International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2166-2174 www.ijprajournal.com ISSN: 2249-7781

# A Review on Analyticalmethod Devlopment and Validation of Amlodipine and Atoravastatin by RP-HPLC

Nilesh S Mhaske1, Pradip R Lokhande[2]
[1,2]*Department of Pharmaceutical Quality Assurance,Dr. VittalraoVikhePatil Foundation College of Pharmacy, ViladGhat; Ahmednagar(MS)-India.*

Submitted: 15-02-2023 Accepted: 25-02-2023

**ABSTRACT**
The determination of drug in pharmaceutical formulations method was developexact, precise, specific, and RP-HPLC approach. The investigation is straightforward because no chemical simulation of the purification stage has been done before using the samples in the study. A typical RP-HPLC test using C8 coloumsymmetry or an equivalent with the mobile phase's pH 4 phosphate buffer: Acetonitrile was used(50:50), with a flow rate of 1.5ml per minute, and UV detection at 254 am. Several ICH metrics, including linearity, detection limit, accuracy, precision, robustness, and applicability of the proposed approach have been validated
**Key word:** RP- HPLC,mobile phase, phosphate buffer, c8 coloum

## I. INTRODUCTION

In order to separate, recognise, and quantify the active chemicals, high-performance liquid chromatography (also known as high pressure liquid chromatography, or HPLC) is a particular type of column chromatography. The primary components of HPLC are a stationary phase (column holding packing material), a mobile phase (or mobile phases) pump, and a detector that displays the retention periods of the molecules. The interactions between the stationary phase, the molecules being studied, and the solvent(s) being utilised affect retention time. A small volume of the sample to be examined is added to the stream of the mobile phase, and it is slowed down by particular chemical or physical interactions with the stationary phase. The nature and composition of the analyte determine the amount of retardation. Retention time refers to the moment an individual analyteelutes (emerges from the end of the column). Any are common solvents that are employed. All Rights Reserved. enhanced miscibility of organic or water-based liquids (the most common are methanol and acetonitrile). Gradient elution refers to the separation process used to change the mobile phase composition during the analysis. Depending on the analyte's affinity for the current mobile phase, the gradient separates the analyte mixtures. The nature of the stationary phase and the analyte determine the choice of solvents, additives, and gradient.

**History**

Prior to HPLC, researchers used conventional liquid chromatographic techniques. Because the flow rate of solvents depends on gravity, liquid chromatographic techniques are inefficient. Separations take many hours, and maybe even days, to complete. Despite the fact that liquid chromatography (LC) at the time was more efficient, it was assumed that gas stage partition and research of extremely polar high atomic weight biopolymers were both impractical. GC wasineffective for some organic chemists because the solutes were thermally unstable. As a result, it was predicted that alternative methods would soon lead to the advancement of HPLC.It was foreshadowed by Cal Giddings, Josef Huber, and others in the 1941 Martin and Synge original work as well as Cal Giddings, Josef Huber, and others predicted in the 1960s that LC might be operated in the high-efficiency mode by reducing the pressed molecule measurement well below the standard LC (and GC) threshold of 150 m and using pressure to increase the flexible stage velocity. Throughout the 1960s and into the 1970s, these expectations underwent extensive research and improvement. The development of Zipax, an externally permeable chemical, was promising for technology as early developmental research began to improve LC particles. Numerous improvements in machinery and instrumentation were made in the 1970s.

