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Review Article

Analytical Progress in Olanzapine Characterization: A Comprehensive Review of RP-HPLC Method Development and Validation for Impurity Analysis

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ARTICLEINFO

ABSTRACT

Olanzapine is an atypical antipsychotic drug, and chromatography is the backbone of separation science. It is utilized in all research laboratories and pharmaceutical industries throughout the world. The current review article focuses on the development and validation of an RP-HPLC method for olanzapine. Method development and validation are important in new drug discovery, development, and manufacturing, as well as a variety of other human and animal investigations. An analytical technique is developed to compare a defined characteristic of a drug substance or drug product to established acceptance criteria. Accuracy, precision, specificity, linearity, range and limit of detection, limit of quantification, robustness, and system suitability testing are all covered by validation of HPLC methods according to ICH Guidelines

Keywords: RP-HPLC; Olanzapine; Analytical Validation; impurity

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1. Introduction

Olanzapine is an atypical antipsychotic that has been approved by the United States Food and Drug Administration (FDA) to treat schizophrenia and bipolar disorder. Olanzapine is a thienobenzodiazepine that is structurally similar to clozapine and quetiapine. Eli Lilly and Company, a pharmaceutical company, manufactures and markets olanzapine formulations; the drug became generic in 2011[1]. Olanzapine is a dopamine receptor antagonist that works on types 1, 2, and 4. Olanzapine's antipsychotic effect is due to antagonistic activity at dopamine and serotonin type 2 receptors, with serotonin 5-HT 2 receptor activity being higher than dopamine type 2 receptor activity. At muscarinic receptors, there is antagonism.H1 receptors, and alpha (1) - receptors also occurs with olanzapine [2]. The half-life of olanzapine ranges from 21 to 54 hours, with an average of 30 hours. In about one week after starting olanzapine on a daily basis, the steady-state plasma concentration is reached. When dosed within the FDA-approved range, olanzapine has linear pharmacokinetics. The drug is disseminated broadly throughout the body, with a volume of distribution of about 1000 litres. It binds to plasma proteins 93 percent of the time, notably albumin and alpha-1 acid glycoprotein. The liver metabolises olanzapine substantially through direct glucuronidation and the cytochrome P450 system. The enzymes that metabolise olanzapine in this system are principally 1A2 and, to a lesser extent, 2D6. Although the CYP1A2 gene is polymorphic, a study found no link between various variants and pharmaceutical pharmacokinetics [3]. Hepatic impairment does not necessitate a dose change. Cirrhosis patients with Child-Pugh Classification A and B had no effect on the medication's metabolism. Because of olanzapine's substantial metabolism, only 7% of the drug stays unaltered after elimination. Olanzapine is mostly excreted in the urine (53 %) and faeces (30 %). Because of its metabolism, patients with renal impairment do not require specific doses for this medicine [4].

1.1 Uses of olanzapine

Oral formulation utilised in the acute and maintenance treatment of Schizophrenia in adults, as well as the acute treatment of manic or mixed episodes linked to Bipolar, I Disorder (monotherapy and in combination with lithium or valproate).In adults with Schizophrenia and Bipolar I Mania, an intramuscular formulation is used to treat acute agitation.

Acute treatment of depressive episodes associated with Bipolar I Disorder in adults, or acute therapy of treatment-resistant depression in adults, with an oral formulation containing fluoxetine.

1.2 Adverse Effects of olanzapine

One of the most common side effects of olanzapine is the possibility of weight gain. Olanzapine induces an increase in hunger, which results in hyperplasia and weight gain. As a result, it should be taken with caution in obese individuals who have limited control over their food consumption and do not exercise on a regular basis to prevent weight gain. The increased risk of metabolic problems is another side effect of olanzapine [5]. Olanzapine has a high risk of causing decreased insulin sensitivity, which can lead to impaired glucose tolerance, especially in young people. Olanzapine's mechanism of action also makes it susceptible to developing dopaminergic blockade-related side effects. Akathisia, extrapyramidal disorders, tardive dyskinesia, and neuroleptic malignant syndrome are all possible side effects of olanzapine use. Due to the loose interaction and quick dissociation of olanzapine with the D2 receptors, the chance of

acquiring these adverse effects is lower than with first-generation antipsychotics.

