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A REVIEW ON HPLC METHOD FOR ESTIMATION OF AZELNIDIPINE

Krishnaphanisri Ponnekanti[1]*, Addanki Anusha[2], Malavika Reddy[3] and Omkar H.[4]

Associate Professor[1*,2], Student [3,4]

Department of Pharmaceutical Analysis, Malla Reddy Institute of Pharmaceutical Sciences, Maisammaguda, Kompally. India.

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***Corresponding Author**
Krishnaphanisri Ponnekanti
Associate Professor,
Department of Pharmaceutical Analysis,
Malla Reddy Institute of Pharmaceutical Sciences,
Maisammaguda, Kompally.
India.

ABSTRACT

Hypertension, also known as high blood pressure, occurs when the force of blood against the artery walls is too high. Azelnidipine (AZEL) is a dihydropyridine (DHP) type of calcium channel blocker (CCB) used for the treatment of hypertension. AZEL has two enantiomers due to an asymmetric carbon at 4th position of the DHP ring. The pharmacological action of AZEL resides in (R) - enantiomer and biological activity resides in (S) – enantiomer. High-Performance Liquid Chromatography (HPLC) is a type of column chromatography that is commonly used in biochemistry and analysis to separate, identify, and quantify active chemicals. A new simple, accurate and precise HPLC method has been developed and validated for estimation of Azelnidipine in its formulation. It is one of the most accurate methods widely used for the quantitative as well as qualitative analysis of drug product and is used for determining drug product stability.

KEYWORDS: Azelnidipine, HPLC, Hypertension.

INTRODUCTION

Pharmaceutical analysis plays a vital role in determining the qualitative and quantitative composition of materials, essential for understanding sample characteristics. Pharmaceutical Analysis is an Analytical Method used in determination of the quality and quantity of pharmaceutical products. It also gives the information about the purity and safety of the products. Briefly it can be described as it identifies, determines, quantifies, purifies and separates the active compound from the mixture.[1]

Hypertension is a condition where blood pressure is elevated to an extent that clinical benefit is obtained from BP lowering. In this condition the force of blood against the artery wall is too high usually systolic blood pressure greater than 140mm Hg or diastolic blood pressure greater than 90mm Hg or both over a sustained period. Antihypertensive drugs are prescribed mainly to reduce the morbidity and mortality caused by hypertension and its complications. Azelnidipine is a new and long-acting antihypertensive drug. This drug has been shown to decrease blood pressure with a similar potency as other dihydropyridines, such as amlodipine, but without increasing pulse rate.[2]

Azelnidipine is dihydropyridine derivative and chemically 3-[1-(Benzyldrylazetidin-3-yl] 5-isopropyl- 2- amino6methyl-4-(3-nitrophenyl)-1,4- dihydropyridine-3,5dicarboxylate. Azelnidipine category is Dihydropyridine calcium channel blocker. The presence of asymmetric carbon at the 4-position of the 1,4 dihydropyridine ring enable the Azelnidipine to exists in two enatiomeric form. Azelnidipine inhibits trans-membrane Ca^{2+} influx through the voltage dependent channels of smooth muscles in vascular walls. It is a vasodilator that induces a gradual decrease in blood pressure in hypertension. When calcium channels are blocked, the vascular smooth muscle does not contract, resulting in relaxation of vascular smooth muscle walls and decreased blood pressure. It is Used in Treatment of Hypertension which lower the Blood Pressure due to Blocked calcium Channel and Decreases Blood Pressure and oral dose is 8 – 16 mg once daily. It metabolized in hepatic cytochrome P450 (CYP) 3A4 and has no active metabolite product.[3]

PHYSICAL AND CHEMICAL PROPERTY

Azelnidipine is light yellow to yellow crystalline powder. IUPAC name is 3-[1-(Benzyldrylazetidin- 3-yl] 5-isopropyl- 2- amino6methyl-4-(3nitrophenyl)-1,4-dihydropyridine-3,5dicarboxylate.[4] Molecular formula of Azelnidipine is $C_{33}H_{34}N_4O_6$. Molecular weight is 582.646 g/mol. It is insoluble in water, slightly soluble in methanol, soluble in ethyl acetate, freely soluble in acetone and in acetic acid.[5]

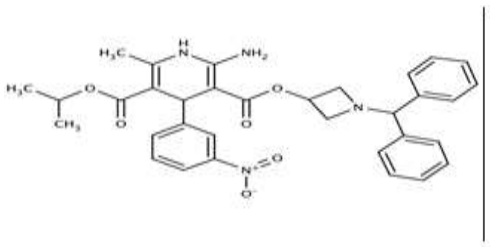

Figure 1: Structure of Azelnidipine.