**Operation**

The mobile phase stream that is permeating the column is introduced with a discrete small volume (typically microliters) of the sample blend that needs to be isolated and dissected.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2166-2174 www.ijprajournal.com ISSN: 2249-7781

Sample segments move through the segment at varying speeds as a result of specific physical contacts with the adsorbent (likewise called stationary stage). Every component's velocity is dependent on its chemical makeup and mobile phase. The retention time of a specific analyte is the time at which it elutes (rises up out of the column). For a given analyte, the retention time measured under particular circumstances serves as a distinguishing normal. There are numerous columns available that are filled with adsorbents with different molecular sizes and surface characteristics ("surface science"). The use of packing materials for tiny molecules necessitates the use of higher operational pressure ("backpressure") and frequently improves chromatographic resolution (i.e. the degree of division between sequential analytes rising up out of the column). The nature of sorbent particles may be polar or hydrophobic. The most common mobile phases are any miscible mixtures of water and several natural solvents (the most widely recognised are acetonitrile and methanol). Some HPLC systems employ mobile phases devoid of water. To aid in the separation of the sample components, the aqueous portion of the mobile phase may contain acids (such as formic, phosphotrifluoroacetic corrosive) or salts.

**Type of HPLC**

Types of HPLC often depend on the process's utilisation of a phase system. the following HPLC types are frequently used in analysis:

**1 Normal phase chromatography (NP-HPLC)** is a technique that combines a polar stationary phase and a non-polar mobile phase to separate analytes depending on polarity. The polar stationary phase reacted with the polar analyte and held it. Adsorption capacities rise withTypes of HPLC often depend on the process's utilisation of a phase system. the following HPLC types are frequently used in analysis
increasedanalyte polarity, as well as the interaction of the polar analyte with the polar stationary phase, enhance theelution period

**2 Reverse phase chromatography (RP-HPLC )** uses an aqueous, moderately polar mobile phase and a non-polar stationary phase. As a result of repulsive forces between a polar eluent, the comparatively non-polar analyte, and the non-polar stationary phase, RPC works on the theory of hydrophobic interactions. Following association with the ligand in the aqueous eluent, the contact surface area around the non-polar section of the analyte molecule determines the analyte's affinity for the stationary phase.

**3 Size exclusion chromatography (SEC**) is a type of chromatography that primarily uses gel permeation or gel filtration to separate particles based on their sizes. Additionally, it is helpful for figuring out the quaternary and tertiary structures of proteins and amino acids. The molecular weight of polysaccharides can be determined using this technique.

**4 Ion exchange chromatograpy**

The attraction between solute ions and charged sites bound to the stationary phase is the basis for retention in lon- exchange chromatography. The equivalent charge in tonnes is not included. This type of chromatography is frequently employed in the purification of water, protein lon-exchange chromatography, high-pH anion-exchange chromatography of carbohydrates and oligosaccharides, among other applications.

**5 Bio-affinity chromatography**:

Proteins and ligands are separated based on a specific, reversible association. Using a bioaffinity column, proteins can eluted in one of two ways: Include a free ligand in the elution solution that competes with column-bound ligands for biospecific elution. Aspecific elution: alteration in pH, salt content, etc. that reduces protein-substrate interaction. because of the interaction's uniqueness. In a single step, bioaffinity chromatography can achieve very high levels of purification.

**PARAMETER USED IN HPLC:**

1. Retention time
2. Retention volume
3. Separation factor
4. Resolution
5. Theoretical plate
6. HET Height Equivalent to a The Original Plate
7. Efficiency (no of theoretical plates)
8. Asymmetry factor- Fronting tailing

**Classification**

**1Column chromatograpy**

chromatography in a column The column is a three-dimensional form model with a geometrical structure that can be either packed or open tubular; when packed, the stationary

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2166-2174 www.ijprajournal.com ISSN: 2249-7781

portion is mostly packed and takes up most of the wall and each column. The fixed segment, however, is with the column sites inside the open tubular .

**2 Thin-layer chromatography**

(TLC) In thin-layer chromatography, the desk-bound phase inacts with a high surface area to generate solid-liquid adsorption while the cell portion is liquid. The cell phase is propelled upward through the stationary part by capillary action (thin plate soaked in fluid).with the solution

**3. In planar chromatography**,

The liquid solution acting as the mobile segment moves through the desk-bound segment, which may also be liquid or cellulosic (paper chromatography)

**4 Gas chromatograpy**

In gas-solid chromatography, a desk-bound segment made of solid adsorbent is employed, and separation is accomplished through an adsorption process, whereas in gas liquid chromatography, a thin layer of nonvolatile liquid is attached to a stable support.aid and separation takes region using the partitioning technique. The most often utilised technology is fuel-liquid chromatography.

