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A REVIEW ON RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF AZLINIDIPINE DRUG IN TABLET DOSAGE FORM

Sarita Sasane*, Bhawar H.S., Dighe A.S. and Kolhe S.D.

Pravara College of Pharmacy, Loni, Tal-Rahta, Dist-Ahmednagar, Maharashtra, India.

***Corresponding Author: Sarita Sasane**

Pravara College Of Pharmacy, Loni, Tal-Rahta, Dist-Ahmednagar, Maharashtra, India.

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ABSTRACT

The stability indicating RP-HPLC method was found to be simple, rapid, sensitive, specific, precise, accurate and cost effective for estimation of azelnidipine in tablet dosage form and bulkdrugs substance. This technique was employed in the present investigation for estimation of Azelnidipine using HPLC with Inertsil ODS-3V, 150 x 4.6 mm, 5µ column, UV/PDA detector at 255 nm wavelength, flow rate was 1 ml/min, injection volume with empower Software was used for the study. The standard and sample solution of azelnidipine were prepared in diluent. the column make injection volume and different pure solvents of varying polarity in different proportions were tried as mobile phase for development of the chromatogram. The mobile phase that was found to be most suitable was Buffer pH 3.2 and Methanol, the wavelength 255 nm were selected for the evaluation of the chromatogram of azelnidipine respectively. The selection of the wavelength was based on the λ max obtained by scanning of standard laboratory mixture in water: methanol. This selected chromatographic condition gave good resolutionand optimum retention time with appropriate theoretical plates, tailing factor.

INTRODUCTION

Quality assurance is a wide ranging concept covering all matters that individually or collectively influence that quality of the product. It plays a central role in determining the safety and efficiency of medicines. Highly specific and sensitive analytical techniques hold the key role to the design, development, standard and quality control of medicinal product.[1] Quality of the drug product is very vital, as it involves life. Proper manufacture and quality control of pharmaceuticals is the vital segment of strong primary healthcare programme worldwide. Quality is the total sum of all factors which contribute directlyor indirectly to the safety; efficacy and acceptability of the product.[2]Pharmaceutical analysis, a branch of pharmacy, plays a very significant role in quality control of pharmaceuticals through a rigid check on raw materials used in manufacturing of formulation and on finished products. Analytical chemistry has since long, occupied an important place in the development of science and technology. It is primarily concerned about determining the qualitative and quantitative composition of material under study. The qualitative analysis gives us the information about the nature of sample by knowing about the presence or absence of certain components. The quantitative analysis deals about the content present in the sample. The development in analytical sciences has been more significant and prominent in recent years than the past. This has really broaden our vistas and helped to develop new methods of analysis. In pharmacy analytical chemistry is responsible for developing sensitive, reliable and more accurate methods for the estimation of drug in pharmaceutical dosage form.[3,4]

1.1. Analytical chemistry

Analytical chemistry is an important part of pharmaceutical analysis. Analytical Chemistry may be defined as the science and art of determining thecomponents of materials in terms of the elements or compound contained. Analytical Chemistry seeks ever improved means of measuring the chemical composition ofnatural and artificial materials. The techniques of this science are used to identify the substances which may be present in a material and to determine the exact amounts of the identified substances.

Classification of Analytical Methods

Analytical methods Classified into two categories; Classical methods andInstrumental methods

- **Classical Methods:** For qualitative analysis the separated compounds are treated with reagents that could be recognized by either colour, by their boiling or melting points, their solubility.
- **Volumetric Methods:** In volumetric, also called titrimetric, procedures, thevolume or mass of a standard reagent required to react completely with theanalyte is measured.
- **Gravimetric Methods:** In gravimetric measurements, the mass of the analyteor some compound produced from the analyte is determined.

The extent of their general application is, however, decreasing with the passage of time and with the advent of instrumental methods to supplant them.

