WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.632
Volume 11, Issue 7, 744-758 Review Article ISSN 2278 – 4357

A REVIEW ON METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC FOR THE ASSESSMENT OF OMEPRAZOLE IN DELAYED-RELEASE TABLETS

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Article Received on
02 May 2022,

Revised on 23 May 2022,
Accepted on 12 June 2022

DOI: 10.20959/wjpps20227-22536

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ABSTRACT

The current study aims to create a new sensitive and selective high-performance liquid chromatography (HPLC) method for evaluating omeprazole in delayed-release tablets. Because it produces very effective separations and, in most situations, great detection sensitivity, RP-HPLC is a prominent separation technology in current pharmaceutical and biological analysis. The key benefit of RP-HPLC over other separation modalities is its high efficiency and ability to differentiate between chemically identical molecules. The antacid omeprazole analysis was performed utilizing a Shimadzu LC-10A system with an RP-C18 column and UV- visible detection at 280nm as delineated during this study. At a rate of 1.5ml/min, the mobile section was diluted with phosphate buffer(pH7.4) and acetonitrile(70:30 v/v). The developed RP-HPLC methodology for omeprazole(Prilosec) analysis was valid in keeping with the ICH (InternationalCouncill for technical needs for prescription drugs for human use) tips. The validated parameters are accuracy, precision, linearity, range, LOD&LOQ, and specificity. The devised method was determined to be satisfactory and adequate for omeprazole quality control in delayed-release tablets.

KEYWORDS: Reversed-phase High-performance liquid chromatography(RP-HPLC), International conference on harmonization(ICH), method development, validation, omeprazole, biomedical analysis.

I. INTRODUCTION

HPLC (high-performance liquid chromatography) is a separation technique that is commonly used in biochemistry and to evaluate active substances. In this approach, a little amount of liquid sample is injected into a tube filled with stationary phase.[1] The various components were driven down the column by high pressure generated by a pump. Physical and chemical interactions between sample component molecules and packing materials contribute to their separation. Elution occurs at various times depending on how the separate components partition.[1-2] The sample compound having a higher affinity for the stationary phase moves slower, whereas the sample compound with a lower affinity moves quicker. The eluted components can be identified at the pump's end.[4]

Classification of HPLC.

1. Based on the principle of separation - Size exclusion chromatography, Affinity chromatography,Ion exchange chromatography, Adsorption chromatography.
2. Based on mode of operation - Normal phase HPLC, Reverse phase HPLC.
3. Based on a scale of operation - Preparative HPLC, Analytical HPLC.[5]

RP-HPLC (Reverse Phase – High-Performance Liquid Chromatography).

Since the initial HPLC separation used a polar stationary phase and a nonpolar eluent, the term "reversed-phase" was coined. The stationary phase in RP-HPLC is non-polar, while the mobile phase is polar. The idea of RP-HPLC is that molecules with similar hydrophobic characteristics, such as proteins, peptides, and nucleic acids, would elute first in the column, resulting in good resolution.[39]

Elution order – Strong Lewis acid< weak Lewis's acid, strong Lewis bases< weak Lewis bases< permanent dipoles< induced dipoles< aliphatic.

The retention increases also with the number of carbon atoms in the molecule.

Pentane< Hexane< heptane.

Branched-chain isomers elute faster than linear chain isomers.

Omeprazole is a proton pump inhibitor with the chemical formula 5-methoxy-3,5-dimethyl-2-pyridinyl-methyl sulfinyl-1H benzimidazole.[25] It has the chemical formula C17H19N3O3S and a molecular weight of 345.4. Omeprazole is soluble in ethanol and methanol, partly soluble in acetone and isopropanol, and completely soluble in water.[11] Omeprazole is used to treat gastric reflux disease, peptic ulcers, and Zollinger-Ellison syndrome. It is also used to prevent upper gastrointestinal bleeding in high-risk patients.[11-19] It may also be used in

conjunction with antibiotics to treat helicobacter pylori-caused stomach ulcers. It inhibits the stomach H+ /K+ ATPase at the secretory surface of gastric parietal cells, decreasing acid secretion.[6] Pharmaceutical dosage forms containing omeprazole provide a greater risk due to the drug's rapid breakdown at acidic pH, heat, moisture, organic solvents, and some light. The antacid samples were stored in two Patra humidity cabinets at 400°C and 75 percent relative humidity. Omeprazole is currently available in a range of capsules and injectable powder.[1,12]

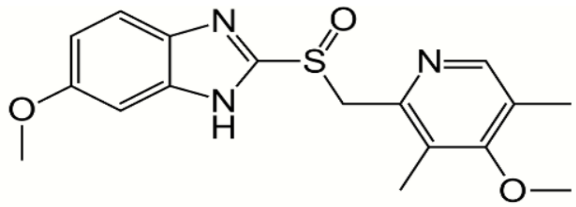


Fig 1: Structure of Omeprazole.