The pattern that needs to be separated is first turned into vapours and mixed with gaseous mobile segments and components as a result. the additives that are less soluble in the desk bound phase of a sample move more quickly than those that are more soluble. As a result, the components are divided according to their partition co-efficient.

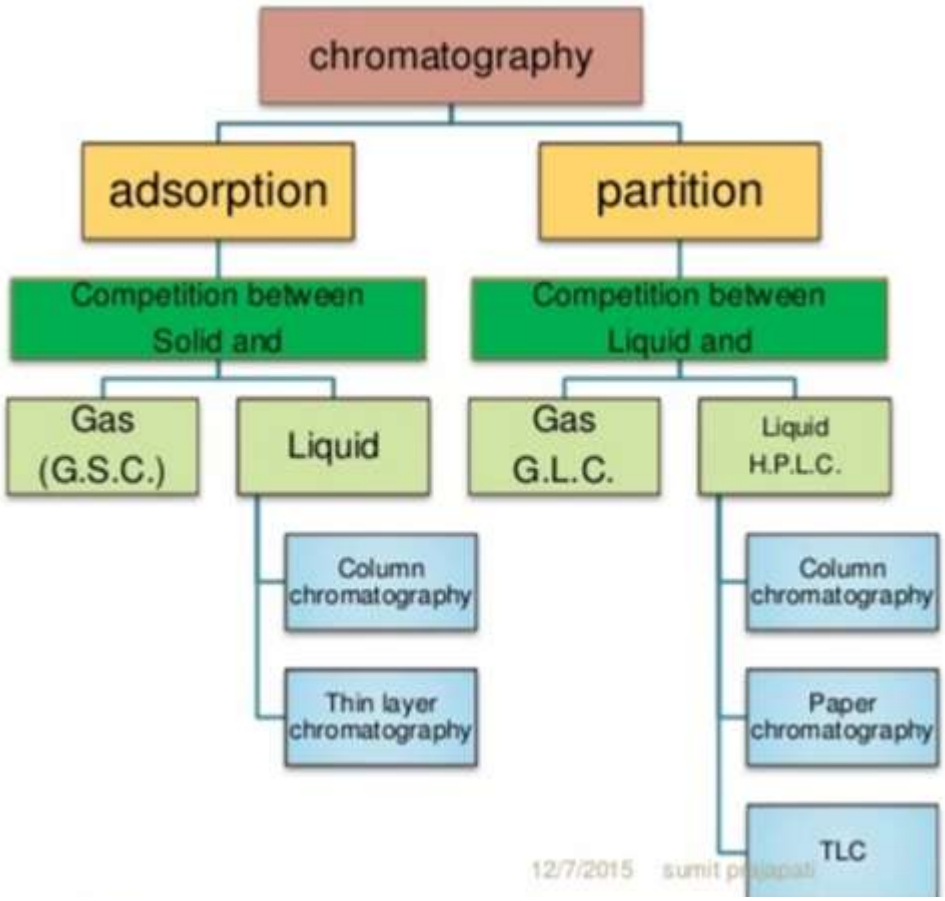



Fig no .1

**5 high-performance thin-layer chromatography** is a stronger form of thin-layer chromatography (TLC), some of upgrades can be made to the fundamental technique of thin-layer chromatography to automate the exclusive steps, to growth the resolution carried out, and to permit more accuratequantitative measurements

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2166-2174 www.ijprajournal.com ISSN: 2249-7781

**Principle of chromatography**

The separation principle of HPLC is based on the distribution of the analyte (sample) between a mobile phase (eluent) and a stationary phase (packing material of the column). Depending on the chemical structure of the analyte, the molecules are retarded while passing the stationary phase.