❖ **Instrumental Methods**

These methods are based upon the measurement of some physical properties asconductivity, electrode potential, light absorption or emission, mass-to-charge ratioand fluorescence of substance. There are many techniques available for the analysis of analytes, which can be broadly classified as,

a) Spectroscopic Techniques

- Ultraviolet and visible spectrophotometer
- Fluorescence and phosphorescence spectrophotometer
- Atomic spectrophotometers (emission & absorption)
- Infra-red spectrophotometer
- Raman spectroscopy
- X-ray spectroscopy
- Radiochemical techniques including activation analysis
- NMR spectroscopy ESR spectroscopy

b) Electrochemical Techniques

- Potentiometry
- Voltametry
- Stripping techniques
- Amperometric techniques
- Coulometry
- Electrogravimetry
- Conductance techniques

c) Chromatographic Techniques

- Gas chromatography (GC)
- High performance liquid chromatography (HPLC)
- High-performance thin layer chromatography (HPTLC)
- Supercritical fluid chromatography (SFC)
- Ultra pressure liquid chromatography (UPLC)

d) Miscellaneous Techniques

- Thermal analysis
- Mass spectrometric
- Kinetic techniques

e) Hyphenated Techniques

- GC-MS
- ICP-MS
- GC-IR
- MS-MS
- CE-MS
- LC-NMR
- LC-MS
- LC-MS-NMR

1.2. Chromatographic techniques

- Chromatography is separation of a mixture into individual components using a stationary phase and a mobile phase. The stationary phase may be solid or a liquidsupported on a solid or gel or may be packed in column. The mobile phase may be gaseous or liquid.
- Chromatographic separation relies on relative movement of two phases. In chromatography one phase is fixed (stationary phase) and other is mobile (mobile phase) the mobile phase passes over the stationary phase.

Classification

1.2.1. Based on the nature of stationary and mobile phase

1.2.1.1. Gas –solid chromatograph.
1.2.1.2. Gas- liquid chromatography.
1.2.1.3. Solid- liquid chromatography e.g. TLC, column chromatography, HPLC.
1.2.1.4. liquid-liquid chromatography e.g. Paper partition chromatography, column partition chromatography.

1.2.2. Based on the principle of separation

1.2.2.1. Adsorption chromatography
1.2.2.2. Partition chromatography

1.2.3. Based on the modes of chromatography

1.2.3.1. Normal phase chromatography
1.2.3.2. Reversed phase chromatography

1.2.4. Other types of chromatography

1.2.4.1. Ion – exchange chromatography
1.2.4.2. Exclusion chromatography

1.3. Spectrophotometry

Spectrophotometric techniques are the most important instrumental techniques available to the pharmaceutical analyst for estimation of complex mixture of drugs. The basis of all instrumental techniques is that they measure the interaction of electromagnetic radiation with matter in quantised, i.e. specific energy levels. In spectrophotometry absorption of the electromagnetic radiation of definite and narrow wavelength range by molecules, ions and atoms of chemical substance is measured. There are various spectrophotometric techniques available as follows,

- UV-Visible absorption spectrophotometry
- Atomic emission and atomic absorption spectrophotometry
- Spectrofluorimetry
- Infrared spectrophotometry
- Nuclear magnetic resonance spectroscopy
- Mass spectrometry
- Raman spectrometry

The study of spectroscopy can be carried out under following two heads,

a) **Atomic spectroscopy:** This spectroscopy is concerned with the interaction of electromagnetic radiation with atoms which are commonly in their lowest energy state.

b) **Molecular spectroscopy:**This spectroscopy deals with the interaction of electromagnetic radiation with molecules. This results in transition between rotational and vibration energy levels in addition to electronic transition.

- The fundamental law that governs the quantitative spectrophotometric analysis is the Beer- Lambert's law which states that,'When a beam of monochromatic light is passed through a transparent cell containing a solution of an absorbing substance, reduction of intensity of the light may occurs; the rate of reduction in intensity with the thickness of the medium is proportional to the intensity of the light and the concentration of the absorbing substances'.

- Mathematically Beer- Lamberts law is expressed as:

A = a.b.c

Where,

A = absorbance or optical density

a = absorptivity or extinction coefficient b = path length in cm

c = concentration of solute in solution.