2. High Performance Liquid Chromatography

High-performance liquid chromatography (HPLC) is the most utilised of all analytical separation techniques, with yearly sales surpassing a billion dollars. The method's wide applicability to substances of primary interest to industry, many fields of science, biomedical applications, and the public is due to its sensitivity, suitability for separating non-volatile or even thermally fragile substances, ready adaptability to quantitative determinations, and above all, its wide spread applicability to substances of primary interest to industry, many fields of science, biomedical applications, and the general public. Amino acids, nucleic acids, hydrocarbons, carbohydrates, medicines, threnodies, pesticides, antibiotics, and metals are examples of such compounds. Although the introduction of open-column methods, such as paper chromatography in the 1940s and thin layer chromatography in the 1950s, substantially increased the speed and resolution of LC, there were still significant limits when compared to modern LC methods[6]. Analysis times were long, resolution was poor, and quantitative analysis, preparative separations, and automation were difficult to accomplish. The development of reliable, reasonably cost instruments and effective columns has aided HPLC's rapid growth. HPLC analysis is now the most extensively used device for tests [7].

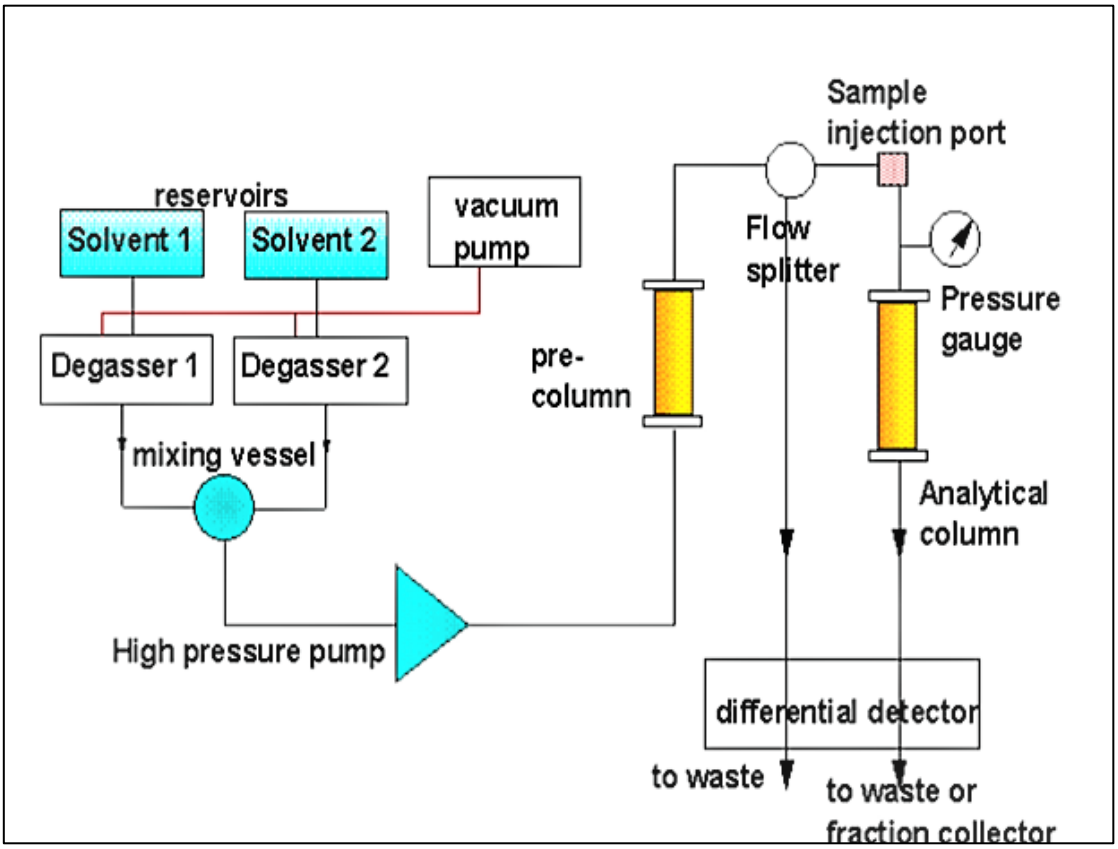

Fig.1: Schematic Diagram of High-Performance Liquid Chromatography

2.1 Reverse phase chromatography

Chromatographers have been chemically treating silica with organic silanes to change the polar character of the silane group since the 1960s. The goal was to make polar solvents less polar or non-polar so that water-soluble polar molecules could be separated using polar solvents. Because the chemically changed silica's ionic nature has been reversed, it is now non-polar or has a reversed phase. Reversed-phase chromatography is the name given to the chromatographic separation carried out with such silica. Commercially, there are a large number of chemically bonded stationary phases based on silica [8]. Chemically modified silica has some functional groups that are bound. Other absorbents based on polymer (styrene-di-vinyl benzene copolymer) are slowly gaining hold in reversed phase chromatography. In general, retention increases as the contact area between the sample molecule and the stationary phase grows, i.e. as the number of water molecules released during a compound's adsorption increases. Branched chain compounds are eluted more rapidly than their corresponding normal isomers. In reversed phase systems the strong attractive forces between water molecules arising from the 3 - dimensional inter molecular hydrogen bonded network, from a structure of water that must be distorted or disrupted when a solute is dissolved. Only higher polar or ionic solutes can interact with the water structure [9]. Non- polar solutes are squeezed out of the mobile phase and are relatively insoluble in it but with the hydrocarbon moieties of the stationary phase, they get adsorbed. Reverse phase chromatography - a bonded phase chromatography technique, uses water as base solvent. Separation is depending on the strength and selectivity of the solvent. Temperature and pH of the column have an impact on separation. More polar chemicals elute faster than less polar molecules in general. The most prevalent detection method is UV detection [10].

2.3 Certain limitations of RP-HPLC are

In the solvent front / empty volume, compounds that are substantially more polar than the compound of interest may be veiled (eluted together).

Compounds with lower UV extinction coefficients or different wavelength maxima may not be detectable at low levels relative to the visibility of the analyte because only one or two wavelengths are monitored.

3. Method Development

A step involved in method development of HPLC is as follows:

1. Understanding the Physicochemical properties of drug molecule.

2. Selection of chromatographic conditions.

3. Developing the approach of analysis.

4. Sample preparations

5. Method optimization

6. Method validation

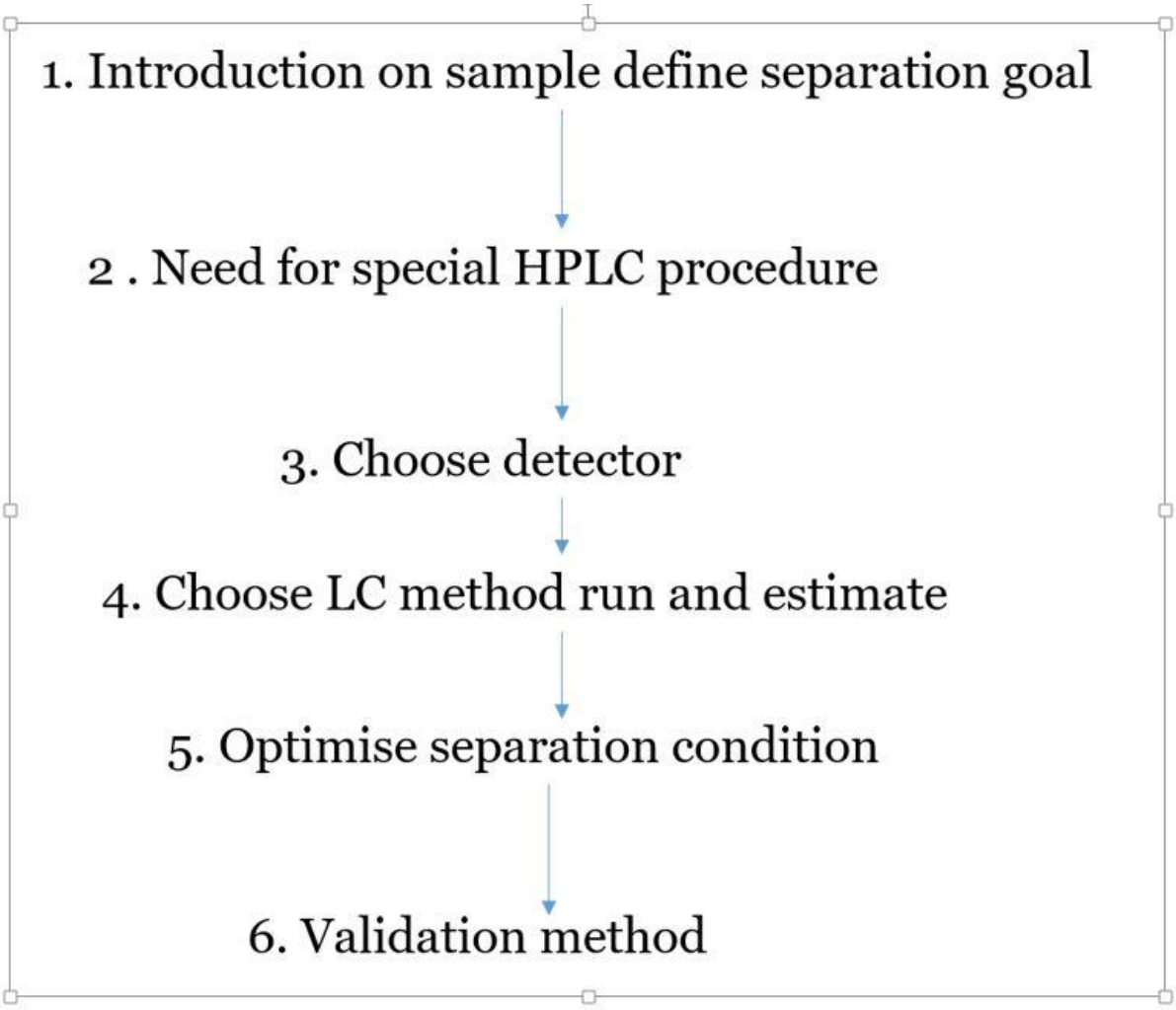

Fig.2: Steps involved in HPLC method validation

3.1. Understanding the Physicochemical properties of drug molecule

The physicochemical characteristics of a therapeutic molecule are crucial in the development of methods. The physical properties of the drug molecule, such as solubility, polarity, pKa, and pH, must be studied for method development. A compound's polarity is a physical property. It helps an analyst, to decide the solvent and composition of the mobile phase. The polarity of molecules can be used to explain the solubility of molecules. Solvents that are polar, such as water, and nonpolar, such as benzene, do not mix. Like dissolves like, which means that materials with similar polarities are soluble in each other. The solubility of the analyte determines the mobile phase or diluents used [11]. The analyte must be diluent soluble and not react with any of its constituents. In the development of HPLC methods, pH and pKa are crucial. The negative logarithm to base 10 of the hydrogen ion concentration is used to get the pH value. pH is equal to - log10[H3O+]. In HPLC, choosing the right pH for ionizable analytes generally results in symmetrical and crisp peaks. In quantitative analysis, sharp, symmetrical peaks are required to achieve low detection limits, low relative standard deviations between injections, and repeatable retention durations [12].

3.2 Selection of chromatographic conditions

