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Review on Development and Validation of Stability Indicating Method RP-HPLC on Marketed Formulation

Dr. Anup Barsagagade[1], Ashwini Bhukya[2], Yogita Kuranjekar[3], Nutan Pustode[4]

[1]Associate Professor, Maharashtra Institute of Pharmacy Betala, Bramhapuri, Gondwana University, Gadchiroli
[2,3,4]Students, Maharashtra Institute of Pharmacy Betala, Bramhapuri, Gondwana University, Gadchiroli

Abstract

The aim of present research work is to "DEVELOP AND VALIDATION OF STABILITY INDICATING METHOD RP-HPLC ON MARKETED FORMULATION " Its a process in which different chemical substances, including the active drug, are combined to produce a final medicinal product. The word formulation is often used in a way that includes dosage form.A tablet (also known as a pill) is a pharmaceutical oral dosage form (oral solid dosage, or OSD) or solid unit dosage form. Tablets may be defined as the solid unit dosage form of medication with suitable excipients. It comprises a mixture of active substances and excipients, usually in powder form, that are pressed or compacted into a solid dose. The main advantages of tablets are that they ensure a consistent dose of medicine that is easy to consume.The high-performance liquid chromatography (HPLC) of Shimadzu SCL-10AVP inbuilt with binary pump (LC-10ATVP), UV detector (SPD-10AVP), Rheodyne 20µl loop capacity manual injector (P/N 77251) was used throughout the analysis. The LC-Solution software was used to interpret the HPLC reports. Zodiac C18, 5µm; 150 x 4.6 mm ID., column purchased from UltraChrom Innovatives Pvt. Ltd was used throughout the analysis. Digital weighing balance (ME-204) purchased from Mettler-Toledo (USA), ultra-sonicator Labman® purchased from UltraChrom Ltd, India. Digital pH meter from Mettler-Toledo was purchased from (Mumbai-India). 50 µ micro-syringe was purchased from Hamilton USA. 0.20µ and 0.45µ nylon membrane filters were purchased from Phenomenex® Mumbai, India. From all above results and discussion, it has been concluded that the developed analytical method for the estimation of tablet formulation has obliged the ICH guidelines. As per the ICH guidelines, the developed method has complied the linearity range (calibration data), accuracy/drug recovery studies (%), repeatability, precision studies (intraday and interday/intermediate), and robustness. Moreover, as per the ICH guidelines, the system suitability test performed for marketed formulation has achieved all guidelines; including, tailing factor (T), separation factors (α), theoretical plates (N), capacity factor (k'), resolution ® and RSD (%). The validated stress degradation studies under thermal, oxidative, alkali and acid ascertained few degradation products for marketed formulation

Hence, this developed and validated method for investigation by reverse phase high performance liquid chromatography (RP-HPLC) can be used for routine analysis of estimation of marketed formulation.

INTRODUCTION

A tablet (also known as a pill) is a pharmaceutical oral dosage form (oral solid dosage, or OSD) or solid unit dosage form. Tablets may be defined as the solid unit dosage form of medication with suitable excipients. It comprises a mixture of active substances and excipients, usually in powder form, that are pressed or compacted into a solid dose. The main advantages of tablets are that they ensure a consistent dose of medicine that is easy to consume

Fig no . 1.tablet formulation

HPLC Introduction

High Performance Liquid Chromatography (HPLC) was derived from the classical column chromatography and, is one of the most important tools of analytical chemistry today. The principle is that a solution of the sample is injected into a column of a porous material (stationary phase) and a liquid (mobile phase) is pumped at high pressure through the column. The separation of sample is based on the differences in the rates of migration through the column arising from different partition of the sample between the stationary and mobile phase.

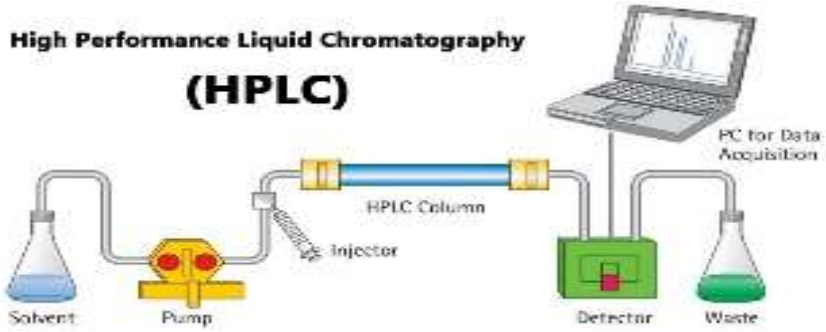

Fig .no.2.schematic representation of RP-HPLC instrument

Method development

Analytical method development and validation studies play an important role in discovery, development and manufacture of pharmaceuticals. These methods used to ensure the identity, purity, potency, & performance of the pharmaceutical drug products. There are many factors to consider when developing methods. The initially collect the information about the analyte's physicochemical properties (pKa, log P, solubility) and determining which mode of detection would be suitable for analysis (i.e., suitable wavelength in case of UV detection)