1.4. Instrumentation and theory of operation of HPLC

The technique of HPLC was developed in the late 1960"s. This technique based on same modes of separation as column chromatography, ion exchange chromatography, and gel permeation but differs from column chromatography in that the mobile phase is pumped through column with high pressure.[5]

a) Solvent delivery system

A mobile phase is pumped under pressure from one or several reservoir and flows through the column at a constant rate. For normal phase separation eluting power increases with increasing polarity of the solvent but for reversed phase separation, eluting power decreases with increasing polarity. A degasser is needed to remove dissolved air and other gases from the solvent. Special grades of solvents are availablefor HPLC and these have been purified carefully in order to remove absorbing impurities and particulate matter to prevent these particles from damaging the pumping or injection system or clogging the column.

b) Pumps

1. Pumps are required to deliver a constant flow of mobile phase at pressureranging from 1 to 550 bar (14.6 to 8000psi).
2. Mechanical pump give a pulsating supply of mobile phase.
3. Flow rate range: 1 to 10ml/min.

The pump is one of the most important components of HPLC, since its performance directly affects retention time, reproducibility and detector sensitivity. Three main types of pumps are used in HPLC to propel the liquid mobile phase through the system.[6]

a) **Displacement pump**: It produces a flow that tends to independent of viscosity and back pressure and also output is pulse free. But it possesses limited capacity (250 ml).

b) **Reciprocating pump:** It has small internal volume (35 to 400 µl), their high output pressure (up to 10,000 psi) and their constant flow rates. But it produces a pulsed flow.

c) **Pneumatic or constant pressure pump**: They are pulse free; suffer from limited capacity as well as a dependence of flow rate on solvent viscosity and column back pressure. They are limited to pressure less than 2000 psi.

c) Injection systems

Insertion of the sample onto the pressurized column must be as a narrow plug so that the peak broadening attributable to this step is negligible. The injection system itself should have no dead (void) volume.There are three important ways of introducing the sample into injection port.

a) **Loop injection:** In which, a fixed amount of volume is introduced by making useof fixed volume loop injector.

b) **Valve injection:** In which, a variable volume is introduced by making use of aninjection valve.

c) **On column injection:** In which, a variable volume is introduced by means of a syringe through a septum.

d) **Column**: HPLC column are made up of high quality stainless steel, polished internally to a mirror finish. Standard column are 4-5mm in diameter and 10-30 cm in length.

Different types of column that are used include

- **Guard columns:** They are placed anterior to the separating column. They are for protective purpose. They are dependable columns designed to filter or remove particles that clog the separation of the column. These are used in thefollowing cases Compounds and ions that could ultimately cause "baseline drift", decreased resolution, decreased sensitivity and create false peaks.
- **Preparative columns:** These columns are utilized when the objective is to prepare bulk of sample for laboratory applications. Accessories important to mention are the backpressure regulator and the fraction collector. Back- pressure regulator is designed to apply constant pressure to the detector outlet, which prevents the formation of air bubbles

within the system. This, in turn, improves chromatographic baseline stability. It is usually designed to operate regardless of flow rate, mobile phase, or viscosity.

- **Capillary columns:** These are also known as micro columns, capillary columns have a diameter much less than a millimeter and there are three types:

i) Open-tubular
ii) Partially packed
iii) Tightly packed

Micro bore and small-bore columns are also used for analytical and small volume assays. A typical diameter for a small bore column is 1-2 mm. However besides the advantage of smaller sample and mobile phase volume, there is a noted increase in mass sensitivity without significant loss in resolution.

e) Detectors

The detection of the separated compounds in the elute from the column is based upon the bulk property of the elute or the solute property of the individual components.Generally, a detector is selected. That will respond to a particular property of the substance being separated.

a. Bulk property detectors: It compares overall changes in a physical property of the mobile phase with and without an eluting solute. e.g. refractive index, dielectric constant or density.

b. Solute property detectors: It responds to a physical property of the solute which is not exhibited by the pure mobile phase. e.g. UV absorbance, fluorescence or diffusion current. Such detectors are about 1000 times more sensitive giving a detectable signal for a few nanograms of sample.

f) Recorder

Recorders are used to record the responses obtained from detector after Amplification.

1.4.1. Applications of HPLC

1.4.1.1. Analytical HPLC

Here the focus is to obtain information about the sample compound, which includes relative comparison, quantification and resolution of a compound.

1.4.1.2. Preparative HPLC

It refers to the process of isolation and purification of compound. Importanceis the degree of solute purity and the throughput, which is the amount of compound, produced per unit time.

1.4.1.3. Identification

For this purpose a clean peak of known sample assay has to be observed from the chromatogram. Selection of column mobile phase and flow rate matter to certain level in this process by comparing with reference compound does identification and it can be assured by combing two or more detection method.