**Instrumentation**

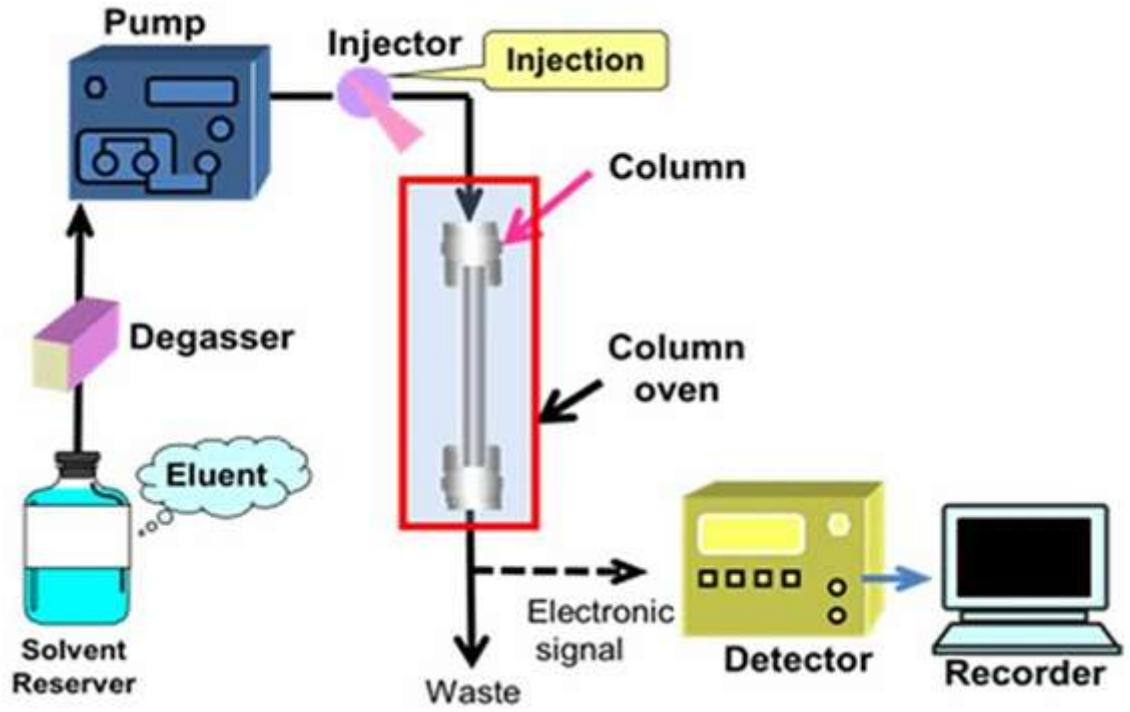



**Fig no .2**

**1 Solvent reservoir**

Mobile phase's content serves as the solvent reservoir.solventare in a glass container present. In HPLC, polar and non-polar liquid components are combined to form the mobile phase, or solvent. The selection of polar and non-polar solvents will vary depending on the sample's makeup.

**2Pump**:

The pump propels the mobile phase into the column from the solvent reservoir before passing it on to the detector. The pump's operating pressure is 42000 KPa. This operating pressure is influenced by the mobile phase's composition, flow rate, and column dimensions.

**3 Sample Injector**:

A single injector may be used.a computerised infusion system, or infusion. An injector for an HPLC framework should provide high-reproducibility, high-pressure infusion of the fluid specimen in the volume range of 0.1 mL to 100 mL. (up to 4000 psi).

**4Columns**:

Typically made of polished stainless steel, columns have an inside diameter of between 2 and 5 mm and range in length from 50 to 300 mm. Typically, the is loaded with stationary phase V molecules that range in size from 3 m to 10 pm. with inner columns

**5 Detector**:

The HPLC detector, which is located towards the column's end, helps identify the analytes as they elute from the chromatographic column. Electrochemical identification, fluorescence, and mass spectrometric detectors are frequently used.

