Review Article

Method Development and Validation of Pharmaceuticals by Different Instrumental Techniques – A Review

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Received: 23-05-2018; **Revised:** 30-06-2018; **Accepted:** 13-07-2018.

ABSTRACT

Analytical methods development and validation assume imperative parts in the discovery, improvement and preparation of pharmaceuticals. Method development is the way toward demonstrating that a analytical strategy is satisfactory for use to measure the concentration of an active pharmaceutical ingredient (API) in a particular formulated dosage form which enables simplified methods to be utilized to check that an analytical procedure, precisely and reliably will convey a reliable estimation of an active ingredient in an compounded preparation. The analytical strategy validation is important for analytical technique improvement and tested widely for specificity, linearity, exactness, accuracy, precision, range, detection limit, quantization limit, and robustness. In outline, analytical method development and validation permits to affirm that an exact and reliable potency estimation of a pharmaceutical preparation can be performed.

Keywords: Method validation, Pharmaceuticals, Specificity, Precision, Accuracy, Validation Parameter, Stability indicating.

INTRODUCTION

The quantity of medications brought into the market is expanding each year. These medications might be either new elements or halfway auxiliary alteration of the current one. The target of any analytical estimation is to acquire predictable, solid and exact information. Validated analytical [1]strategies assume a noteworthy part in accomplishing this objective. Validation of analytical strategies is additionally required by most directions and quality norms that effect research facilities. All the time there is a period slack from the date of presentation of a medication into the market to the date of its consideration in pharmacopeias. This happens due to the conceivable vulnerabilities in the constant and more extensive use of these medications, reports of new toxicities (bringing about their withdrawal from the market), advancement of patient protection and presentation of better medications by contenders. Under these conditions, benchmarks and logical methods for these medications cannot be measurable in the pharmacopeias [2, 3]. There is an extension, accordingly to develop more up to date analytical techniques for such medications. Analytical method development and validation assume important parts in the revelation, advancement, and preparation of pharmaceuticals. Pharmaceuticals formulated with more of one medication, regularly alluded to as combination product, are proposed to meet previously unmeet patients, require analytical method development and validation by consolidating the remedial impacts of at least two medications in a single product. These combination products can show amazing difficulties to the analytical chemist responsible for the improvement and approval of analytical method. The official test strategies that outcome from these procedures are utilized by quality control research facilities to guarantee the integrity, pureness, effectiveness, and execution of drug products. Identification and assessment of debasements is an essential assignment in pharmaceutical process development for quality and safety. Regulatory authorities like ICH [4, 5], USFDA, and MHRA has their own guidelines for method development & validation for APIs & impurity profiling [6, 7] in APIs. Essential criteria for new method development for drug analysis [8]

- The drug or drug combination may not be legitimate in any pharmacopeias.
- An appropriate analytical methodology for the drug may not be available in the literature because of patent regulation.
- Analytical strategies may not be approachable for the drug as a formulation because of the obstruction caused by the formulation excipients.
- Analytical techniques for the quantization of the medication in biological solution may not be available
- Analytical techniques for a drug in blend with different drugs may not be available
- The existing analytical procedure may require costly reagents and solvents. It might also include bulky extraction and isolation techniques and these may not be decisive.

Analytical method development

Analytical chemistry deals with methods for labeling, isolation, and measurement of the chemical components of innate and unreal materials.[9] The selection of analytical methodology is depending on many attention, such as: chemical properties of the test sample and its concentration sample matrix, the agility and expense of the analysis, type of measurements i.e., determinable or qualitative and the number of samples. A qualitative approach yields data of the chemical identity of the species in the sample. A quantitative approach gives numerical information regarding the relative quantity of one or more of the analytes in the sample. There are five general types of analytical methods, each with its own set of validation requirements Identification tests, Potency assays, Quantitative assay for impurities Limit test for the control of impurities and Specific tests. The first four tests are universal tests, but the exact tests such as particle-size analysis and X ray diffraction are used to control distinct properties of the active pharmaceutical ingredient (API) or the formulation.[10, 11]

