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Open Access Review Article

A review on analytical method validation and its regulatory perspectives

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ABSTRACT

Analytical methods plays vital role in the process of identification, separation and then quantification of chemical components in natural materials or synthetic materials based on their chemistry. The main purpose of the analytical method development and validation is to prove that proposed analytical method is accurate, specific, precise and robust in the pharmaceutical industry for analysis of a drug moiety. Analytical method development gives important information in the pharmaceutical industry, on the potency of a drug, the drug's bioavailability, the drug's stability and also its effects. The analytical method validation is essential for analytical method development and tested for specificity, linearity, accuracy, precision, range, detection limit, quantitation limit and robustness. In summary, analytical method development and validation confirms that an accurate, precise and reliable potency measurement of a pharmaceutical preparation can be performed.

Keywords: HPLC, HPTLC, UPLC, GC, MS, SOP

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INTRODUCTION

It may be defined that Analytical chemistry is the study of separation, identification and quantification of chemical components of natural and artificial materials constituted with one or more compounds or elements. Analytical chemistry is divided into two main categories based on analysis, qualitative analysis that is to say the identification with regard to the chemical components exist in the sample, where as quantitative analysis estimates the numerical amount or concentration of certain element or compound present in the substance i.e., sample.

Pharmaceutical analysis [1-3] plays a very outstanding role in the examination of pharmaceutical formulations and bulk drugs regarding the quality control and assurance. Number of drugs or compounds (New entities or partial structural modification of the existing one) introduced in the market, rapid increase in pharmaceutical industries [4], advancement in analytical instruments and production of drug around the world bring forward a rise in inevitable demand to seek novel and systematic analytical techniques in the pharmaceutical industries. The improvements of the analytical method development and analytical instruments have reduced the time and cost of analysis [5] and enhanced precision and accuracy. Techniques pertaining to analysis are developed and validated for active pharmaceutical ingredients, excipients, related substances, drug products, degradation products and residual solvents etc. Resulting which become an integral part of the required necessities for regulatory organization[6]. Analytical method development finally results in official test methods[7]. Consequently quality control laboratories used these methods to check the efficacy, identity, purity, safety as well as performance of products of the drug. Regulatory authorities give utmost importance on analytical methods in manufacturing. Drug approval by regulatory authorities requires the applicant to prove control of the entire process of drug development by using validated analytical methods [8].

As there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias, therefore it becomes necessary to develop novel analytical methods for such drugs.

Modern pharmaceutical analysis needs the following key points [9]

1. Validation should address the performance of the analytical procedure under conditions of routine use.
2. Suitability is strongly connected with both the requirements and the design of the individual analytical procedure,

3. Consequently, the analyst has to identify relevant parameters which reflect the routine performance of the given analytical procedure, to design the experimental studies accordingly and to define acceptance criteria for the results generated.
4. Absolute, preferably normalised parameters should be selected as acceptance criteria. These can be defined from regulatory requirements, statistical considerations or experience. Statistical significance tests should be applied with caution; they do not take into consideration the practical relevance.
5. Validation must not be regarded as a singular event. The analyst is responsible for the continued maintenance of the validated status of an analytical procedure.

I) TYPICAL TECHNIQUES AND INSTRUMENTATION IN ANALYTICAL CHEMISTRY[10]

The first instrumental analysis was flame emissive spectrometry developed by Robert Bunsen and Gustav Kirchhoff who discovered rubidium (Rb) and caesium (Cs) in 1860[11].

Currently there are many techniques and instrumentation available for qualitative and quantitative determination of drugs based on their physical (solubility, transparency or degree of turbidity, color, density or specific gravity for liquids, melting, freezing, boiling points and moisture content) chemical, physicochemical [12,13](physical phenomena that happened as a result of chemical reactions).

Some of the techniques are mentioned below:

1. Titrimetric techniques
2. Chromatographic techniques
3. Spectroscopic techniques
4. Electrochemical methods
5. Kinetic method of analysis
6. Electrophoretic methods
7. Flow injection and sequential injection analysis
8. Hyphenated techniques

1. Titrimetric techniques:

Origin of the titrimetric method of analysis goes back to somewhere in the middle of the 18th century. It was the year 1835 when Gay–Lussac invented the volumetric method which subsequently leads to the origin of term titration. Although the assay method is very old yet there are signs of some modernization, i.e., spreading of non-aqueous titration method, expanding the field of application of titrimetric methods to (very) weak acids and bases as well as potentiometric end point detection improving the precision of the methods. With the development of functional group analysis procedures titrimetric methods have been shown to be beneficial in kinetic measurements which are in turn applied to establish reaction rates. There are many advantages associated with these methods which include saving time and labour, high precision and the fact that there is no need of using reference standards.