1.4.1.4. Chemical Separation

This can be accomplished using HPLC by utilizing the fact that, certain compounds have different migration rates given at a particular column and mobile phase. The extent or degree of separation is mostly determined by the choice of stationary phase and mobile phase.

1.4.1.5. Quantification

It is the analyte confirmation by using the known reference standards. Quantification of known and unknown areas with respect to the principle peak by various methods like,

1.4.1.5.1. Area normalization method.
1.4.1.5.2. Internal standard method.
1.4.1.5.3. External standard method.

HPLC is used to estimate the concentration of API as well as dosage formulation.

1.5 Method development by HPLC

The development of a method of analysis of any compound is usually based on existing literature, using same or quite similar instrumentation. But now days HPLC based method is not similar as compare to existing literature based approaches. The development of new or any improved method should be beneficial in any way than existing method. Method development usually requires selecting the method requirement and deciding the instrumentation to utilize for what purpose.

There are different kinds of reasons for developing new method for analysis

- There is no any suitable method for a particular compound or combination of thatcompound.

The exiting method may be too error, contamination prone or may be not well suitable.

a) Hydrolytic conditions

Hydrolysis is one of the most common degradation chemical reactions over a wide range of pH. Hydrolysis is a chemical process that includes decomposition of a chemical compound by reaction with water. Hydrolytic study under acidic and basic condition involves catalysis of ionizable functional groups present in the molecule. Acid or base stress testing involves degradation of a drug substance by exposure to acidic or basic conditions which generates primary degradants in desirable range. The selection of the type and concentrations of acid or base depends on the stability of the drug substance.[7,8] Hydrochloric acid (HCL) or sulfuric acids (0.1–1 M) for acid hydrolysis and sodium hydroxide or potassium hydroxide (0.1–1 M) for base hydrolysis are suggested as suitable reagents for hydrolysis.29, 30 If the compounds for stress testing are poorly soluble in water, then co-solvents can be used to dissolve them in HCl or NaOH. The selection of co-solvent is based on the drug

substance structure. Stress testing trial is normally started at room temperature and if there is no degradation, elevated temperature (50–70°C) is applied. Stress testing should not exceed more than 7 days. The degraded sample is then neutralized using suitable acid, base or buffer, to avoid further decomposition.[9]

b) Oxidation conditions

Hydrogen peroxide is widely used for oxidation of drug substances in forceddegradation studies but other oxidizing agents such as metal ions, oxygen, and radical initiators (e.g. azobisisobutyroni-trile, AIBN) can also be used. Selection of an oxidizing agent, its concentration, and conditions depends on the drug substance. It is reported that subjecting the solutions to 0.1–3% hydrogen per-oxide at neutral pH and room temperature for seven days or up to a maximum 20% degradation couldpotentially generate relevant degradation products.[10] The oxidative degradation of drug substance involves an electron transfer mechanism to form reactive anions and cations. Amines, sulfides and phenols are susceptible to electron transfer oxidation to give N-oxides, hydroxylamine, sulfones and sulfoxide.32The functional group with labile hydrogen like benzylic carbon, allylic carbon, and tertiary carbon or α-positions with respect to hetero atomis susceptible to oxidation to form hydro peroxides, hydroxide or ketone.[11]

c) Photolytic conditions

The photo stability testing of drug substances must be evaluated to demonstrate that a light exposure does not result in unacceptable change. Photo stability studies are performed to generate primary degradants of drug substance by exposure to UV or fluorescent conditions. The most commonly accepted wavelength of light is in the range of 300– 800 nm to cause the photolytic degradation.35 Light stress conditions can induce photo oxidation by free radical mechanism. Functional groups like carbonyls, nitro aromatic, N-oxide, alkenes, aryl chlorides, weak C–H and O–H bonds, sulfides and polyenes are likely to introduce drug photosensitivity.[12,13]

d) Thermal conditions

Thermal degradation (e.g., dry heat and wet heat) should be carried out at more strenuous conditions than recommended ICH Q1A accelerated testing conditions.**[**36**]**Samples of solid-state drug sub-stances and drug products should be exposed to dry and wet heat, while liquid drug products should be exposed to dry heat. Studies may be conducted at higher temperatures for a shorter period. Effect of temperature on thermal degradation of a substance is studied through the Arrhenius equation:

K ¼ Ae _Ea=RT

Where, k - is specific reaction rate,

A is frequency factor,

Ea - is energy of activation,

R - is gas constant (1.987 cal/deg mole) and

T - is absolute temperature.