Method validation

The necessity to validate an analytical procedure is come across by analyst in the pharmaceutical industry on an almost daily basis, because sufficiently validated methods are necessity for approval of regulatory filings. The document contains definition of different validation parameters. The United States Environmental Protection agency (US EPA), Resource Conservation and Recovery Act (RCRA), The American Association of Official Analytical Chemist (AOAC), and other scientific organizations provide procedures that are validated through multi-laboratory studies[12]. The US FDA has scheduled protocol on submitting sample and examining data for methods validation. The United States Pharmacopoeia (USP) has announced particular guidelines for method validation and compound evaluation [13]. The goal of validation of analytical method is to show that it is appropriate for its intended purpose. The discussion of the conformation of analytical procedures is aimed to following four common types [14] such as identification tests, quantitative tests for impurities content, limit tests for the control of foreign substances, and quantitative tests of the active component in samples of drug substance or formulated product or other selected components in the drug product. Methods may need to be validation and revalidation[15].The various parameters for analytical methods are selectivity/Specificity ,precision and Reproducibility, accuracy and Recovery ,stability, range, limit of Detection, limit of Quantization, repeatability, reproducibility, measurement Uncertainty, sensitivity and ruggedness.

Typical instrumental techniques

The methods of estimation of drugs are divided into physical, chemical, physicochemical and biological categories. In these methods, generally physical and physicochemical methods are applied and the most of the physical methods pertaining to analysis immerse the studying of the different physical properties of a substance. They are examining of the solubility, clearness or degree of turbidity, color, density or specific gravity (for liquids), melting, freezing, boiling points and moisture content. Physicochemical methods [16, 17] are utilized to test the physical changes that happened as a result of chemical reactions. In the Physicochemical Methods, the ocular properties like Refractometry, Polarimetry, Emission Spectrophotometry and Nephelometry or Turbidometry, Electrochemical properties such as Potentiometry, Amperometry and Polarography and Chromatography like Paper, Column, Thin Layer[18], Gas Liquid Chromatography[19], High Performance Liquid Chromatography[20,21] methods are usually preferable. Methods involving nuclear reaction like Nuclear Magnetic Resonance to be more popular. GC-MS analysis is one of the prominent tools. The chemical methods include the stoichiometric and gravimetric procedures, which are depend on complex formation, acid – base and redox reactions. Titrations like complexometry and non-aqueous have been extensively applying in pharmaceutical analysis whenever the sensitivity at milligram level is adequate and the conflict are negligible. The current techniques like HPLC, UPLC, GLC, GC-MS/MS, LC-NMR and Liquid chromatography–mass spectrometry are the convenient choices for assay involving sophisticated equipment, which are highly sensitive, accurate and require very small amount of samples for analysis.

UV-Visible spectrophotometry

UV-Visible spectrophotometry[22,23] is a common attenuated technique used in pharmaceutical investigation. It based on estimating the measure of ultraviolet or visible radiation absorbed by a substance in solution. Instrument which measure the proportion, or function of proportion, of the intensity of two light emissions in the U.V-Visible locale are called Ultraviolet-Visible spectrophotometers. In quantitative examination, original compound can be distinguished by use of spectrophotometer, if any recorded information is available, and quantitative spectrophotometric investigation is applied to ascertain the amount of molecular species absorbing the radiation. Spectrophotometric method is simple, quick, reasonably particular and relevant to little amounts of compounds.