Literature Review

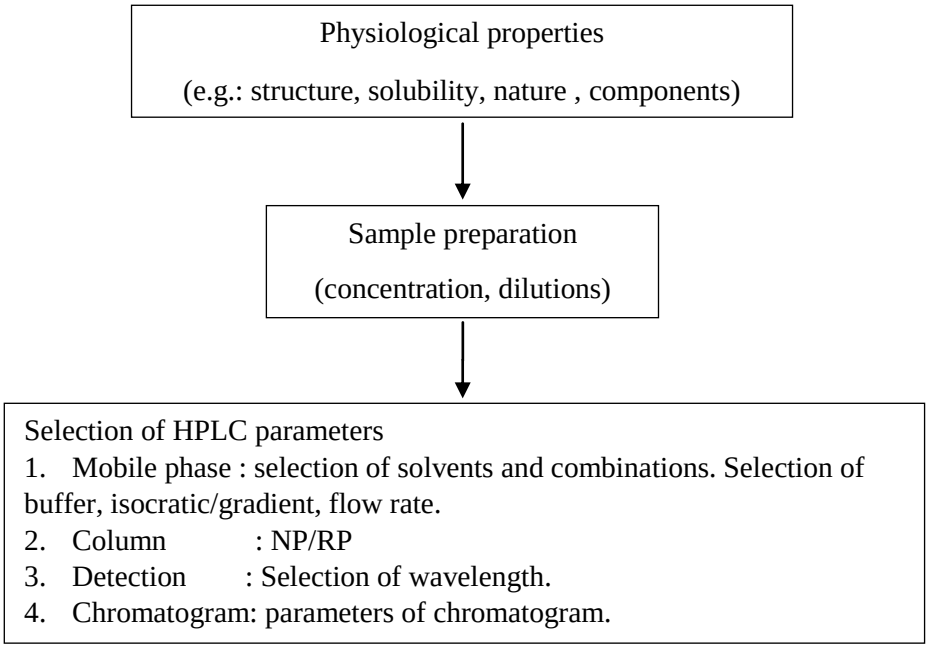
Numerous HPLC systems have been documented for the assessment of omeprazole in pharmaceutical formulations, as per a literature review. According to ICH requirements, the current work involves implementing RP-HPLC equipment with a simple mobile phase and sample preparation. It was sensitive for the quantification of omeprazole in delayed-release tablets.

II. Apparatus and materials: The chromatographic separation was accomplished using a Shimadzu LC-10A system, an associated LC-10AD pump, and an SCL-10AVP control unit. The RPC18 column with a UV-Visible detector was used for the analysis. The mobile section was diluted in isocratic mode with phosphate buffer (pH-7.4) acetonitrile (70:30v/v) at a flow rate of 1.5ml/min-1 and injection volume of 20l for all reference and sample solutions.[7-11] CLASS-VP software was used to administer data acquisition by measuring observed peaks. To validate the approach using FDA and ICH criteria for accuracy, linearity, specificity, and repeatability. This paper provides an approach for estimating omeprazole in capsule dosage form using RP-HPLC that has been developed.[7-11]

III. Method development: Analytical technique development demonstrates that a developed chromatographic method is suitable for its intended use in the development and production of pharmaceutical medication components and therapeutic products. There are several strong reasons to develop a novel method of examination for determining a chemical or drug:[13-14]

1. It is possible that a suitable method for a given analyte in a certain sample matrix does not exist.
2. Existing techniques may be very error-prone, prone to artifacts, or lack accuracy or precision.
3. Existing methods in relevant samples may lack sensitivity or analyte sele**ctivity.**

Overview of method development steps.



Pre method validation

1. Selection of upper and lower limits of quantification
2. Impurity profiling.
3. Accuracy and precision
4. Linearity
5. Selectivity(LOD, LOQ)
6. Stability.

The following are the steps concerned in HPLC technique development.

1) Understanding the physiochemical properties of medicinal compounds.