The majority of the analytical development effort goes into validating a stability indicating HPLC–method. The goal of the HPLC-method is to try & separate quantify the main active drug, any reaction impurities, all available synthetic inter-mediates and any degradants. Steps involve in method development are:

1. Understand the physicochemical properties of drug molecule.
2. Set up HPLC conditions.
3. Preparation of sample solution for method development.
4. Method optimization.
5. Validation of method

Method Validation studies

Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for its intended use. The methods validation process for analytical procedures begins with the planned and systematic collection by the applicant of the validation data to support analytical procedures. All analytical methods that are intended to be used for analyzing any clinical samples will need to be validated. The validation of analytical methods is done as per ICH guidelines. Components of method validation studies which have to be evaluated during methods validation are as follows

System Suitability test

- Repeatability
- Precision studies (Intraday and Interday/intermediate)
- Linearity/Calibration studies
- Detection limit (LOD)
- Quantitation limit (LOQ)
- Robustness
- Accuracy/Drug recovery studies
- Forced degradation/Stability indicating studies
- Peak retention time,
- Peak tailing,
- Capacity factor (k´),

1. Repeatability

Repeatability is the variation experienced by a single analyst on a single instrument. It does not distinguish between variation from the instrument or system alone and from the sample preparation process. During validation, repeatability is performed by analyzing multiple replicates of an assay composite sample by using the analytical method [63,65]. The recovery value is calculated. Intermediate precision is the variation within a laboratory such as different days, with different instruments, and by different analysts. The precision is then expressed as the relative standard deviation.

2. Precision studies

The precision of an analytical method is the degree of agreement among individual test results obtained when the method is applied to multiple sampling of a homogenous sample. Precision is a measure of the reproducibility of the whole analytical method (53). It consists of two components: repeatability and intermediate precision or system alone and from the sample preparation process. During validation, repeatability is performed by analyzing multiple replicates of an assay composite sample by using the analytical method. The recovery value is calculated.

3. Linearity

Linearity is the ability of analytical procedure to obtain a response that is directly proportional to the concentration (amount) of analyte in the sample. If the method is linear, the test results are directly or by well-defined mathematical transformation proportional to concentration of analyte in samples within a given range. Linearity is usually expressed as the confidence limit around the slope of the regression line. Range is defined as the interval between the upper and lower concentrations of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity.

4. Detection limit (LOD)

The detection limit (DL) or limit of detection (LOD) of an individual procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value ( ). In analytical procedures that exhibit baseline noise, the LOD can be based on a signal-to-noise (S/N) ratio (3:1), which is usually expressed as the concentration of analyte in the sample.(book) The signal-to-noise ratio is determined by: s = H/h Where H = height of the peak corresponding to the component. H = absolute value of the largest noise fluctuation from the baseline of the chromatogram of a blank solution.

5. Limit of Quantification (LOQ)

The limit of Quantitation (LOQ) or Quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy [54]. For analytical procedures such as HPLC that exhibit baseline noise, the LOQ is generally estimated from a determination of S/N ratio (10:1) and is usually confirmed by injecting standards which give this S/N ratio and have an acceptable percent relative standard deviation as well.

6. Robustness studies of HPLC

Robustness is defined as the measure of the ability of an analytical method to remain unaffected by small but deliberate variations in method parameters (e.g. pH, mobile phase composition, temperature and instrumental settings) and provides an indication of its reliability during normal usage. Determination of robustness is a systematic process of varying a parameter and measuring the effect on the method by monitoring system suitability and/or the analysis of samples.

7. Accuracy or Drug recovery studies

Accuracy is the nearness of a measured value to the true or accepted value. Accuracy indicates the deviation between the mean value found and the true value (43). Lt is determined by applying the method to samples to which known amounts of analyte have been added. These should be analysed against standard and blank solutions to ensure that no interference exists. The accuracy is then calculated from the test results as a percentage of the analyte recovered by the assay. It may often be expressed as the recovery by the assay of known, added amounts of analyte.

8. Forced degradation and stability indicating studies

Chemical stability of pharmaceutical molecules is a matter of great concern as it affects the safety and efficacy of the drug product. The FDA and ICH guidance state the requirement of stability testing data to understand how the quality of a drug substance and drug product changes with time under the influence of various environmental factors.