3.2.1 Selection of column

Selection of the stationary phase/column is the first and the most critical stage in method development. Without the availability of a stable, high-performance column, developing a tough and repeatable procedure is impossible. It's critical for columns to be stable and repeatable during method development to avoid concerns with irreproducible sample retention [13]. For all samples, a C8 or C18 column manufactured from particularly purified, less acidic silica and built specifically for the separation of basic chemicals is generally appropriate and strongly recommended. The key ones include column diameters, silica substrate qualities, and bonded stationary phase characteristics. Due to many physical features, silica-based packing is preferred in most current HPLC columns [6].

3.2.2 Buffer Selection

The pH that is desired determines the buffer to use. pH 2 to 8 is the normal pH range for reversed phase on silica-based packaging. Because buffers control pH best at their pKa, it is critical that the buffer possess a pKa near to the desired pH. A general rule is to select a buffer with a pKa value less than 2 units higher than the intended mobile phase pH.

3.2.3 Buffer Concentration

For small compounds, a buffer concentration of 10-50 mm is usually sufficient. In general, a buffer should not contain more than 50% organic material. This will vary depending on the buffer and its concentration. The most frequent buffer systems for reversed-phase HPLC are phosphoric acid and its sodium or potassium salts. When analysing organophosphate chemicals, sulfonate buffers can be used instead of phosphonate buffers [14].