Method development is littered with the physiological potions of a pharmaceutical substance. Physical features of the drug molecule, such as solubility, polarity, pKa, and pH, must be assessed for procedure development. The polarity of molecules can be used to demonstrate their solubility. Like dissolves like, and substances with comparable properties dissolve in one another. Polar and nonpolar solvents, such as water and benzene, are incompatible. pH

and pKa are critical in the development of RP-HPLC methods. In most circumstances, choosing the correct pH for ionizable analytes in HPLC results in symmetrical and crisp peaks. Sharp, symmetrical peaks are essential in quantitative analysis to achieve low detection limits, low relative standard deviations between injections, and consistent retention durations.[1-2]

2) Selection of chromatographic conditions

When the analytical method is chosen, various chromatographic settings are chosen. The concentration of solvent in the mobile phase affects analyte flow over the column. The concentration of solvent is typically used to change the retention time. The mobile phase pH and ion-pairing reagents also affect the sample retention time. To avoid a long retention time, the gradient system is used to evaluate samples with a large number of components, whereas the isotonic system is utilized to examine samples with one or two components.[15] Buffer capacity refers to a buffer's ability to survive a pH change. The pH range for reversed-phase on silica-based packaging is commonly 2 to 8.

Concerns about buffer selection in general.

1. Phosphate dissolves more easily in methanol than in acetonitrile.
2. There are absorbent salt buffers. As a result, chromatographic changes may occur. (enhanced basic compound tailing)
3. In general, ammonium salts are more soluble in organic/water mobile phases.
4. At pH greater than 7, phosphate buffer promotes silica dissolution and substantially lowers HPLC column life. If at all possible, organic buffers should be used at pH levels higher than 7.

Detector Choice

The detector is an essential component of HPLC. The chemical makeup of the analytes, interference potential, required detection limit, and detector value are all factors to consider when selecting a detector. PDA detectors and UV-visible detectors are frequently utilized.[15-18]

Sample preparation for method development

The drug substance under investigation should be stable in the presence of a diluent. During the early phases of methodology development, solution preparation in amber flasks should be done until the active component is proven to be stable at room temperature and does not

degrade under typical laboratory conditions. For particle removal, a 0.22 or 0.45 m pore size filter is typically recommended.[14]

3) Method optimization

The mobile phase and stationary phase compositions must be evaluated to obtain acceptable selectivity (peak spacing). Only the parameters that are predicted to significantly affect selectivity in the optimization should be explored to reduce the number of trail chromatograms involved. It is crucial to consider the nature of analytes before picking them. After determining the analyte types, the associated optimization parameters can be chosen.[27] The most common method is to change the composition of the mobile or stationary phase. Changing the mobile phase or stationary phase composition is the most effective technique to improve selectivity, however, particle size, pore size, column length, temperature, and mobile phase strength have significantly less of an impact.

4) Method Validation

The process of creating a documented proof that a certain process will consistently create a product fulfilling its set parameters and quality features is known as validation of an analytical procedure. The process of method validation is performed to ensure that the analytical approach utilized for a specific test is appropriate for its intended usage. Regulatory bodies now require analytical method validation for marketing authorizations, and guidelines have been issued. All analytical procedures intended for use in the analysis of clinical samples must be validated. Analytical methods are validated by ICH criteria.

IV. Chemicals and reagents

Omeprazole (antacid) reference standard with 100% purity, Omeprazole raw material, HPLC grade ultrapure water, methanol, HPLC grade acetonitrile, potassium phosphate, and sodium hydroxide. Analytical grade solvents and reagents were employed as alternatives.[11]

Omeprazole formulation

The formulation had a target weight of 180 mg and contained 20 mg of the drug (omeprazole sodium) and 160 mg of excipient. The tablets were coated with a sub-coating of Opadry II at a concentration of 6-8 percent before being coated with an acryl-EZE enteric polymer system to achieve a weight gain of 10-12 percent.