RP-HPLC (Reverse Phase High Pressure Liquid Chromatography)

Reversed-phase chromatography (RP-HPLC) [24] isolates particles based on contrasts in their hydrophobicity. The segments of the analyte mixture pass over stationary-phase particles bearing pores sufficiently substantial for them to enter, where interaction with the hydrophobic surface expels them from the streaming mobile phase stream. The quality and nature of the interaction

between the sample particles and the stationary phase relies upon both hydrophobic interaction and polar interaction. As the concentration of organic solvent in the eluent increases, it achieves a critical value for each analyte which desorbs it from the hydrophobic stationary-phase surface and enables it to elute from the column in the streaming mobile phase**.** The mobile phase has to be chosen in terms of solute retention and solvent solute separation. Solvent polarity is the key term in chromatographic separations since a polar solvent will give rise to low solute retention in normal phase and high solute retention in reverse phase LC.

Steps for Analytical Development [25, 26]

Analyte Standard Characterization

a) All data about the analyte i.e., physical and chemical properties, lethality, virtue, hygroscopic nature, solubility and stability.

b) The standard analyte (100% purity) is acquired. Made an arrangement for the best possible storage (refrigerator, desiccators and freezer).

c) When numerous components are to be investigated in the sample natrix, the quantity of components is noted, information is assembled and the accessibility of standard for everyone is resolved.

METHOD REQUIREMENTS

The objectives of the analytical method that should be developed are considered. The limit of detection, selectivity, linearity, range, accuracy and precision are characterized.

Literature Search and Prior Methodology

The data related with the analyte is overviewed. For synthesis, physical and chemical properties, solubility and pertinent analytical methods. Books, periodicals and USP/NF, and publication are checked on. Concoction Abstracts Service (CAS) automated computerized literature searches are convenient.

Choosing a Method

Utilizing the data in the literatures, methodology is adjusted. The methods are adjusted wherever necessary. At times it is important to gain extra instrumentation to replicate, change, enhance or approve existing methods for in-house analytes and samples. There is generally one compound for which analytical technique as of now exist that is like the analyte of interest.

Instrumental Setup and Initial Studies

The required instrumentation is setup installation, operational and performance qualification of instrumentation and SOP's are checked. Continuously new solvents, filter are utilized the analyte standard in an appropriate injection/introduction solution and in known concentration and solvents are readied. It is imperative to begin with, known standard rather than with a complex sample matrix.

Optimization

During optimization one parameter is changed at once, and set of conditions are secluded, instead of utilizing an experimentation approach. Work has been done from a sorted out orderly plan, and each progression is recorded (in a lab journal) if there should arise an occurrence of dead ends.

Documentation of Analytical Figures of Merit

The originally decided analytical figures of merit limit of quantization (LOQ), Limit of detection (LOD), linearity, time per analysis, cost, and test preparation and so on are reported.

Evaluation of method development with actual Samples

The sample solution should prompt unequivocal, total recognizable proof of the analyte peak of intrigue apart from all other matrix components

Method Validation [27]

It can be done by calculating these parameters such as precision, intermediate Precision/Ruggedness, accuracy, linearity, LOD, LOQ, and robustness.

Ultra-Performance Liquid Chromatography

The UPLC working principle depends on the primary of utilization of stationary phase comprising of particles less than 2 µm. The basic principle of this separation are represented by the van Deemter equation, which is an experimental formula that depicts the relationship between flow rate and plate hight (HETP or column efficiency) [28-30].In this separation mechanism the foremost apply is Van Deemeter equation, with which any understudy of chromatography is intimately familiar.

$H=A+B/v+Cv$

Where A, B and C are constants. V is the linear velocity, the carrier gas flow rate.

Method Development

Facts for basic compound

1. Alkaline pH rises retention of alkaline analytes
2. Methanol rises retention of all components related to acetonitrile
3. Similar alkaline analytes differ little in selectivity, respective to one another, when they are either fully charged or uncharged
4. Largest selectivity differences between bonded phases occur with methanol and analytes in their unionized state

Facts for acid compound

1. Acidic pH improves retention of acidic analytes

2. Methanol improves retention of all components compared to acetonitrile
3. Large differences in selectivity are observed when change in pH alters charge state
4. Largest selectivity differences between bonded phases occur with methanol and analytes in their unionized state

Sample injector

Sample injection is of much importance in UPLC[31]. Intense pressures to be attained in UPLC are not promoted by conventional injection valves. To avoid the hazardous effects of intense pressure fluctuations, the process of sample injection need to be pulse – free and swept volume also should be minimum for reducing band spreading.