3.2.4 Isocratic and Gradient Separations

Constant eluent composition is part of the isocratic mode of separation, which means the equilibrium conditions in the column and the actual velocity of compounds passing through the column are both constant. The peak capacity is minimal, and the bigger the resultant peak is the longer the component is held on the column. Gradient mode of separation greatly enhances a system's separation power, result of an increase in perceived efficiency (decrease of the peak width). Peak width varies with the pace at which the eluent composition changes. An initial gradient run is done to determine whether a gradient or isocratic is required, and the ratio between the total gradient duration and the difference in gradient time between the first and last component is computed. A calculated ratio of 0.25 gradient would suffice [7].

3.2.5 Internal Diameter

In gradient elution, the internal diameter (ID) of an HPLC column is a critical parameter that determines detection sensitivity and separation selectivity. It also influences how much analyte may be placed into each column [15].

3.2.6 *Particle size*

The stationary phase is usually connected to the surface of tiny spherical silica particles in classical HPLC. These silica beads come in a variety of sizes, with 5 m beads being the most prevalent. The inverse of the particle diameter squared increases the pressure required for the optimum linear velocity. Smaller particles usually provide

more surface area and better separations, but the pressure required for the optimum linear velocity increases by the inverse of the particle diameter squared. Larger particles are utilised in preparative HPLC and non-HPLC applications such as solid-phase extraction, where column diameters range from 5 cm to >30 cm.

3.2.7 Pore size

The capacity of analyte molecules to enter inside the particle and interact with its inner surface is determined by the pore size of the column.

3.3 Selection of Mobile Phase

Resolution, selectivity, and efficiency are all affected by the mobile phase. In RP-HPLC separation, the mobile phase composition (or solvent strength) is critical. Acetonitrile (ACN), methanol (MeOH), and tetrahydrofuran (THF) are three typical RP-HPLC solvents with UV cut-offs of 190, 205, and 212nm, respectively. These solvents are water miscible. During technique development, a mixture of acetonitrile and water is the ideal first choice for the mobile phase [16].

3.4 Selection of detectors

The detector is an essential component of HPLC. The chemical nature of the analyses, potential interference, needed detection limit, availability, and/or cost of the detector all influence detector selection. For HPLC, the UV visible detector is a versatile dual wavelength absorbance detector. This detector has the sensitivity needed for routine UV-based impurity identification and quantitative analysis at low levels Array of Photodiodes (PDA). For Waters analytical HPLC, preparative HPLC, or LC/MS system solutions, Detector provides advanced optical detection. Its integrated software and optics innovations deliver high chromatographic and spectral sensitivity. This detector has a high refractive index, chromatographic and spectral sensitivity, stability, and reproducibility, making it ideal for analysing components with little or no UV absorption. For quantitating tiny amounts of target substances, the Multi-wavelength Fluorescence Detector provides excellent sensitivity and selectivity fluorescence detection.

4. Developing the approach for analysis

The selection of various chromatographic parameters, such as mobile phase, column, flow rate of mobile phase, and pH of mobile phase, is the first step in developing an analytical method on RP-HPLC. All of these parameters are chosen based on trials, and then the system suitability parameters are taken into account. For example, the retention period should be greater than 5 minutes, the theoretical plates should be greater than 2000, the tailing factor should be less than 2, and the resolution between two peaks should be greater than 5% R.S.D In standard chromatograms, the area of analyte peaks should not be greater than 2.0 percent. In the situation of simultaneous estimation of two components, the detection wavelength is usually an isosbestic point. After this the linearity of the drug is analysed in order to know the range of concentrations up to which the drug follows the linear pattern. Analysis of the laboratory mixture is also carried out in order to know practicability of proposed approach for

simultaneous estimation. Then, by diluting the marketed formulation up to the linearity concentration range, an examination of the marketed formulation is performed [9].

5. Sample preparation

Sample preparation is an important aspect of HPLC analysis because it ensures a consistent and homogeneous solution that can be injected onto the column. The goal of sample preparation is to provide a sample aliquot that is reasonably free of interferences, does not harm the column, and is compatible with the planned HPLC procedure, meaning the sample solvent dissolves in the mobile phase without impacting sample retention or resolution. Sample preparation begins with the collection of the sample and continues with the injection of the sample onto the HPLC column [17].

6. Method optimization

Identify the method's "weaknesses" and improve the method using experimental design. Understand how the approach performs under various settings, with various instrument setups, and with various samples [18].

7. Method Validation

Validation is the process of confirming that the specified requirements for a given intended use have been met through inspection and the supply of objective evidence. A way of evaluating its performance and demonstrating that it satisfies a specific requirement. In other words, it understands what your method is capable of, especially at low doses [10].

