IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

A Review: Method Development and Validation of Antipsychotic Drug by HPLC Method

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Abstract

High Performance Liquid Chromatography (HPLC) is a well-known analytical technique for separating, isolating, and quantifying each component of a mixture. In recent times, it is the most advanced and robust technique of liquid chromatography. Method Development and Validation by HPLC is done on atypical antipsychotic Cariprazine Hydrochloride. The demand for new analytical techniques has risen quickly as a result of the pharmaceutical industries rapid expansion and the consistent production of pharmaceuticals throughout the world. As a result, the development of analytical methods has taken centre stage in a quality control laboratory. A validation study is intended to offer sufficient proof that the analytical method achieves its goals. As per the ICH guidelines, the validation parameters are, linearity, accuracy, precision, robustness, Limit of Detection, Limit of Quantification, specificity, range. Analytical method development and validation play a crucial role in discovering, generating and development of pharmaceuticals.

Keywords: Analysis, Method development, accuracy, precision, validation, Cariprazine Hydrochloride.

1 INTRODUCTION

Atypical antipsychotic Cariprazine Hydrochloride is prescribed to treat bipolar disorder and schizophrenia. It has a high selectivity for the D3 receptor and functions as a partial agonist for both the D2 and D3 receptors. It is most beneficial as an adjunct therapy for treating major depressive illness.[1]

Schizophrenia is a severe condition of the central nervous system that affects 1% of people worldwide.

It is characterized by a variety of symptom domains, including positive symptoms (such as hallucinations and delusions), negative symptoms (such as social and emotional withdrawal and anhedonia), cognitive dysfunctions (such as attention deficit disorder and executive function impairment), and comorbid affective symptoms (such as anxiety and depression). Different receptor binding characteristics of antipsychotics lead to various side effect profiles. [2]

Table no. 1: Drug bank of Cariprazine Hydrochloride

ATTRIBUTES	DESCRIPTION
NAME OF THE DRUG	Cariprazine Hydrochloride
CATEGORY	Atypical Antipsychotic
MOL FORMULA	$C_{21}H_{33}Cl_3N_4O$
MOL WEIGHT	463.9 g/mol
IUPAC	3,3-dimethyl-1-[(1r,4r)-4-{2-[4-(2,3-dichlorophenyl)piperazin-1-yl]ethyl}cyclohexyl]urea hydrochloride
HALF LIFE	32 to 68 hours
Pka VALUE	7.91
MECHANISM OF ACTION	Cariprazine potently binds to both of D2 and D3 receptors, more preferably to D3 receptors with higher affinity. Cariprazine's mechanism of action is hypothesized to be mediated through the partial agonism of dopamine D2 / D3 receptors, and antagonism of serotonin receptors.

The area of science known as analytical chemistry is concerned with the separation, identification, and quantification of the chemical components of manufactured and natural goods. Analytical chemistry is crucial to the process sector as well as research labs. Qualitative and quantitative procedures make up the fundamental two methods of analytical chemistry. In addition to being employed in pharmaceutical products, analytical data is also used in fields like biology, clinical diagnosis, art, etc. [3] The analytical technique created employing tools like the GC, TLC, HPTLC, and Spectrophotometer has several uses in determining the amount and quality of the completed product or raw material. [4] A highlight among the most often used techniques in pharmaceutical research is UV-Visible spectrophotometry. It entails calculating the amount of brilliant or noticeable radiation a substance in an arrangement absorbs. [5-8]

In all the above techniques, Hplc is the most robust and advanced one with promising results. [One of the most significant tools in analytical chemistry nowadays is High Performance Liquid Chromatography (HPLC), which was developed from the traditional column chromatography. In the modern pharmaceutical industry, high performance liquid chromatography (HPLC) is the major and integral analytical tool applied in all stages of drug discovery, development, and production. The Goal of HPLC method is to try & separate, quantify the main drug, any reaction impurities, all available synthetic intermediates and any degradants. Any material that can dissolve in a liquid can have its constituents separated, identified, and quantified using this technique. The most precise analytical techniques, such as HPLC, are frequently employed to assess the stability of pharmacological products as well as their quantitative and qualitative composition. The stationary phase, or solution of the sample, is injected into a porous column, and the mobile phase, or liquid phase, is pumped through the column at a higher

pressure. The adsorption of solute on stationary phase based on its affinity towards stationary phase is the separation principle that is used. [9,10,11]