2. Chromatographic techniques:

The objective of chromatography is to separate the various substances that make up a mixture. The applications range from a simple verification of the purity of a given compound to the quantitative determination of the components of a mixture. The chromatographic system consists of a fixed phase and a moving phase. The mixture to be analyzed or solute is introduced into the system through the mobile phase, and it is the affinity of the solute for one phase over the other which governs its separation from the other components. Each component is retained to a different degree in the system and retention is based on various attraction forces. A chromatogram is a graphical representation of the compounds eluting from a chromatographic system. Chromatography can be used for qualitative and quantitative determination of various food constituents. Paper chromatography, Thin layer chromatography[14], Gas liquid chromatography[15], High performance liquid chromatography [16,17] are the available choices for assay involving sophisticated equipment, which are highly sensitive, accurate and consume very tiny amount of samples for analysis.

3. Spectroscopic techniques:

3.1 Spectrophotometry:

Another important group of methods which find an important place in pharmacopoeias are spectrophotometric methods based on natural UV absorption and chemical reactions. Spectrophotometry is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength.

The advantages of these methods are low time and labour consumption. The precision of these methods is also excellent. The use of UV–Vis spectrophotometry especially applied in the analysis of pharmaceutical dosage form has increased rapidly over the last few years. The colorimetric methods are usually based on the following aspects:

- Complex-formation reaction
- Oxidation-reduction process
- A catalytic effect

3.2 Near infrared spectroscopy (NIRS)

Near infrared spectroscopy (NIRS) is a rapid and non-destructive procedure that provides multi component analysis of almost any matrix. In recent years, NIR spectroscopy has gained a wide appreciation within the pharmaceutical industry for raw material testing, product quality control and process monitoring. The growing pharmaceutical interest in NIR spectroscopy is probably a direct consequence of its major advantages over other analytical techniques, namely, an easy sample preparation without any pre-treatments, the probability of separating the sample measurement position by use of fibre optic probes, and the expectation of chemical and physical sample parameters from one single spectrum. The major pharmacopoeias have generally adopted NIR techniques.

3.3 Nuclear magnetic resonance spectroscopy (NMR)

Since the first report appeared in 1996 describing the use of NMR spectroscopy to screen for the drug molecules, the field of NMR based screening has proceeded promptly. Over the last few years, a variety of state-of-the art approaches have been presented and found a widespread application in both pharmaceutical and academic research. Recently NMR finds its application in quantitative analysis in order to determine the impurity of the drug, characterization of the composition of the drug products and in quantitation of drugs in pharmaceutical formulations and biological fluids.

3.4 Fluorimetry and phosphorimetry

The pharmaceutical industries continuously look for the sensitive analytical techniques using the micro samples. Fluorescence spectrometry is one of the techniques that serve the purpose of high sensitivity without the loss of

specificity or precision. A gradual increase in the number of articles on the application of fluorimetry and phosphorimetry in quantitative analysis of various drugs in dosage forms and biological fluids has been noticed in the recent past.

4. Electroanalytical techniques or methods:

Electrochemistry is the relationship between electrical properties and chemical substances in reactions. In its application to analytical chemistry, this generally involves the measurement of some electrical property under conditions which, directly or indirectly, allow an association between the magnitude of the property measured and the concentration of some particular chemical species. Both direct potentiometry and the potentiometric titration method require the measurement of emf between an indicator electrode system and a reference electrode system, the two comprising a cell system. In many present-day applications, the indicator and the reference electrodes are combined in a single electrode system called a "combination electrode. The application of electrochemical techniques in the analysis of drugs and pharmaceuticals has increased greatly over the last few years. The renewed interest in electrochemical techniques can be attributed in part to more sophisticated instrumentation and to increase the understanding of the technique themselves.

5. Kinetic methods of analysis

Kinetic method of analysis has been developing since 1950s and yet in modern days it is taking a major resurgence in activity. The repetitive interest in the kinetic methods can be credited to the advancements made in principles, in automated instrumentation, in understanding the chemical and instrumentation, in data analysis methods and in the analytical application. From the literature it is evident that the kinetic approach to analytical chemistry is rather general with several advantages over traditional equilibrium approach. Essentially, kinetic methods trust the measurements of concentration changes (detected via signal changes) in a reactant (which may be the analyte itself) with time after the sample and reagents have been mixed manually or mechanically.