Thermal degradation study is carried out at 40–80ºC.

2. Materials and Methods

2.1. Drug

Table 2.1: Drug and drug product samples suppliers and manufacturers.

Name of drug and product	Supplier and manufacturer by
Azelnidipine	J B chemicals & pharmaceuticals ltd. mumbai
Azelnidipine Tablet 16 mg	J B chemicals & pharmaceuticals ltd. mumbai

2.2. Reagents

Table 2.2: List of Reagent.

Sr.No	Chemical	Make
1	Water	Rankem
2	Methanol	Merck life science
3	Phosphoric acid 88%	Merck life science
4	Potassium dihydrogen phosphate	Merck life science
5	Hydrochloric Acid	Merck life science
6	Sodium Hydroxide	Merck life science
7	Peroxide Solution	Merck life science
8	0.45 µ Nylon membrane disc filter	Mdi

2.3. Instruments

2.3.1. HPLC

Make	Waters e2695
Pump	Reciprocating Water-510
Detector	Waters 2998 (PDA)
Software	Empower PRO

2.3.2. Spectrophotometer: Double beam UV-visible spectrophotometer with 10mm Matched quartz cells.

Model	UV1900i
Make	shimadzu

2.3.3. Analytical Balance: Digital Analytical balance.

Model	XSE205DU
Make	Mettler Toledo

2.4. Ph Meter: Digital pH Meter.

Make	Thermo Scientific
Model	Orian Star A211

2.5. Uv Spectroscopic Method

2.5.1.1. Selection of solvent

Prepare a mixture of Water and Methanol in the ratio 10:90 v/v respectively and mix. Sonicate to degas.Used as solvent for dissolving azelnidipine.

2.5.1.2. Selection Of Wavelength Preparation of standard solution

An accurately weighed quantity about 27 mg of Azelnidipinestandard was transferred to 250 mL volumetric flask. Add 200 mL of diluent, sonicate to

dissolve and dilute up to the mark with diluent and mixed.

2.5.1.3. Determination Of Λ Max (Selection Of Wavelength)

The standard solutions were scanned separately between 400nm to 200nm. From thespectrum at 255 nm show high absorbance, so 255 nm λ max of azelnidipinewere selected for estimation drugs.

3. RESULT AND DISCUSSION

The stability indicating RP-HPLC method was developed on the base of physical and chemical properties of drug molecule and validated as per ICH guidelines by using variousvalidation parameters such as Linearity, accuracy, precision, specificity and robustness.

3.1. Selection of Wavelength

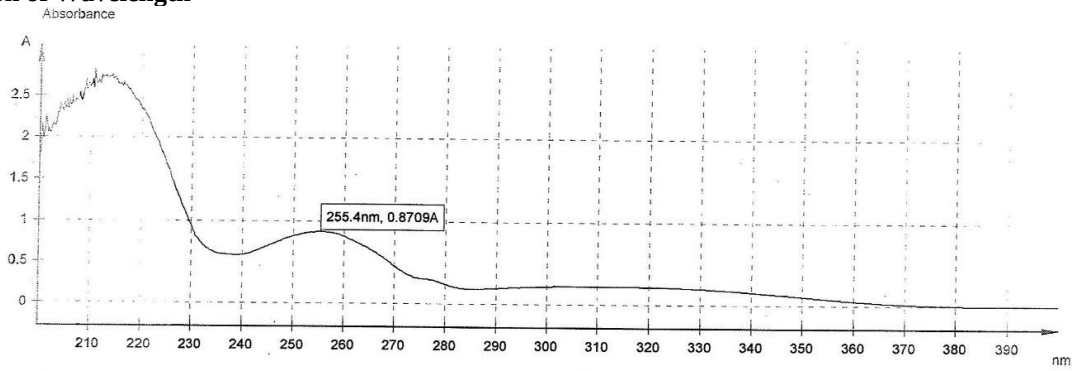


Figure 3.1 Spectra showing λ max of Azelnidipine.

Table 7.1: Determination of λ max of Azelnidipine.