Mechanism of Action of Azelnidipine

Azelnidipine inhibits trans-membrane Calcium influx through the voltage-dependent channels of smooth muscles in vascular walls. Calcium channels are classified into various categories, including L-type, T-type, N-type, P/Q-type, and R type Calcium channels. Normally, calcium induces smooth muscle contraction, contributing to hypertension. When calcium channels are blocked, the vascular smooth muscle does not contract, resulting in the relaxation of blood vessels.[6]

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

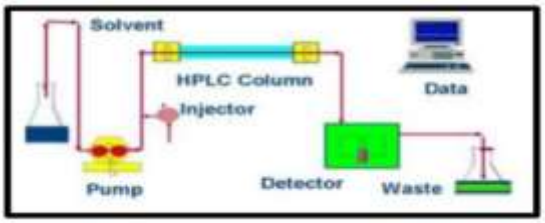

Figure 2: Schematic Representation of HPLC System.

High-Performance Liquid Chromatography (HPLC) was developed in the late 1960s and early 1970s. Today it is widely applied for separations and purifications in a variety of areas including pharmaceuticals, biotechnology, environmental, polymer and food industries.[7]

High-performance liquid chromatography (or High-pressure liquid chromatography, HPLC) is a specific form of column chromatography generally used in biochemistry and analysis to separate, identify, and quantify the active compounds. HPLC mainly utilize a column that holds packing material (stationary phase), a pump that moves the mobile phase(s) through the column, and a detector that shows the retention times of the molecules. Retention time varies depending on the interactions between the stationary phase, the molecules being analysed, and the solvent(s) used. The sample to be analysed is introduced in small volume to the stream of mobile phase and is retarded by specific chemical or physical interactions with the stationary phase.[8] The amount of retardation depends on the nature of the analyte and composition of both stationary and mobile phase. The time at which a specific analyte elutes (comes out of the end of the column) is called the retention time. Common solvents used include any miscible combinations of water or organic liquids. Separation has been done to vary the mobile phase composition during the analysis; this is known as gradient elution. The gradient separates the analyte mixtures as a function of the affinity of the analyte for the current mobile phase.[10]

Principle of HPLC

HPLC is a separation technique that involves: The injection of a small volume of liquid sample into a tube packed with tiny particles (3 to 5 micron (μm) in diameter called the stationary phase) where individual components of the sample are moved down the packed tube (column) with a liquid (mobile phase) forced through the column by high pressure delivered by a pump. These components are separated from one another by the column packing that involves various chemical and/or physical interactions between their molecules and the packing particles. These separated components are detected at the exit of this tube (column) by a low-through device (detector) that measures their amount. Output from this detector is called an "HPLC".[11]

METHOD DEVELOPMENT OF HPLC

Method development involves the following steps

1. Understanding the Physicochemical Properties of the drug molecule.
2. Selection of chromatographic conditions.
3. Developing the approach of analysis.
4. Sample preparations
5. Method optimization
6. Method validation[12]

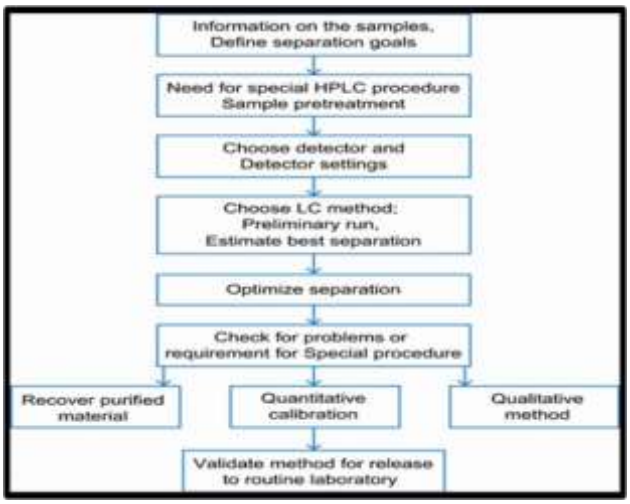

Figure 3: flow diagram of HPLC.

TYPES OF HPLC

1. **Normal Phase Chromatography:** This technique, also known as Normal phase HPLC (NP-HPLC), divides analyses according to polarity. Polar stationary phase and non-polar

mobile phase are both used in NP-HPLC. The polar stationary phase reacted with the polar analyte and held it. Increased analyte polarity results in stronger adsorption forces, and the interaction of the polar analyte with the polar stationary phase lengthens the elution time.

2. **Reversed phase chromatography:** Reversed phase HPLC (RP-HPLC or RPC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase. RPC operates on the principle of hydrophobic interactions, which result from repulsive forces between a polar eluent, the relatively non-polar analyte, and the non-polar stationary phase. The binding of the analyte to the stationary phase is proportional to the contact surface area around the non-polar segment of the analyte molecule upon association with the ligand in the aqueous eluent.[13]
3. **Ion exchange Chromatography:** The attraction between solute ions and charged sites bound to the stationary phase drives retention in ion-exchange chromatography. Same-charge ions are not included. This type of chromatography is frequently employed in the ion-exchange chromatography of proteins, the ligand-exchange chromatography of proteins, the high-pH anion-exchange chromatography of carbohydrates and oligosaccharides, and other processes for the purification of water.
4. **Size exclusion chromatography:** Size exclusion chromatography (SEC), also called as gel permeation chromatography or gel filtration chromatography mainly separates particles on the basis of size. It is also useful for determining the tertiary structure and quaternary structure of proteins and amino acids. This technique is widely used for the molecular weight determination of polysaccharides.
5. **Bio affinity chromatography:** Separation based on a particular, reversible interaction between ligands and proteins. A bio-affinity matrix has ligands covalently bonded to a solid support that holds onto proteins that interact with the ligands connected to the column.[13]

INSTRUMENTATION

1. **Solvent reservoir:** Mobile phase contents are contained in a glass reservoir. The mobile phase, or solvent, in HPLC, is usually a mixture of polar and non-polar liquid components whose respective concentrations are varied depending on the composition of the sample. The type and composition of the mobile phase affect the separation of the components.

