time may cause this problem
3. Homogeneity: The sample to be analyzed gets partitioned**.**

The detector must then be selected to provide the required sensitivity, the necessary. These are some of the basic options, but there are many others to make, such as an internal or external standard, the sampling process, the need for gradient elution or temperature programming, sensitivity to detectors, etc. The efficient implementation of the system includes expertise in chromatographic science and comprehensive practical experience.

***Spectrophotometric methods***

These approaches are reliable and consistent with good reproducibility but due to costly instrumentation, reagent, and expertise the cost of the study is quite high. Such methods are reliable and consistent with good reproducibility, but the pharmaceutical analyst also takes into consideration a situation where concentration of one or more substances is required in samples known to contain other absorbent substances that may interfere with the assay.

If the sampling method is known, the interfering material's identity and concentration is known, and the degree of interference may be measured in the study. The analyst has a number of modifications to the basic spectrophotometric technique which can eliminate some sources of interference and allow all absorbing components to be measured accurately. Some changes to the basic procedure can be made if certain criteria are met. The basis of all the spectrophotometric techniques for multicomponent samples is the possessions that at all wavelengths:

- The sum of absorption of the individual components is the absorption of a solution.

or

- Precise absorption is the difference between the overall absorption of the sample cell solution and that of the reference cell solution.

There are various spectrophotometric methods available that can be used to test a mixture sample. They can be used according to methods:

- Difference spectrophotometry
- Derivative spectrophotometric method
- Method for absorbing the ratio (Q-Absorbance method)
- Simultaneous equation method

***Assay bias and factor for the response of analytes***

All analytical procedures are associated with a number of biases, particularly biological assays, that test for biopharmaceutical purity, potency, and molecular interactions. Adequate reference criteria may also not be readily available, as the commodity may be one of a kind. The most difficult part of the production and testing process can be determining the accuracy and bias of the assay. Comparing the findings of the new method with those of the old method often only makes sense when controlling for bias in the test.

***Stability***

The detection limit for a single analytical technique is the lowest analyte quantity in a sample that can be detected. The standard deviation of the response and the slope can be measured visually, by signal to noise ratio. Only analytical procedures that show baseline noise can be added to the detection limit signal to the noise method. Comparison of measured signals from samples with identified analyte concentrations with those from blank samples and the minimum level at which the analyte can be detected reliably. A signal-to-noise ratio of 3 or 2:1 is generally deemed appropriate for the detection limit estimate. The Detection Limit (DL) can be represented as DL=3.3 π/S where, while the answer is standard deviation, the calibration curve slope is S. The slope S can be estimated from the analyte's calibration curve. It can be measured in a variety of ways, depending on the standard deviation of the blank and the calibration curve.

**The USP eight steps of method validation[8]**

- Detection limit
- Quantitation limit
- precision
- linearity & size
- robustness
- Accuracy

**Validation parameters for ICH method[8,9]**

- Accuracy
- Detection limit
- Quantitation limit
- Specificity
- Linearity
- Distance
- Robustness
- Adaptability to the device

The assay validation carried out as per the parameters mentioned below[10,11,12].

The process type and its intended use determines the parameter to be investigated. The USP divide analytical method into:

1. Determination of performance characteristics.
2. Main components or active ingredients quantified
3. The determination of impurities or products for degradation

**Table 1:** Various analytical parameters and assay categories

| Analytical performance parameter | Assay category 1 | Assay category 2 Q | Assay category 2 L | Assay category 3 |
|---|---|---|---|---|
| Accuracy | Y | Y | | * |
| Precision | Y | Y | N | Y |
| Specificity | Y | Y | Y | * |
| LOD | N | N | Y | * |
| LOQ | N | Y | N | * |
| Linearity | Y | Y | N | * |
| Range | Y | Y | * | * |
| Ruggedness | Y | Y | Y | Y |

(Y=YES, N=NO)

Q = quantitative

L= limit test

*= Can be required according to the nature of the particular test

**Accuracy**

It is the calculation of an empirical method's precision, or the consistency of agreement between the calculated value and the true or agreed reference value.

**Precision**

It is the estimate of the degree of repeatability in the normal operation of a methodical process.

**Specificity**

In the being thereof other components that may be assumed to be present in the sample matrix, it is the ability to calculate accuracy and precisely the analysis value.

**LOD**

It is identified in a sample as the lowest analyte concentration that can be detected, but not necessarily quantitated.

**LOQ**

It is defined as the lowest concentration of analytes in a sample that can also be measured with appropriate precision and accuracy under the operating conditions specified by the method.

**Linearity and range**

Linearity is the system's ability to produce test results within a given range which are directly proportional to the concentration of the analyte.

**Range**

Using the method the interval between the upper and lower analyte levels was shown to be accurate, accurate and linear.

**Ruggedness**

It is the degree of reproducibility of experiments, expressed as a percentage Relative Standard Deviation (RSD), which has been obtained under various conditions.

**Robustness**

The method's robustness is assessed using various method parameters such as organic solvent percentage, temperature, organic strength, and the effect on the method's performance.

**Conclusion:**

The analytical method development is the need of pharmaceutical industries. It usually needs the set of requirements for the system and the decision on the instrumentation type. It includes a system of study of herbal products, new processes and reactions, new chemicals, active ingredients (macro analysis), residues (microanalysis), profiling of impurities, and so on.

**References**

1. Analytical method development & validation by Michael Swartz & Ira Krull, first edition, published by Marcel Dekker page no.39-50
2. Gary D Christian, analytical chemistry, sixth edition2007 page no. 6-8
3. P.D.Sethi, HPLC quantitative analysis of pharmaceutical formulations 1st edition, page no.11
4. Willard HH, Meritt LL, instrumental method of analysis, CBS publishers & distributors, New Delhi 7th edition page no. 2-6.
5. Swartz ME, Krull IS. Analytical method development and validation. New York: Marcel Dekker Inc; 1997.
6. Huet S, Jolivet E, Messean A, La regression non lineaire, method et applications en biologie, Paris: Editions INRA; 1992.
7. G. Oliver, R. Gerrit, and VZ. Maxmilian, Leading Pharmaceutical Innovation, Trends, and Drivers for Growth in the pharmaceutical industry, (2nd Ed., Springer, 2008) page no.12-15.
8. ICH Q2 (R1), Validation of analytical procedures (definitions and terminology); 2005; page no. 9-10.
9. ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology, note for guidance on validation of analytical procedures: Text and methodology (CPMP/ICH/381/95), 1995.
10. Dadgar D, Burnett PE, Choc MG, Gallicano K, Hooper JW. Application issues in bioanalytical method validation, sample analysis, and data reporting. J Pharm Biomed Anal 1995;13:89-97.
11. Breaux J, Jones K, Boulas P. Understanding and implementing efficient analytical methods development and validation. Pharm Technol Anal Chem Test 2003; 5:6-13.
12. Shabir GA. Validation of HPLC chromatography methods for pharmaceutical analysis. Understanding the differences and similarities between the validation requirements of the FDA, the US Pharmacopeia and the ICH. J Chromatogr 2003;987:57-66.