**5 Data Collection Devices or Integrator**:

Signals from the detector may be collected on graph recorders or electronic integrators, which differ in their multifaceted quality and ability to

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2166-2174 www.ijprajournal.com ISSN: 2249-7781

handle, store, and reprocess chromatographic data. The PC coordinates the indicator's response to each component and inserts it into an easily readable chromatograph.

A sampler, pumps, and a locator are typically included in the schematic illustration of an HPLC device. The sample is introduced into the mobile phase stream by the sampler, which then transports it into the column. The mobile phase is moved through the column by the pumps. The detector produces a signal corresponding to the size of the sample component emerging from the segment, so taking into account a quantitative analysis of the example components. The HPLC device is controlled by a digital microchip and software, which also provides information. A few mechanical pump models in an HPLC device can mix a variety of solvents in amounts that change over time, creating a synthetic slope in the portable stage. The majority of HPLC devices also incorporate a column broiler that takes into account changing the temperature at which the partition is

## II. APPLICATION

The identity, quantity, and resolution of a compound are among the pieces of information that can be discovered via HPLC. The isolation and purification of chemicals is known as preparative HPLC. This contrasts with analytical HPLC, where learning more about the sample substance is the primary goal.

**1 Chemical separation**

Chemical Discriminations The extent or degree of separation is largely influenced by the choice of stationary phase and mobile phase since various compounds migrate at varying rates depending on the column and mobile phase used.

**2 Purification**

Purification is the process of separating or removing the desired product from a mixture of impurities or chemicals. Under specific chromatographic circumstances, the Fach compound displayed a distinctive peak. In order for the pure desired compound to be collected or extracted without causing any other undesirable compounds, the migration of the compounds and contaminants through the column needs to differ sufficiently from one another.

**3.Identification**

Identification commonly assay ofCompounds are transported through HPLC. The assay's settings should allow for a clear peak of the known sample to be seen on the chromatograph. At the detection levels where the assay will be run, the identifying peak should have a respectable retention time and be clearly distinguished from unrelated peaks.

**4Other applications**

**Pharmaceutical application**

Determinations of the shelf-life of pharmaceutical dosage forms.pharmaceuticalitems identification of dose form active ingredients.Control of pharmaceutical quality

**Applications for the Environment**:

Monitoringof contaminants and identifying elements in water.

**Forensic applications** such as the examination of textile colours and the estimation of steroid and drug levels in biological samples.

**Applications in food and flavour** include sugar analysis of fruit juices, polycyclic chemical detection in vegetables, and preservative analysis.

**Cinical applications**

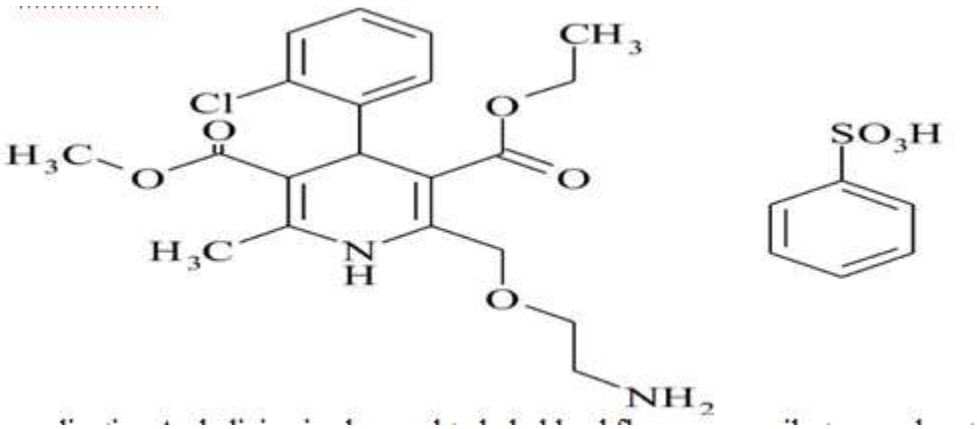
Clinical applications include the detection of endogenous neuropeptides and the examination of bodily fluids like blood and urine.