2. **Injection of the sample:** Septum injectors are available; using which sample solution is injected. Sample can be injected when the mobile phase is flowing or it is stopped. A new advanced rotary valve and loop injector can be used to produce reproducible results.
3. **Pump**: A pump aspirates the mobile phase from the solvent reservoir and forces it through the system's column and detector. Depending on a number of factors including column dimensions, the particle size of the stationary phase, the low rate and composition of the mobile phase, operating pressures of up to 42000 kPa (about 6000 psi) can be generated. The role of the pump is to force a liquid (called the mobile phase) through the liquid chromatography at a specific low rate, expressed in millilitres per min (mL/min). Normal low rates in HPLC are in the 1-to 2-mL/min range.
4. **Columns:** Columns are usually made of polished stainless steel, are between 50 and 300 mm long, and have an internal diameter of between 2 and 5 mm. They are commonly illed with a stationary phase with a particle size of 3–10 μm. Columns with internal diameters of less than 2 mm are often referred to as micro bore columns.
5. **Detectors:** The HPLC detector, located at the end of the column detects the analyses as they elute from the chromatographic column. Commonly used detectors are evaporative light scattering, UV-spectroscopy, Fluorescence, mass-spectrometric and electrochemical detectors. The detector can see (detect) the individual molecules that come out (elute) from the column. A detector serves to measure the amount of those molecules so that the chemist can quantitatively analyse the sample components.
6. **Interpreting the output from the detector:** The output is recorded as a series of peaks, each one representing a compound in the mixture passing through the detector and absorbing UV light. The area under the peak is proportional to the amount of substance, which is passed through detector, and this area can be calculated automatically by the computer linked to the display.[14]

Validation Parameters

Accuracy: The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.

Retention time: Retention time is the difference in time between the point of injection and the appearance of peak maxima. It is also de ined as the time required for 50% of a component to be eluted from a column. It is measured in minutes and seconds.

Retention volume: Retention volume is the volume of carrier gas required to elute 50% of the component from the column. It is the product of retention time and low rate.

Specificity: Specificity now refers to how clearly; we can tell if a test has the right properties

Precision: The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Repeatability: This refers to the degree of consistency in measurements made rapidly and repeatedly throughout the same procedure.

Intermediate precision: This degree of precision verifies accuracy in a variety of scenarios, such as various analysts, items, or days. It illustrates how several conditions cause the lab to alter. • Reproducibility: This indicates how well data from several labs match up.

Linearity: When you measure something in a sample, such as the amount of a chemical present, linearity means that the test findings shift in a straight line as the item's concentration varies.

Limit of Detection: The least amount of a chemical that can be found in a sample using the analytical method, even if the measurement isn't exact, is known as the detection limit.

Limit of Quantisation: The smallest quantity of a material that can be accurately determined in a sample is known as the limit of quantisation in analytical procedures.

Robustness: The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Asymmetry factor: A chromatographic peak should be symmetrical about its centre. But in practice due to some factors, the peak is not symmetrical and shows tailing or fronting. Fronting is due to saturation of stationary phase and can be avoided by using less quantity of sample. Tailing is due to more active adsorption sites and can be eliminated by support pre-treatment. Asymmetry factor (0.95 to 1.05) can be calculated by AF = b/a (b, a calculated by 5% or 10% of the peak height). Broad peaks occur due to the more conc. of the sample, large injection volume, and column deterioration.[16]

LITERATURE REVIEW OF AZELNIDIPINE

SL NO	COLUMN	MOBILE PHASE	RESULT	REFERENCE
01.	C18 column	75:25methanol:water and 0.1%glacial acetic acid	Retention time: 6.130 mins Linearity:10-60µg/mL %RSD: <2	[17]
02.	Hypersilgold C18	Methanol:Acetonitrile: Water (40:40:20)	RetentionTime:8.56min Linearity:2-48 µg/ml	[18]
03.	IntersilC_{18} column	Acetonitrile: phosphate buffer	Linearity:10-50µg/ml Accuracy: 98 to 102 % % RSD: 1.5%s	[19]

CONCLUSION

The HPLC method provides a reliable and efficient tool for the quantitative analysis of azelnidipine in pharmaceutical products. The methods advantages, including short analysis time, low solvents consumption, and high sensitivity, make it suitable for routine quality control analysis.

The results of this study demonstrate the potential of HPLC as a powerful analytical technique for the estimation of azelnidipine. The methods accuracy, precision, and specificity make it a valuable tool for pharmaceutical industries, research institutions, and quality control laboratories.

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