6. Electrophoretic methods

Another important instrument essential for the analysis of pharmaceuticals is capillary electrophoresis (CE). CE is a relatively new analytical technique based on the separation of charged analytes through a small capillary under the impact of an electric field. In this technique solutes are perceived as peaks as they pass through the detector and the area of individual peak is proportional to their concentration, which allows quantitative estimations.

7. Flow injection and sequential injection analysis

The basis of Flow injection analysis (FIA) is injection of a liquid sample into a moving, non-segmented uninterrupted carrier stream of a suitable liquid. The injected sample forms a zone, which is then transported toward a detector that uninterruptedly records the changes in absorbance, electrode potential, or other physical parameter resulting from the passage of the sample material through the flow cell. The stages of flow injection analysis have been shown in Fig. 1.

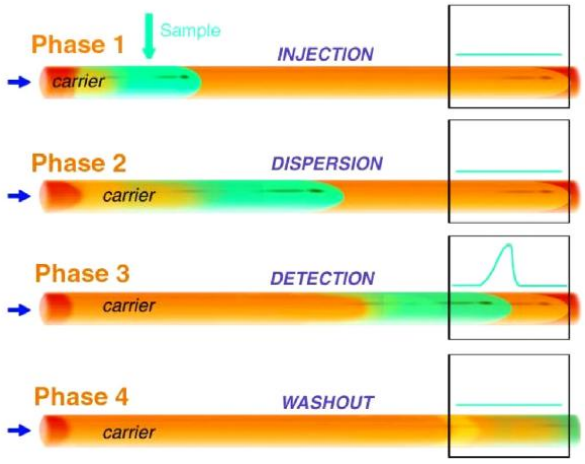

Figure 1: Stages of flow injection analysis. Source: http://ww2.chemistry.gatech/edu/class/analyst/fia/pdf

8. Hyphenated techniques

The coupling of a separation technique and on-line separation technique leads to the development of a hyphenated technique. The last two decades saw a remarkable advancement in the hyphenated techniques and its application in pharmaceutical analysis. A variety of hyphenated techniques such as LC-MS, GC-MS, LC-NMR, CE-ICP-MS and CE-MS have been applied in the analysis of pharmaceuticals.

II) ANALYTICAL METHOD DEVELOPENT[18-21]

When there are no compendial methods are available, new methods are being developed for analysis of novel products. To analyze the existing either pharmacopoeial or non-pharmacopoeial products novel methods are developed to reduce the cost besides time for better precision and ruggedness. These methods are optimized and validated through trial runs. Alternate methods are proposed and put into practice to replace the existing procedure in the comparative laboratory data with all available merits and demerits.

Regulatory perspectives on development of novel methods of drug analysis are:

a) When there is no official drug or drug combination available in the pharmacopoeias.

b) When there is no validated analytical process for the existing drug in the literature due to patent regulations.

c) When there are no analytical methods for the formulation of the drug due to the interference caused by the formulation excipients.

d) Analytical methods for the quantitation of the analyte in biological fluids are found to be unavailable.

Steps for analytical method development:

The procedure for analytical method development follows a set of steps as below:

1. Purpose of Analytical Method Development:

In the pharmaceutical industry, analytical method development gives important information on the potency of a drug, the drug's bioavailability, the drug's stability and also its effects. In the very first step, the purpose of conducting any analytical method development is established.

2. Highlighting of Steps

In the second step of Analytical Method Development, the steps involved in the development are recorded in a laboratory book or online database.

3. Characterization of the Analyte

In this step, both the biological and chemical properties in addition to the physical properties of the analyte are collected. After that, the analyte is obtained and stored according to its specific requirements. The methods for analysis are then recorded with an example being the chromatography technique which employs different methods such as the High Performance Liquid Chromatography.

4. Arrangement and definition of Requirements

5. Requirements for the method development of the analysis are done and recorded. All the materials, reagents and instruments are procured those are required for the analysis of the sample.

6. Review of Literature and existing Methods

All literature information related to the specific analyte e.g. a specific drug is assessed for any biological, chemical and chemical properties regarding the analyte. Reference is then taken from journals, books and any other publications.

7. **Choosing an Analytical Method**

From the information obtained from the literature during the literature review, a specific methodology is modified to cater for accurate output and also because methods change with the requirements of the analyte. If there are no previous methods in the literature being reviewed regarding the analyte, the procedure goes on uninterrupted.