**Drug profile**

**Amlodipine**

Amlodipine is used to lower your blood pressure. It can be used alone or in combination with other heart medication.Amlodipine is also used to help blood flow more easily to your heart when the arteries in your heart are blocked.Amlodipine is also used to treat coronary artery disease and angina (chest pain).

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2166-2174 www.ijprajournal.com ISSN: 2249-7781




**Stucture**

**Molecular formula:**C20H25ClN2O5
**Solubility:** methanol
**Meltingpoint :203°c**

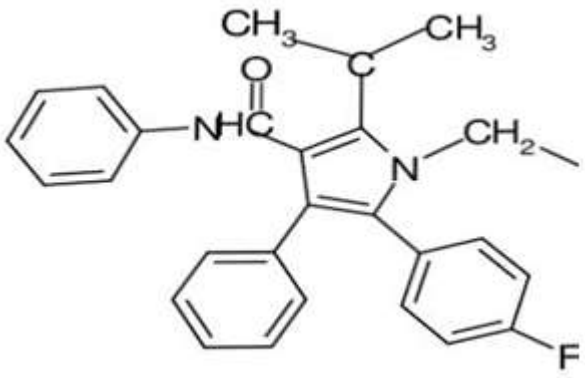
**Mechanism of Action (9)**

Amlodipine is in a class of medications called calcium channel blockers. It lowers blood pressure by relaxing the blood vessels so the heart does not have to pump as hard. It controls chest pain by increasing the supply of blood to the heart

**Atoravastatin**

Atorvastatin is a statin medication used to prevent cardiovascular disease in those at high risk and to treat abnormal lipid levels. For the prevention of cardiovascular disease, statins are a first-line treatment. It is taken by mouth.

**structure**




**Molecular formula:**C33H35FN2O5
**Solubility:**methanol
**Melting point:155°c**

**Mechanism of Action (10)**

Atorvastatin competitively inhibits 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. By preventing the conversion of HMG-CoA to mevalonate, statin medications decrease cholesterol production in the liver.

**Reverse phase high pressure liquid chromatography (RP-HPLC)**

By using a technique called reverse phase chromatography (RP-HPLC), molecules are divided according to how hydrophobic they are. the elements a stationary phase particle with pore size large enough to allow the test mixture to pass over it, where interactions with the hydrophobic Remove them from the moving mobile phase stream at the surface. The degree and kind of particle-particle interaction in the sample and the

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2166-2174 www.ijprajournal.com ISSN: 2249-7781

stationary phase rely on strong polar and hydrophobic interactions. As the amount of organic solvent in the eluent increases
As the concentration rises, it reaches a critical point for each analyte that causes it to desorb from the stationary phase's hydrophobic surface and allowsit to remove the column's flowing mobile phase by eluting it.
1.Hydrophobic interactions,
2. Non-polar packaging materials, such as polymer and silica-C18
3. Mobile phase: Polar Ex: MeOH/H20, MeOH/Sol, Buffer.
4. Sample: having a different carbon chain length. Mobile phase solvents: 5. Main solvent MeOH-H20, CH3CN-H20
6. Low solvent: EtOH, IPA, THF, DMF

**ANALYTICAL DEVELOPMENT STAGES**
a)Theanalyte's typical characteristics are as follows:A complete description of the analyte, Inluding its solubility, stability, hygroscopicity, purity, and physical and chemical properties.
b) Acquire the pure standard analyte. arranged for appropriate storing (refrigerator, dryer, and freezer), e) When more than one component in the sample matris is to be tested, the number of components is noted, the data is compiled, and it is established whether standards are available for each.