The technique of HPLC has following features

- High resolution
- Small diameter, Stainless steel, Glass column
- Rapid analysis
- Relatively higher mobile phase pressure
- Controlled flow rate of mobile phase [12,13]

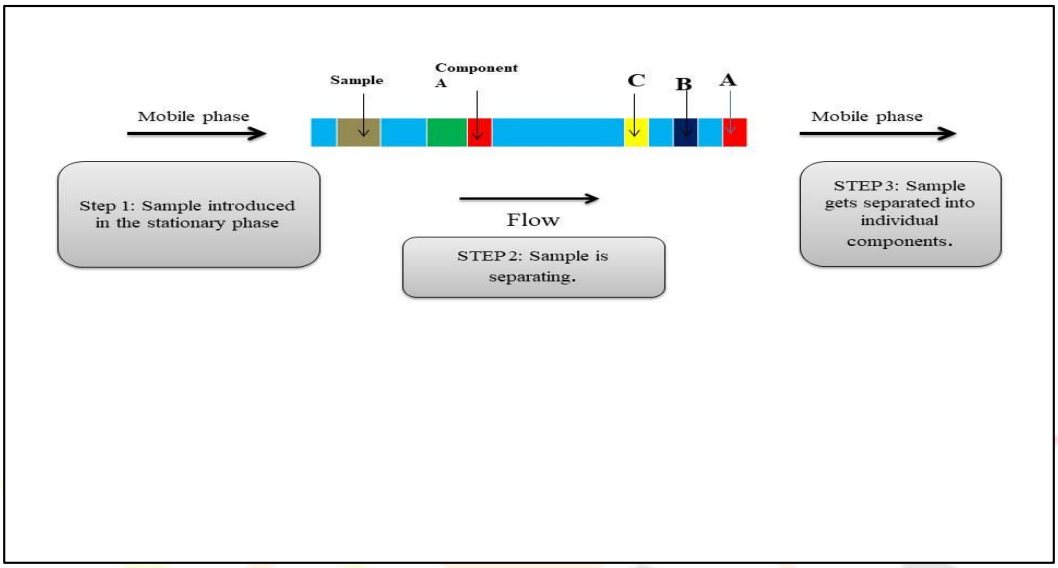

Figure no.1: HPLC working

1.1 Types of HPLC:

- Normal phase chromatography: Silica gel, for example, is a strongly polar stationary phase, and water is a non-polar mobile phase (such as n-hexane or tetrahydrofuran). As a result, more polar samples are kept on the polar surface of the column packing than less polar ones.
- Contrarily, reverse-phase chromatography: The mobile phase, such as combinations of water and acetonitrile or methanol, is a polar liquid, whereas the stationary bed is nonpolar (hydrophobic) in nature. Here, the item will be kept for a longer period of time the more nonpolar it is. Nearly 90% of all chromatographic applications fall under one of the aforementioned groups. All types of HPLC depend most on eluent polarity.[14,15,16]

1.2 Instrumentation

The HPLC includes

a. Solvent reservoir, mixing system and degassing system
b. High pressure pump
c. Sample injector
d. Column
e. Detector
f. Data Recording System[17,18]

Solvent reservoir, mixing system and degassing system: The solvent is kept in a reservoir (mobile phase). These containers are made of glass or stainless steel. Glass bottles are the most popular kind of solvent reservoir. In addition to dispensing mobile phase, the pump must mix solvents precisely and accurately. Low pressure mixing and high pressure mixing are two different types of mixing units. A degassing system eliminates air bubbles that have become trapped in the solution. Ultra sonication and filtration are two degasser procedures that are used. [19]

High pressure pump: A pump's function is to pressurize a liquid and provide a particular flow rate. Milliliters per minute (ml/min) are the unit used to measure flow rate. A flow rate of 1-2 ml/min is typical. The pump operates between 6000 and 9000 psi (400 and 600 bar). [6, 7] Constant pressure pumps, syringe pumps, and reciprocating piston pumps are the three most popular types of pumps.