Preparation of the standard and sample

Standard preparation: Dissolving a 20mg omeprazole reference standard in 5ml methanol and 50ml phosphate buffer at pH-11, then transferring to a 100ml volumetric flask and stirring for 10 minutes in an ultrasonic bath, yielded a 200g/ml stock standard solution. To get habituated to the volume, a comparable buffer was utilized.[11]

Preparation of the sample: Ten delayed-release omeprazole tablets were finely ground. A portion of one pill was weighed precisely and quantitatively transferred to a 100ml volumetric flask. Phosphate buffer (50 mL) The volume was reconstituted using the same buffer (200g/ml) and agitated for 10 minutes. A 5ml amount of this solution was diluted with the mobile section in a 50ml volumetric flask.[11]

Sample preparation for content uniformity: Separately powdered 10 delayed-release omeprazole tablets were added to a 100ml volumetric flask with 50ml phosphate buffer (pH-7.4) and swirled in an ultrasonic bath for around 20 minutes. A similar buffer is used to compensate for the volume. The solution was filtered using a 0.45µ nylon membrane, and 5ml of the filtered solution was diluted in an exceedingly 50ml volumetric flask with a mobile phase.[11]

V. METHOD VALIDATION

Validation of analytical techniques is a mandatory regulatory requirement in pharmaceutical analysis. Method validation provides documented evidence and a high level of assurance that an analytical method employed for a particular test is suitable for its intended use. The International Conference on Harmonisation released validation recommendations for analytical procedures (ICH). Analytical procedures must be validated. The four most common types of analytical processes are the subject of the issue of analytical procedure validation.

- Tests for identification.
- Impurity quantification tests
- Limit tests for contaminants in control
- Quantitative testing for active moiety in drug substance or drug product samples, as well as other drug product components.

Components of methodology validation: The following are typical analytical performance characteristics that will be tested throughout method validation.

1. Accuracy.
2. Precision.
3. Linearity and range.
4. Limit of detection.
5. Limit of quantitation.
6. Specificity.
7. Robustness.

1. Accuracy

The degree to which a measured value corresponds to the true or accepted value is referred to as accuracy. It expresses the distinction between the true and discovered mean values. It is computed by applying the procedure to samples containing known analyte concentrations. The accuracy was estimated as a percentage of analyte recovered by an assay based on the test findings. One of four methods is typically used to determine accuracy.

a) To begin, evaluate a sample of known concentration (reference materials) and compare the measured value to the true value.
b) The second alternative is to compare the new method's results to those of a well-known and accurate procedure.
c) The third technique is based on the recovery of analyte concentrations that are known. To accomplish this, the analyte is spiked in blank matrices. For testing methods, spiked samples are prepared in triplicate at three levels ranging from 50 to 150 percent of the target concentration. The percentage recovery should then be determined.
d) The fourth method is to use standard additions, which can also be used to monitor analyte recovery after spiking. When preparation is impossible, this method is adopted. The blank sample matrix contains no analyte.[40]

The ICH standards recommend that a minimum of three concentration levels covering the prescribed range be employed, with three replicates of each concentration investigated (for a total of 33= 9 determinations).

Acceptance Criteria

The average recovery for non-regulated items will be between 90 and 110 percent of the theoretical value. The US pharmaceutical industry requires an assay of an active ingredient in a medicinal product at concentrations ranging from 80 to 120 percent of the target concentration.

Three known quantities of reference standards were added at three separate concentration levels to a 10 g/ml omeprazole sample solution: lower, medium, and upper. In triplicate analysis, the recovery of the additional standard was measured and approximated using the formula.

$R\% = (F_S - S_t / S_s) \times 100$

Where,

R – recovery, Fs – Fortified solution, Ss – Sample solution, St - Standard solution.

2. Precision

The degree to which multiple measurements of the same homogenous substance taken under specified conditions agree. For a statistically significant number of samples, it is given as the percent relative standard deviation.[40] Precision can be achieved on three levels.

i) Repeatability

ii) Intermediate precision.

iii) Reproducibility.[26]

i) Repeatability (intra assay precision)

It expresses precision over a brief length of time while functioning under conditions. At least nine determinations covering the procedure's prescribed range (3 levels, 3 repetitions) or at least six determinations at 100% of the test or goal concentration ought to be accustomed to confirm it. To assess the analytical method's repeatability, six sample solutions of Omeprazole 20g/ml were tested on an equivalent day, underneath identical experimental conditions.

ii) Intermediate precision: concentrationss

It reflects differences in the laboratory on different days, different analyzers, different equipment, and so on. An assay method's accuracy criterion is that the intra-assay precision is less than 2%. To establish intermediate precision, the solutions were tested on three distinct days. Precision was expressed as a percentage relative standard deviation (RSD).[40]

iii) Reproducibility.

It can be determined by analyzing homogenous samples in several laboratories, which is commonly done in the context of interlaboratory crossover investigations.