7.1 Validation

Validation of an analytical technique is the process of determining whether the method's performance characteristics fulfil the requirements for the intended analytical applications through laboratory tests [19].

Validation procedures were carried out in the following steps:

1. Validation techniques or parameters are proposed and established.

2. Experimentation is carried out.

3. The outcomes of the analysis are assessed.

4. A statistical analysis is performed.

5. A report is written outlining all of the findings.

7.2 Objective and Parameters of Analytical Method Validation

The goal of validation of an analytical technique is to show that it is suitable for the task at hand. The following are typical analytical performance parameters that should be addressed in the validation of different types of techniques, according to ICH guidelines.

7.3 Validation Procedures for Analytical Procedures

The topic of analytical procedure validation is focused on the four most prevalent types of analytical procedures: Quantitative tests for impurity content, Limit tests for impurity control, Quantitative testing of the active moiety in samples of drug substance or drug product or other specified component(s) in the drug product [20].

7.4 Components of method validation: The following are typical analytical performance characteristics which may be tested during methods validation:

7.4.1 Accuracy

The closeness of a measured value to the true or accepted value is defined as accuracy. In practise, accuracy refers to the difference between the found mean value and the genuine value. lt is calculated by applying the technique to samples containing known levels of analyte. To confirm that there is no interference, these should be compared to standard and blank solutions. The accuracy is then estimated as a percentage of the analyte recovered by the assay using the test findings. It is frequently expressed as the recovery of known, added amounts of analyte by test [18].

7.4.2 Precision

It expresses the degree of agreement (stretch) between a set of measurements acquired from multiple sampling of the same homogenous sample under the same set of conditions. Precision is a measure of the analytical method's reproducibility as a whole. There are two parts to it: repeatability and intermediate precision. The fluctuation encountered by a single analyst on a single instrument is known as repeatability [21]. It makes no distinction between variation caused solely by the instrument or system and variation caused by the sample preparation process. Repeatability is determined during validation by analysing numerous replicates of an assay composite sample using the analytical procedure. The value of recovery is calculated. Variation inside a laboratory, such as on different days, with different instruments, and by different analysts, is referred to as intermediate precision. The relative standard deviation is then used to express the precision [22].

7.4.3 Linearity

Linearity refers to an analytical procedure's capacity to produce a result that is proportional to the analyte concentration (amount) in the sample. If the procedure is linear, the test findings are proportional to the concentration of analyte in samples within a given range, either directly or via a well-defined mathematical transformation. The confidence limit around the slope of the regression line is commonly used to express linearity [23].

7.4.4 Limits of detection and quantitation:

The lowest concentration of an analyte in a sample that can be identified but not measured is known as the limit of detection (LOD). LOD is defined as a concentration at a given signal-to-noise ratio, which is commonly 3:1. The lowest concentration of an analyte in a sample that can be detected with acceptable precision and accuracy under the method's stated operational conditions is known as the limit of quantitation (LOQ). The ICH recommends a signal-to-noise ratio of 10:1 for LOQ. The standard deviation of the response (SD) and the slope of the calibration curve(s) at values near the LOD can also be used to determine LOD and LOQ using the formulae below.

$$LOD = 3.3 \times S / SD \text{ and } LOQ = 10 \times S / SD \ldots\ldots\ldots\ldots\ldots \quad (1)$$

The ability to assess the analyte definitively in the presence of components that may be present is known as specificity. Impurities, degradants, matrix, and other substances are common examples. A particular analytical procedure's lack of specificity may be compensated by additional supporting analytical procedures [24]. The following are the implications of this definition: To ensure the identity of an analyte, it must be identified. Purity Tests: to verify that all analytical procedures performed, such as related substances testing, heavy metals testing, residual solvents content, and so on, allow an accurate assessment of the presence of impurities in an analyte. Assay (content or potency): to produce a precise result that allows a precise statement of the analyte's content or potency in a sample.

7.4.5 Range

The interval between the highest and lower levels of an analyte that have been determined with acceptable precision, accuracy, and linearity is the method's range. It's calculated using a linear or nonlinear response curve (i.e., when more than one range is involved, as shown below), and it's usually given in the same units as the test results [9].

7.4.6 Robustness

The robustness of an analytical procedure is a measure of its ability to remain unaffected by modest but deliberate changes in method parameters, and it indicates its reliability in routine use [14].