2. Conditions for the Method:
The goals of the analytical technique under development are taken into consideration. Limits of detection, selectivity, linearity, and runge.Precision and accuracy are defined.

3. Research Methodology and Prior Literature:
Study is done on the analyte's information. physical and chemical characteristics for synthesis

4. Select A Technique:
The methodology is modified using data from the literature. When needed, the procedures are modified. It is occasionally required to invest in additional equipment to duplicate, adapt, enhance, or validate current procedures for analytes and internal samples. Usually, the analyte of interest is analogous to a chemical for which an analytical technique already exists.

5. Instrumental setup and preliminary studies:
The necessary instrumentation is set up. Utilizing laboratory SOPs, instrument installation, operation, and performance qualifications are confirmed. Filters for the standard analyte in an appropriate injection introduction solution and in known concentrations are always used, and fresh solvents are prepared. Starting with a well-known standard is crucial instead of a challenging sample matrix.

6. Improvement:
Instead than utilising a trial-and-error method, optimization involves changing one parameter at a time and isolating a set of conditions. Each step of the process was recorded (in a lab notebook) in case there was a dead end. The work was carried out according to an organised, methodical plan.

7. Documenting the merit of analytical figures:
Analytical parameters for limit of detection (LOD), limit of quantification (LOQ), linearity, and time per
Documentation is provided for sample preparations, pricing, and analysis.
8. Real-Sample Evaluation of the Method Development:
The sample solution must result in the complete and unambiguous identification of the target analyte peak. additional elements of the matrix.

## III. METHODOLOGY

Preparation for the Mobile Phase
Phosphate buffer (50%) with 400 ml of HPLC acetonitrile (50%) before degassing for five minutes in an ultrasonic bath. Under vacuum filtration, filter through a 0.45 a filter.

Making the Standard Solution:
Transfer 50 mg of the working standard drug, accurately weighed, to a 50 ml volumetric flask. Both medications are dissolved in 50 ml of mobile phase using a sonicator to ensure thorough dissolution and to get the desired volume using the same solvent (stock silution). additional 0.5 ml of the aforementioned stock solution into a 10 ml pipette

**VALIDATION OF THE METHOD:**
This can be done by
1. Precision
2. Intermediate / Resistant Accuracy
3. Linearity
4. Limit of Detection
5. Limit of Quantification

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2166-2174 www.ijprajournal.com ISSN: 2249-7781

**1.Precision**

Precision measures how well repeated measurements of the same quantity agree.

**2.Accuracy**

Accuracy is a measure of the closeness of the experimental value to the actual amount of the substance in the matrix. Precision measures of how close individual measurements are to each other.

**3.Linearity**

Linearityis a mathematical relationship between two variables quantities (they may be same unit), which are directly propertional to each other**.**

**4. Limit of Detection**

The limit of detection (LOD) is defined as the lowest concentration of an analyte in a sample that can be consistently detected with a stated probability (typically at 95% certainty)

**5.Limit of Quantification**

Limit of quantification expresses the lowest concentration of analyte that can be quantified precisely and accurately.

## REFERENCE

[1]. Jena AK. HPLC: highly accessible instrument in the pharmaceutical industry for effective method development. Pharm Anal Acta. 2011; 3.

[2]. Hamilton RJ, Sewell PA. Introduction to high-performance liquid chromatography. Springer. 1982; 1-12.

[3]. Gokul S. Sanap, Nilesh S. Zarekar, Sarita S. Pawar Review On Method Development And Validation International Journal Of Pharmaceutics & Drug Analysis; Vol.5; Issue 5, 2017; 177 – 184.

[4]. Malviya R, Bansal V, Pal OP, Sharma PK. High-performance liquid chromatography: a short review. J Glob Pharma Technol. 2010; 2(5): 22-26.

[5]. Ravisankar S, HPLC, Textbook of Pharmaceutical Analysis, 3rd Ed, 2006, 18(1)-18(15) Skoog DA, Holler FJ, Crouch SR, Principles of Instrumental Analysis, 6th Ed, Baba barkahanath printers, Haryana, 2007, 816-855.