RSD precision was calculated as a percentage of RSD. This acts as a daily check of the system's repeatability. At the start of each series of analyses, the relative standard deviation is

frequently estimated as percent RSD for five or six replicate injections of a reference standard. The formula for calculating standard deviation is.

$$s = \sqrt{\frac{\sum(x-\bar{x})^2}{N-1}}$$

Where,

S = standard deviation, X = each value in sample, $\bar{x}$ = mean of the values , N = no of values.

3. Linearity and range

Procedures are typically characterized as linear when the technique response and the concentration of analyte in the matrix are perfectly proportionate over the vary of analyte concentration of interest.[38] It can be determined by analyzing homogeneous samples in several laboratories, as is finished halftimes within the context of interlaboratory crossover studies.

At the beginning of every series of analyses, the relative standard deviation is often calculable as percent RSD for five or six replicate injections of a reference standard. Standard deviation is calculated using the following formula: $\sqrt{\frac{\sum_{i-1}^{n}(x_i-\bar{x})^2}{N-1}}$

Linearity: Linearity is the ability of an analytical technique to produce test results that are proportional to the concentration of analyte in the sample (within a specified range). The linearity response of omeprazole was evaluated between 10 and 30 g/ml.

Range: The distance between the greatest and lowest analyte concentrations where precision, accuracy, and linearity are acceptable in an analytical procedure. It is computed using a linear or nonlinear response curve and is always given in the same units as the test results.[27]

Omeprazole's linearity response was measured between 10 and 30 g/ml. Stock solutions were diluted with mobile phase to obtain concentrations of 10,15,20,25, and 30 g/ml, respectively. Every injection was done thrice. Standard compound peak area ratios were shown versus theoretical standard concentrations. Linear regression analysis[26] commonly expresses linearity as the correlation coefficient.

4. Limit of detection

The limit of detection (LOD) is defined as a concentration at a given signal-to-noise ratio, which is typically 3:1. Alternatively, it can be defined as the smallest amount of analyte in a

sample that can be identified but not measured. The tests are carried out on samples with extremely low analyte concentrations.[26] The slope of the calibration curve and the standard deviation of the response (SD) for values matching the LOD can be calculated alternatively.[1]

LOD = 3.3 S/SD.

The LOD is not a stable or robust metric, and it can be affected by minor changes in the analytical system (for example, temperature, reagent purity, matrix effects, and experimental conditions).[28]

5. Limit of Quantitation

There are numerous techniques for calculating the quantitation limit depending on whether the procedure is instrumental or non-instrumental. Alternatives to those listed below may be acceptable as well.

a) Signal-to-noise ratio-based analyte quantification: this is accomplished by comparing observed signals from samples with known low analyte concentrations to blank samples and finding the concentration at which the analyte can be simply quantified. The signal-to-noise ratio is typically 10 to one.

b) Based on the standard deviation of the responses.[29-30]

LOQ = 10×S/ SD.

6. Specificity

Specificity is an important attribute to assess in a validation program since it ensures the method's ability to detect the analyte response in the presence of all possible sample components. These include impurities, degradants, and matrices. To assess the specificity, a placebo tablet and a delayed-release tablet lacking omeprazole were utilized. Each shot was administered three times. Because no interference from excipients or degradation products was identified, the method was determined to be specific for omeprazole determination.[36]

7. Robustness

The robustness of an analytical method is a measure of its resilience to minor changes in experimental conditions, as well as its dependability in real-world applications. Changes in mobile phase composition, pH, gradient profile, buffer concentration, column temperature, and injection volume are all crucial to monitor.[37]

CONCLUSION

This study provides a simple, innovative RP-HPLC technique that has been successfully developed and validated for estimating Omeprazole in delayed-release tablets. The key advantage of RP-HPLC over alternative separation modalities is its high potency and capability to discriminate between the chemically identical substances as well as its specificity, directness, accuracy, precision, phenomenal sensitivity, and robustness. All these important aspects demonstrate that this method is more profitable than alternative styles The sample preparation is easy and the analysis time is short. This technique represents a simple analytical procedure for the estimation of omeprazole in delayed-release tablets. The choice of column, buffer, detector, wavelength, and alternative condition compositions plays a dramatic role in separation property. This can be a wonderful technique for the routine quality control of omeprazole in raw materials and finished products. This established technique was validated using ICH criteria and demonstrated to be accurate, exact, and specific.

ACKNOWLEDGEMENT

The author is grateful to their teachers for their encouragement and support in doing this work.

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