Sample injector: The sample injector is used to introduce the liquid sample into the mobile phase. Between the pump and the column is a sample valve. The sample can be injected into the continuously flowing mobile phase stream, which transports the sample to the HPLC column, using an injector (auto sampler). Sample quantities typically range from 5 to 20 microliters. [6] There are two different types of injectors: manual and automated.

Column: The real separation of components occurs in the column. Stainless steel makes up the column. Its inside diameter is 2.46 cm and it is 5 to 25 cm long.

Detector: The detector may turn the data into an electrical signal by identifying each component that elutes from the column. Specific detectors and bulk property detectors are the two types of detectors that are employed. UV-VIS detectors, photo diode array detectors, fluorescence detectors, and mass spectrometric detectors are examples of specific detectors. Refractive index detectors, electrochemical detectors, and light scattering detectors are examples of bulk property detectors.

Data recording system: The output is recorded as a series of peaks, and the computer connected to the display can automatically calculate the area beneath each peak. [20]

2 ANALYTICAL METHOD DEVELOPMENT AND VALIDATION

Development and validation of analytical methods are essential to the creation of pharmaceutical goods. When there are no established ways for analyzing innovative products, new approaches are created. To lower the cost and improve precision and robustness while improving the method's accuracy, new methods are developed through optimization and validation through test runs. While substitute techniques are created to replace the current process with comparable laboratory data. [21]

Steps involved in method development are as follows

1. Physicochemical Properties of drug molecule
2. Selection of Chromatographic conditions
3. Developing the analytical strategy
4. Sample preparation
5. Optimization of method
6. Method validation[22]

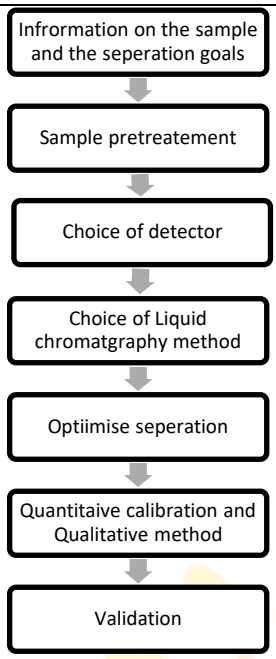

Figure no.2: Flowchart of method development

2.1 Physicochemical Properties of drug molecule:

It plays a crucial role in development of a method for a drug molecule. In order to build a method, one has to study certain properties such as pKa, log P and pH of the drug molecule. Upon studying these physicochemical properties the solvent and composition of the mobile phase can be finalized. The polarity of the molecules can be used to explain their solubility. Solvents that are nonpolar, like benzene, and polar, like water, do not combine. Like generally dissolves like, which means that substances with comparable polarities can be dissolved in one another. The choice of diluents depends on how soluble the analyte is. The pH value is often used to determine whether a substance is acidic or basic. In HPLC, choosing the right pH for ionizable analytes frequently results in symmetrical and sharp peaks. [23,24]

2.2 Automation adaptability: For procedures that are likely to be utilised in a high sample volume application, the term "automatability" is crucial. The manual sample preparation process should be simple to complete. This will guarantee that the sample preparation process can be automated at standard sample preparation workstations. [25]

2.3 Recognize the chemistry: A global literature review of the physical and chemical characteristics of analytes, similar to another research project, is crucial to the project's success.

2.4 Selection of Chromatographic conditions

To get the first "scouting" chromatograms of the sample, a set of basic settings (detector, column, mobile phase) are chosen during initial technique development. On reversed-phase separations on a C18 column with UV detection, they are typically based. At this point, a choice should be taken regarding whether to develop an isocratic or a gradient methodology.[26]

2.4.1 Column selection

The choice of columns is the most crucial phase in the HPLC development process. For the separation of a basic substance, silica that has been specifically purified and made to be less acidic is used in C8 or C18 columns. [27,28,29]

2.4.2 Selection of chromatographic mode

2.4.3 Mobile phase Optimization

- Effect of pH: If analytes can be ionized, the appropriate mobile phase pH must be selected based on the analyte's pKa so that the target analyte is in one predominant ionization state, either ionized or neutral. One of the best techniques in the "chromatographer's toolbox" for changing both retention and selectivity between important pairs of components simultaneously is adjusting the pH of the mobile-phase.
- Selection of buffer: The desired pH scale frequently influences the buffer choice. For reversed-part silica-based packing, the normal pH scale ranges from two to eight. Since buffers control pH scale best at their pKa, it is crucial that the buffer has a pKa on the desired pH scale. A general rule is to select a buffer whose pKa value is less than two units of the required mobile phase pH.[30,31,32]