8. Setting up of Instruments

Appropriate instruments for the analytical method development are set up in the laboratory by each of the instruments standard operating procedures. Standard Operating Procedures usually abbreviated as SOP's are a set of instructions or steps to aid in performing a specific procedure in a laboratory set up. They are usually universal and standardized for ease of use in any laboratory set up.

9. Optimization of the Method

In carrying out the optimization of the analytical method, parameters are changed individually depending on the arising interests. Optimization of an analytical method is done in reference to a systematic and procedural plan while making sure to critically follow all the documented steps.

10. Analytical Figures of Merit Documentation

Documentation of the analytical figures of merit decided upon is done. These analytical figures of merit include quantification limits, detection limits, analysis time frame, operational costs and sample preparation.

11. Development Method Evaluation

The resultant product of analysis should give a desirable result as expected in the identification of the analyte.

12. Sample Estimation, Quantitative Demonstration and Analysis of Samples

Estimation of an analyte with an example being a drug in a matrix sample containing the analyte is done here.

III) ANALYTICAL METHOD VALIDATION [22-26]

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. Guidelines from the USP, ICH, US-FDA etc., can provide a framework for validations of pharmaceutical methods. Results from the method validation can be considered to judge its quality, reliability as well consistency pertaining to analytical results.

Types of Analytical Procedures to be validated

The discussion of the validation of analytical procedures is directed to the four most common types of analytical procedures:

- Identification tests;

- Quantitative tests for impurities' content;

- Limit tests for the control of impurities;

- Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product.

Parameters of Analytical Method Validation

Analytical methods have been validated in pursuance of ICH guidelines of Q2 (R1). Validation parameters are:

1. System suitability
2. Specificity
3. Linearity
4. Precision
5. Accuracy
6. LOD
7. LOQ
8. Robustness

As a regulatory perspective, minimum numbers of determinations along with different types of test are presented in table 1.

Table 1: Validation characteristics normally evaluated for the different types of test procedures and the minimum number of determinations required.

Validation characteristic	Minimum number	Analytical procedure			
		Identity	Impurities		Assay[1]
			Quantitative	Limit	
1. Specificity [2]	Not applicable	Yes	Yes	Yes	Yes
2. Linearity	5	No	Yes	No	Yes
3. Range	Not applicable	No	Yes	No	Yes
4. Accuracy	9 (e.g. 3 × 3)	No	Yes	No	Yes
5. Precision					
Repeatability	6 or 9 (e.g. 3 × 3)	No	Yes	No	Yes
Intermediate precision/ Reproducibility [3]	(2 series)[4]	No	Yes	No	Yes
6. Detection limit	Approach dependent	No	No [5]	Yes	No
7. Quantitation limit		No	Yes	No	No

Yes / No normally evaluated / not evaluated
1 including dissolution, content/potency
2 lack of specificity of one analytical procedure could be compensated by other supporting analytical procedure(s)
3 reproducibility not needed for submission
4 no number given in [1b], logical conclusion
5 may be needed in some cases

Common method validation issues from regulatory perspective are:

- Wide validation acceptance criteria that is not well justified
- Incomplete system suitability criteria
- Inappropriate method controls
- Linearity assessed using serial dilution
- No product/assay control sample in bioassay
- Plate effects not evaluated
- LOQ is not within the validated linear range
- Specification does not fall within the validated linear range
- Validation conditions/equipment are different than the method validation SOP
- Method is validated for purity but not impurities
- Specificity does not include other products manufactured at the same site
- Stressed samples not used for validation of impurity detection
- Impurities levels detected below the LOQ reported numerically
- Method fails robustness evaluation and parameters not reflected in the method SOP
- Robustness not assessed properly.

IV) CONCLUSION

This review article provides a structured way to perform method validation as per regulatory perspective for its intended purpose and to assure the capabilities of the test method. Although the requirements of validation have been clearly documented by regulatory authorities, the approach to validation is varied and opened to interpretation, and validation requirements differ during the development process of pharmaceuticals. Validation is an important procedure in the pharmaceutical industry and it is utilized to ensure that quality is built into the processes by following CGMP and standard procedures.