[6]. United States Pharmacopoeia, national formulary, section; "Validation of compendial methods", US Pharmacopoeial Convention, Rockville, 2000. United States Pharmacopoeia, section "system suitability", US Pharmacopoeial Convention, Rockville, 2004.

[7]. Kumar S. Spectroscopy of organic compounds. Cosmic rays. 2006; 10: 4.

[8]. Luxminarayan L, Sharma N, Viswas A, Khinchi MP. A review of chromatography techniques.Asian Journal of Pharmaceutical Research and Development. 2017; 1-8.

[9]. RajendraPatil, TusharDeshmukh, Vijay Patil, and KishanchandKhandelwal; Review on Analytical Method Development and Validation; Research and Reviews; Journal of Pharmaceutical Analysis; Volume 3; Issue 3; July-September, 2014; 01-10.

[10]. Ashok Kumar, NavpreetKaur, Lalit Kishore, Anoop Nair; Method development and validation; Skills and tricks Article in Chronicles of Young Scientists; March 2012; 03-11.

[11]. Raising Swati, PatilMansi and Patil Aditya; Analytical Method Development and Validation; A Concise Review International Journal of Pharmacy and Biological Sciences-IJPBSTM (2021) 11 (1): 09-16.

[12]. Suyash Gupta, PreetiVerma, AbhinavPrasoon Mishra, Nainshi Omar, RiyaMathur; A Review on Novel Analytical Method Development and Validation by RP-HPLC Method; Indian Journal of Forensic Medicine & Toxicology, October-December 2021, Vol. 15, No. 4 3476-3486.

[13]. DhanashriParasramFand; A Review On Analytical Method Development; International Journal of creative research thoughts; Volume 10, Issue 1 January 2022; ISSN: 2320-2882.

[14]. Kirthi, R. Shanmugam, M. Shanti Prathyusha, D. Jamal Basha; A Review On Bioanalytical Method Development And Validation By RP – HPLC; Journal of Global Trends Pharmaceutical Sciences (2014); Volume 5(4) - 2265 – 2271.

[15]. Analytical Procedures and Methods Validation for Drugs and Biologics Guidance for Industry S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER) Center for Biologics Evaluation and Research

(CBER) July 2015 Pharmaceutical Quality/CMC.

[16]. AshishChauhan, BhartiMittu and PriyankaChauhan; Analytical Method Development and Validation: A Concise Review; Journal of Analytical & Bioanalytical Techniques 2015, Volume 6; Issue 1; 6:1.

[17]. RoshanBhagat, RavindranathSaudagar; A Review On Analytical Method Development And Validation; Journal of Drug Delivery And Therapeutics 2019; 9(3-S):1064-1067.

[18]. HetalBhenjaliya, RohanBarse Review Article On Analytical Method Development And Validation: Requirements In Pharmaceutical Field Manipal Journal Of Pharmaceutical Sciences; March 2020; Volume 6 ; Issue 1; 60-65.

[19]. SenthilSelvanPerumal, SanmugaPriyaEkambaram, Samundeswari Raja; Review Article on Analytical Method Development And Validation Of Simultaneous Estimation Of Rabeprazole, Pantoprazole, And Itopride By Reverse-Phase High-Performance Liquid Chromatography; Journal Of Food And Drug Analysis 22 (2014) 520 -526.

[20]. RishabhaMalviya, Pramod Sharma, VipinBansal; High-performance liquid chromatography: A short review Article in Journal of Global Pharma Technology; June 2010; 2(5). 22-26.

[21]. Abdu Hussein Ali Short Review on High-Performance Liquid Chromatography (HPLC) HSPI: A review; Annals of Advance in Chemistry; 2022; 10-20.

[22]. Kissinger PT. Instant Notes: Analytical Chemistry. Clin Chem. 2002; 48(12): 2303.