9. Tailing factor

Our treatment of chromatography in this section assumes that a solute elutes as a symmetrical Gaussian peak, such as that shown in Figure 1 as dotted line. This ideal behaviour occurs when the solute's partition coefficient, KD is the same for all concentrations of solute

KD = [Ss] / [Sm]
[S]s= concentration of solute in the stationary phase,
[S]m = concentration of in the mobile phase,

Material and method

1. Reagents

HPLC grade acetonitrile and deionised water were purchased from Merck (Mumbai, India). 0.20µ and 0.45µ nylon membrane filters were used and purchased from UltraChrom Innovatives Pvt. Ltd. (India). All other chemicals and reagents were used of analytical grade.

2. Standard stock solutions

Standard stock solution of marketed formulation was prepared by dissolving 10 mg of the drug in 10 ml of acetonitrile-methanol-water (4:4:2 v/v) in a 20 mL volumetric flask. Furthermore, freshly prepared sample solution was sonicated for 10-20 minutes and filtered through 0.20µ nylon filters. Required serial dilution was made for evaluating the validation studies

3. Chromatographic conditions

Chromatographic separation was achieved on Acclaimed mix-mode HILIC-1 (150 mm × 4.6 mm, 5µm) applying an isocratic elution based on water- acetonitrile (40:60, v/v) as a mobile phase. The ultraviolet detector was operated at 230 nm. The buffer solution was filtered through 0.2 µm nylon membrane filter and degassed for 10-20 min in an ultrasonic bath prior to its use. The mobile phase was pumped through the column at a flow rate of 1.1 mL min-1. The column temperature was adjusted to 28◦C and the injection volume was 20 µL.

4. Sample preparation for drug recovery studies

Exactly 10-20 tablets of marketed formulation was containing 500 mg of were weighed separately and then crushed to the fine powder. An accurately weighed 10 mg amount of the finely powder was transferred to 25 mL volumetric flask. It was then mixed with 10 mL of equal volume of acetonitrile-methanol-water (4:4:2, v/v) and sonicated for 20 mins. Furthermore, the solution was filtered through 0.20µ filter and then analysed with HPLC technique.

5. Sample preparation for Linearity/Calibration studies

Accurately measured aliquots of stock solutions equivalent to 32.15-500 µg, of REM, respectively were transferred separately into a series of 10 mL volumetric flasks. The final volume was adjusted with same mobile phase, and then 20 µL were injected into HPLC. A calibration curve (linearity graph) was plotted by calculating peak area against concentration.

6. Precision studies of the proposed method

Nine similar concentrations of the marketed formulation (100 ppm) was analyzed within the same day (intraday precision), using the chromatographic condition Similarly, the same concentrations (100 ppm) of marketed formulation were analyzed in 3 successive days using the same chromatographic condition to determine the intermediate precision.

7. Robustness for the chromatographic method

The flow rate of the mobile phase was changed by ±1 decimal from 1 mL/min to 1.1 mL/min and to 0.9 mL/min to evaluate the effect of the flow rate; similarly, the variation of organic modifier used as acetonitrile was changed by ±2% from 70% to 72% and 68% to monitor the peak area and retention time. Finally, the effect of wavelength was monitored by making deliberate variation from 230 to 228 and 232

nm and the differences in system suitability parameters such as retention time, peak tailing, capacity factor, resolution and theoretical plates were tested and evaluate**Discussion**

System suitability parameters

S.No	Parameter name	Acceptance criteria
1	Number of theoretical plates or Efficiency (N)	> 2000
2	Capacity factor (K)	< 1
3	Separation or Relative retention (α)	> 1
4	Resolution (Rs)	> 1.5
5	Tailing factor or Asymmetry(T)	< 2
6	Relative Standard Deviation (RSD)	< 2

Conclusion

It has been concluded that the developed analytical method for the estimation of tablet formulation has obliged the ICH guidelines. As per the ICH guidelines, the developed method has complied the linearity range (calibration data), accuracy/drug recovery studies (%), repeatability, precision studies (intraday and interday/intermediate), and robustness. Moreover, as per the ICH guidelines, the system suitability test performed for marketed formulation has achieved all guidelines; including, tailing factor (T), separation factors (α), theoretical plates (N), capacity factor (k'), resolution ® and RSD (%). The validated stress degradation studies under thermal, oxidative, alkali and acid ascertained few degradation products for marketed formulation RP-HPLC offers improved run times and increased sensitivity Over conventional HPLC based methods. In High Sensitivity – Low limit of detection, Excellent

Hence, this developed and validated method for investigation by reverse phase high performance liquid chromatography (RP-HPLC) can be used for routine analysis of estimation of marketed formulation.

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