REFERENCES

[1] David Watson G, *Pharmaceutical Analysis* (3rd Ed., Churchill Livingstone, London: Harcourt Publishers Limited, Essex CM 20 2JE, 2012).
[2] Beckett AH, and Stenlake JB, *Practical Pharmaceutical Chemistry* (4th Ed., Vol. I & II. CBS Publishers and Distributors, New Delhi: 2007).
[3] Higuchi T, and Brochman-Hansen, *Pharmaceutical Analysis,* (3rd edition, CBS Publishers and Distributors pvt. Ltd., New Delhi:1997).
[4] Oliver G, Gerrit R, and Maxmilian VZ, *Leading Pharmaceutical Innovation, „Trends and drivers for Growth in the pharmaceutical industry*, (2nd Ed., Springer, 2008)12-15.
[5] Br. Jay, J. Kelvin, and B. Pierre, *Understanding and Implementing Efficient Analytical Methods Development and Validation*, 2003.
[6] R.M. Christopher, and W.R. Thomas, *Quality Systems approach to Pharmaceutical cGMP Development and validation of Analytical Methods*, (1st Ed., 2005) 147-152.
[7] R. Lloyd Snyder, J. Joseph Kirkland and L. Joseph Glajah, *Practical HPLC method development* (2nd Ed., 1997) 179-184.
[8] Sharma BK, *Instrumental method of chemical analysis* (29th Ed., Meerut, Chromatography, HPLC, Goel Publishing House, 2013) 286-385.
[9] Joachim E, John HM. Miller (Eds.), *Method validation in pharmaceutical analysis: A guide to best practice* (Wiley-VCH verlag Gmbh & co., Weinheim: 2005).

[10] Siddiqui MR, Alothman ZA, Rahman N, Analytical techniques in pharmaceutical analysis: A review,Arabian Journal of chemistry, 2017; 10:S1409-S1421.
[11] Yoshiko A, Basic Education in Analytical Chemistry (*Analytical Sciences*, **17** Supplement: 2014).
[12] Willard HH, Merrit LL, Jr. Dean JA, and Jr. Settle FA, *Instrumental Methods of Analysis* (CBS Publishers, New Delhi: 1986).
[13] Day RA, and Underwood AL, *Quantitative Analyses*, (5th Ed., Prentice Hall, New Delhi: 1986).
[14] Macek and Karel, *Pharmaceutical Applications of Thin Layer and Paper Chromatography*, 1972; 62(6):1032.
[15] Ramana Rao G, Murthy SSN, and Khadgapathi P, Gas Chromatography to Pharmaceutical Analysis, *Eastern Pharmacist*, 1987; 30(353):35.
[16] Ramana Rao G, Murthy SSN, and Khadgapathi P, High Performance Liquid Chromatography and its Role in Pharmaceutical Analysis, *Eastern Pharmacist*, 1986; 29(346):53.
[17] Sastry C.S.P., Prasad T.N.V., and Rao E.V., Recent applications of High Performance Liquid chromatography in Pharmaceutical analysis, *Indian J. Pharm. Education*, 21 (37),1987.
[18] Ravisankar P, Gowthami S, and Devala Rao G, A review on analytical method development, *Indian journal of research in pharmacy and biotechnology*, 2014; 2(3):1183-1195.
[19] Ravisankar P, Rajyalakshmi G, Devadasu Ch, and Devala Rao G, Instant tips for right and effective approach to solve HPLC trouble shooting, *Journal of chemical and pharmaceutical sciences*. 2014; 7(3):259-274.
[20] Jay Breaux, Kevin Jones, and Pierre Boulas, "Development services analytical method development and validation" *Pharmaceutical technology,* 2003; 27(1):6-13.
[21] E. Michael Swartz, and Iras Krull, *Analytical method development and validation*, CRC press, Marcel dekker, Inc., Madison Avenue, New York: 1997.
[22] Carr GP and Wahlichs JC. "A practical approach to method validation in pharmaceutical analysis", J. Pharm, Biomed. Anal, 1990; 8:613-618.
[23] United States Pharmacopoeia,24, National Formulary 19, section <1225> „Validation of compendial methods". US Pharmacopoeial convention, Rockville, Validation of analytical procedures text and methodology Q2 (R1), November, 2000: 2005.
[24] International conference on harmonization (ICH) of technical requirements for registration of pharmaceuticals for human use, *Validation of analytical procedures: Text methodology*, (Q2 (R1) Geneva, 2005) 6-13.
[25] Draft guidance analytical procedures and method validation, US food and drug administration, Centre for drugs and biologics, Department of Health and Human Services. http://www.fda.gov/cder/guidance/2396 dft.htm#111, 2000.
[26] Orr J.D, Krull I.S and Swartz M.E, *Validation of impurity methods Part II*, (LC North Am; 21, 2003) 1146-1152.

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