2.4.4 Selection of detector

The detector is a crucial component of HPLC. The choice of detector is influenced by the chemical makeup of the analysis, any potential interference, the desired limit of detection, and the detector's cost and/or availability. The dual wavelength UV visible detector is a flexible absorbance detector for HPLC. This detector provides the high sensitivity necessary for routine UV-based applications to identify and quantify low-level impurities. Photodiode Array (PDA). For solutions involving analytical HPLC, preparative HPLC, or LC/MS systems from Waters, Detector delivers superior optical detection. High chromatographic and spectral sensitivity is provided by its integrated software and optics improvements. This detector is the best choice for examination of components with little to no UV absorption due to its refractive index chromatographic and spectral sensitivity, stability, and reproducibility. High sensitivity and selectivity fluorescence detection are provided by multi-wavelength fluorescent detectors for quantifying target molecules at low concentrations.[33,34]

2.5 Developing the analytical strategy

The selection of several chromatographic parameters, such as the mobile phase, column, flow rate, and pH of the mobile phase, is the initial step in the development of an analytical technique for RP-HPLC. Following testing, all of these parameters are chosen, and the system suitability parameters are then taken into account. [35]

2.6 Preparation of Sample

It is a crucial step in method development. A sample aliquot that is largely free of interferences, won't harm the column, and is compatible with the desired HPLC method is the goal of sample preparation. To achieve this, the sample solvent must dissolve in the mobile phase without impacting sample retention or resolution. Beginning at the time of collection, sample preparation continues through sample injection into the HPLC column. [36, 37, 38]

2.7 Optimization of method

The improvement of HPLC conditions has received the majority of attention during HPLC technique development optimization. It is necessary to consider the compositions of the fixed and mobile phases. Mobile phase parameter optimization is always prioritized since it is more convenient and straightforward than stationary phase optimization. Only the parameters that are likely to have a significant impact on selectivity in the optimization must be looked at in order to reduce the amount of trial chromatograms required. The various elements of the mobile phase defining acidity, solvent, gradient, flow rate, temperature, sample quantities, injection volume, and diluents solvent type are the primary control variables in the optimization of liquid chromatography (LC) procedures. Following satisfactory selectivity, this is utilized to identify the ideal balance between resolution and analysis time. The variables include flow rate, column packing particle size, and column dimensions. Changes to these parameters won't have an impact on selectivity or capacity factor. [39, 40]

2.8 Method Validation

Validation is the process of examining something and providing unbiased proof that it meets the requirements for a certain intended usage. a procedure for assessing a method's performance and proving that it satisfies a specific condition. In essence, it is aware of the delivery potential of your approach, especially at low concentrations.[41,42]

Steps in method validation

1) Create a validation master plan, a validation methodology, or an operating procedure for the validation.
2) Describe the method's scope, goal, and applications.
3) Specify the specifications for the performance and its acceptance standards.
4) Specify validation studies.
5) Examine the equipment's relevant performance parameters.
6) Make materials suitable, such as standards and reagent.
7) Conduct pre-validation studies.
8) If necessary, modify the method's parameters or acceptance criteria.
9) Carry out comprehensive internal (and external) validation experiments.
10) Create SOPs for incorporating the technique into the routine.
11) Specify the revalidation criteria.
12) Specify the kind and frequency of the routine's Analytical Quality Control (AQC) checks and system suitability testing.
13) Keep track of validation experiments and their outcomes. [43]

Components of method validation are:

a. Specificity
b. Linearity
c. Accuracy
d. Precision
e. Range
f. Robustness
g. Limit of detection and limit of quantification
h. System suitability[44]

Specificity:

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. [In each case, the peak purity value (for Agilent systems) or purity angle (for Waters systems) must be more than the threshold or less than 0.999, respectively. [45,46]

Linearity:

It is important to assess a linear relationship over the entire analytical procedure. By diluting a standard stock solution of the drug product's constituent parts according to the suggested process, it is directly proven on the drug substance. The confidence limit surrounding the slope of the regression line is typically used to express linearity. If the procedure is linear, the test findings are directly proportional to the analyte concentration in samples falling within a specified range, or they can be transformed mathematically in a specific way. The confidence limit surrounding the slope of the regression line is typically used to express linearity. [46, 47]

Accuracy:

The degree of agreement between the value acknowledged as either a conventional true value or a recognized reference value and the value discovered is expressed as an analytical procedure's accuracy. Accuracy is defined as the discrepancy between the mean value discovered and the actual value. These should be contrasted with standard and blank solutions to ensure that there is no interference. The accuracy is then determined based on the test results as a percentage of the analyte recovered by the assay. A typical statement is the recovery of known, added amounts of analyte by test. [43, 48]

Precision:

The degree of scatter between a set of measurements obtained from multiple sampling of the same homogenous sample under the specified conditions is expressed as the precision of an analytical method. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. [43]

Repeatability: The inconsistency that a single analyst encounters on a single instrument is known as repeatability. It makes no distinction between variation resulting from the instrument or system or from the method used to prepare the sample. Repeatability is assessed during validation by employing the analytical procedure to examine numerous replicates of an assay composite sample. A recovery value calculation is made.

Intermediate precision: It 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.

Reproducibility: Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology).[49]

Range:

The interval between the higher and lower concentration (amounts) of analyte in the sample (containing these concentrations) for which it has been shown that the analytical technique has a sufficient level of precision, accuracy, and linearity is the range of an analytical procedure.[50,51]

Robustness:

An analytical procedure's robustness is a measure of its ability to be unaffected by minor but intended changes (e.g. pH, mobile phase composition, temperature and instrumental settings) to the technique parameters and provides a clue as to its dependability under typical conditions. Determining robustness is a systematic process that involves changing a parameter and evaluating the impact on the methodology through system suitability monitoring and/or sample analysis. [52]

Limit of detection

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. 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. [53, 54]

LOD = 3.3 × S /SD

Limit of quantification

The smallest amount of analyte in a sample that can be quantitatively identified with adequate precision and accuracy is known as the limit of quantitation (LOQ) or quantitation limit of a specific analytical process. The LOQ is typically estimated from a determination of S/N ratio (10:1) for analytical processes like HPLC that exhibit baseline noise, and is typically confirmed by injecting standards that yield this S/N ratio and have an acceptable percent relative standard deviation as well. [55]

LOQ = 10 × S /SD

System Suitability:

Tests for system compatibility are an essential component of liquid chromatographic techniques. They are used to confirm that the chromatographic system's detection sensitivity, resolution, and reproducibility are sufficient for the intended analysis. The tests are founded on the idea that the tools, electronics, analytical processes, and test samples make up a whole system that may be assessed as such. To assess the suitability of the employed approach, variables like peak resolution, theoretical plate count, peak tailing, and capacity have been assessed.

In the system suitability tests, parameters are as follows:

1. Number of theoretical plates or Efficiency (N).
2. Capacity factor (K).
3. Separation or Relative retention (α).
4. Resolution (Rs).
5. Tailing factor (T).
6. Relative Standard Deviation (RSD) [56,57]

CONCLUSION

The development of analytical methods for drug identification, purity assessment, and quantification has attracted a lot of attention recently in the field of pharmaceutical drug analysis. This review describes about RP-HPLC method development

and validation. The creation of an HPLC method for the separation of substances was presented using a general and extremely straightforward methodology. Prior to the development of any HPLC process, understanding the primary compound's physiochemical characteristics is crucial. The choice of buffer and mobile phase (organic and pH) composition has a significant impact on separation selectivity. The gradient slope, temperature, flow rate, type, and concentration of mobile-phase modifiers can all be altered for the final optimization. According to ICH criteria, the optimized method is verified using a variety of factors such as specificity, precision, accuracy, detection limit, linearity, system suitability.

ACKNOWLEDGEMENT

I appreciate Shri D.D Vispute College of Pharmacy and Research Center's Principal, Management, and My Research Guide for giving me with all the resources I needed to finish my review task.

CONFLICT OF INTEREST

None

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