Pumping System[32]

Decrease in particle size, requires greater pressure range. Therefore, pumps should be arranged in such a way that it is able to transfer solvent smoothly and reproducibly at such high pressures, which can operate in both isocratic as well as gradient separation modes.

UPLC columns[33]

Design of particles < 2.0 µm is a challenging task. The followings are specification about some column-

ACQUITY UPLC BEH T M C18 and C8 (straight chain alkyl columns)

ACQUITY UPLC BEH Shield RP 18 (embedded polar group column)

Zorbax stable Bond C8 and C18 is designed for low pH range.

Zorbax Extend C18 is suitable for high pH range.

Zorbax XDB-C8 and C18 for general purpose.

Zorbax-SB CN which provides different reversed phase polarity

Alltima HP HILIC for hydrophilic interaction chromatography separations and is a non bonded, silica column.

Pro sphere HP ZAP C18 for high speed reversed phase separation.

Pronto PEARL

TPP-C8

ACE EPS (8 % carbon loading)

C18 EPS (16 % carbon loading)

Liquid chromatography–mass spectrometry

LCMS is a coupling strategy of both liquid chromatography and mass spectroscopy. Liquid chromatography (LC) isolates the components of a sample in view of contrasts in their affinity (or retention strength) for the stationary phase or mobile phase, at that point identifies the isolated segments utilizing UV, fluorescence, or electrical conductivity in view of their properties. Mass spectrometry (MS) offers a very delicate strategy that ionizes the sample components utilizing different techniques, at that point isolates the subsequent particles in vacuum based on their mass-to-charge proportions and measures the intensity of every particle. Since the mass spectra gave by MS can demonstrate the concentration level of ions that have a given mass, it is to a great degree accommodating for qualitative analysis. In this manner, LC-MS system join the remarkable separation resolution of liquid chromatography with the exceptional outstanding capacities of mass spectrometry. The mass spectra acquired from these scan estimations gives molecular mass and basic data for eluted components, which supplements the quantitative data in view of retention times got utilizing other LC detectors.[34, 35]

LC–MS method development

In developing an LC–MS method[36] these important aspects to consider are:

1. There are a very large number of parameters in LC–MS methods that can be optimized.
2. Some of the key performance characteristics – most especially the ability of ionization of the analyte in the ion source – are either crucial to control or are very sensitive to small changes in system parameters.
3. MS detector in general shows lesser repeatability compared to most other detectors (in their respective working concentration ranges), specially the UV–vis absorbance detector. The repeatability standard deviation of MS signal, even if replicate samples are analyzed within a short time period, can be quite high. The adherence of the quantitative MS results is decreased first of all by different ion source related phenomena, such as ionization suppression/enhancement. This has important implications for determining trueness, precision and accuracy.
4. MS as a detector is mostly used for measuring of very small quantities of analyte. Therefore, a number of problems, such as incomplete selectivity, non-ideal sample preparation, etc. can be even further amplified.

Parameters of LC–MS methods[37, 38]

The parameters for LC-MS method are selectivity, specificity, confirmation of identity, ruggedness/robustness, limit of detection, limit of quantitation, decision limit and detection capability.

CONCLUSION

Analytical method validation plays a fundamental role in pharmaceutical industry for releasing the commercial batch and long term stability data. Hence the data must be produced to acceptable scientific standards. Therefore the need to satisfy regulatory authority requirements all analytical methods should be properly validated and documented.

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Source of Support: Nil, **Conflict of Interest:** None.