8. Method development and validation by RP-HPLC method for olanzapine

Jain et al, for the estimation of olanzapine, an RP-HPLC method was devised. Author creates for the analysis of Olanzapine in marketed formulations, a simple, accurate, precise, and speedy stability indicating RP-HPLC method was designed and validated. The analysis was carried out on a C-18 (250mm x 4.60mm, 5 m) column with a mobile phase of Potassium di-hydrogen phosphate Buffer (pH 6): Acetonitrile (60:40) (v/v) at a flow rate of 1ml/min at constant room temperature with UV detection at 258 nm. The injection volume was 20 l, with a 5-minute chromatographic duration. With a correlation coefficient of 0.998, the proposed approach was found to be linear in the range of 5-25 g/ml. Recovery study was used to evaluate the validity and reliability of the proposed approach. The recovery of increased criteria ranged from 99.58 percent to 100.50 percent (80 percent, 100 percent,120 percent). Changing the temperature, flow rate, and mobile phase ratio tested the robustness of the developed approach [25].

Prameela rani.et al., developed a phase of reversal for the determination of olanzapine in pharmaceutical dosage forms, an HPLC method has been devised. Chromatography was performed at a flow rate of 1 ml/min on an inertsil C18 column with a mobile phase of ammonium phosphate buffer and methanol (70:30 v/v). The detection wavelength was 220 nm.

The medication had a retention time of 3.447 minutes. In the concentration range of 2 to 10g/ml of olanzapine, the approach produced linear results. The approach was shown to be useful for determining the drug content in tablets [26].

Kanakapura Basavaiah et al., developed for the measurement of olanzapine (OLZ) in pharmaceutical formulations, a new high-performance liquid chromatographic (HPLC) method in reverse phase was developed and validated. A reversed phase Intersil ODS column (150 mm 4.6 mm, i.d., particle size 5 mm) was used to achieve optimal separation in less than 10 minutes, and elution was carried out at a rate of 0.5 mL/min. A UV detector with a 271 nm wavelength was used for detection. With a detection limit of 3.0 mg/mL and a quantization limit of 8.0 mg/mL, a rectilinear relationship between mean peak area and OLZ concentration was seen in the range 10-200 mg/mL. Intra-day and Inter-day Precision, and accuracy of the methods have been established according to the current ICH guidelines.

Basavaiah et.al and Baldanla et.al., developed For the determination of olanzapine in tablet dosage forms, a simple, specific, accurate, and exact reverse phase liquid chromatographic approach was developed and validated by author. The mobile phase consisted of acetonitrile: methanol: 25mM potassium dihydrogen orthophosphate (60: 20: 20, v/v) and was run on a Phenomenex Gemini C-18, 5mcm column with a 250 x 4.6 mm i.d. in isocratic mode. The effluents were measured at 256 nm at a flow rate of 1.3 mL/min. As an internal control, calcium atorvastatin was used. The olanzapine and atorvastatin calcium retention times were 2.3 and 3.3 minutes, respectively. Linearity, accuracy, precision, specificity, limit of quantification, limit of detection, robustness, and solution stability were all validated for the method. The LOD and LOQ for olanzapine estimation were determined to be 100 and 450 nanograms per millilitre, respectively. The olanzapine recovery rate was determined to be between 99.02 and 101.68 percent. The proposed method was used to determine the quantity of olanzapine in tablet formulations with excellent results [27].

9. Conclusion

This article discusses the development and validation of Olanzapine methods utilising the RP-HPLC technique. Method development and validation are two processes that work together to ensure that a parameter is measured correctly and that the measurement's performance constraints are satisfied. The composition of the column, buffer, detector, and wavelength, as well as other parameters (organic and pH), has a significant impact on separation selectivity. High selectivity, sensitivity, economics, less time consuming, and low limit of detection were all advantages of the HPLC technique. The gradient slope, temperature, and flow rate, as well as the type and concentration of mobile-phase modifiers, can all be changed for final optimization. As per ICH guidelines, the improved technique is validated using several variables (e.g., specificity, precision, accuracy, detection limit, linearity, and so on).

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authorship contribution statement

Rutuja Desai: Supervision, Validation, Methodology, Investigation, Writing – original draft, **Pratiksha Dhumal:** Conceptualization, Administration, Funding, Data Curation.

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