Sr. No.	Wavelength (nm)	Absorbance
1.	255	0.8709 A

3.1.1. Reverse

Different trials taken were as follows

Trial: 1

Chromatographic Conditions

Mobile Phase	:	**Water: Methanol**
Mobile Phase ratio	:	70:30
Column	:	Zodiac C18, 150 mm, 4.6 mm 3 µm
Flow Rate	:	1.0 mL/min
Injection Volume	:	5 µL
Wavelength	:	255 nm
Column Temp	:	25°C
Sample Temp	:	25°C
Run time	:	15 min

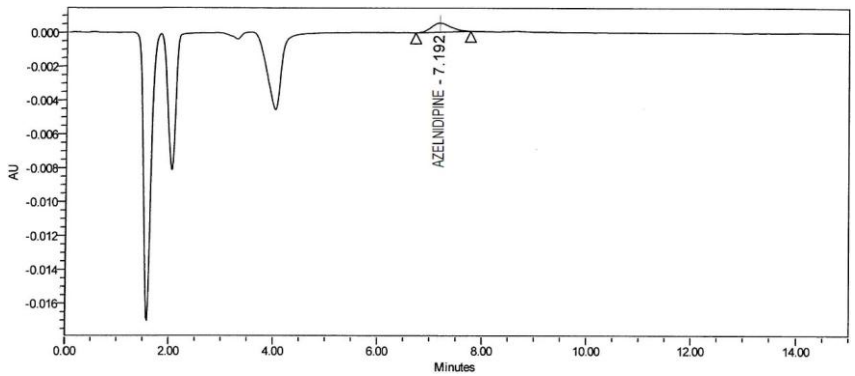


Fig. 3.2: Typical chromatogram for Trial- 1.

Conclusion

The azelnidipine peak in chromatogram observed at 7.1 min. the base line in negative side observed. Hence method need to optimize.

Trial: 2

Chromatographic Condition

Mobile Phase	:	**Water: Methanol**

Mobile Phase ratio	:	70:30
Column	:	Zodiac C18, 250 mm, 4.6 mm 5 µm
Flow Rate	:	1.0 mL/min
Injection Volume	:	10 µL
Wavelength	:	255 nm
Column Temp	:	25°C
Sample Temp	:	25°C
Run time	:	25 min

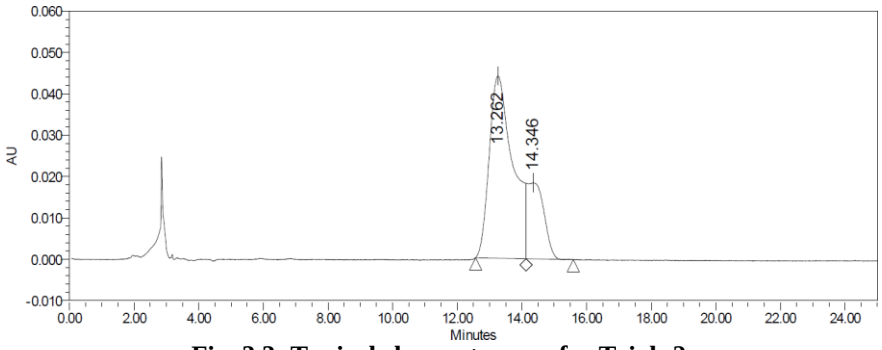


Fig. 3.3: Typical chromatogram for Trial- 2.

Conclusion:-The azelnidipine peak in chromatogram observed at 13.2 min. the system suitability failed. Hence method need to optimize.

Trial: 3

Preparation of Buffer pH 3.2

Dissolve 2.73 g of potassium dihydrogen ortho-phosphate in 1000 mL of water and mix well. Adjust to pH 3.2 ± 0.05 using diluted orthophosphoric acid. Filter the solution through a 0.45µm nylon membrane filter.

Chromatographic Condition

Mobile Phase	:	**Buffer pH 3.2:Methanol**
Mobile Phase ratio	:	30:70
Column	:	Inertsil ODS-3V, 150 x 4.6 mm, 5µ
Flow Rate	:	1.0 mL/min
Injection Volume	:	10 µL
Wavelength	:	255 nm
Column Temp	:	25°C
Sample Temp	:	25°C
Run time	:	25 min

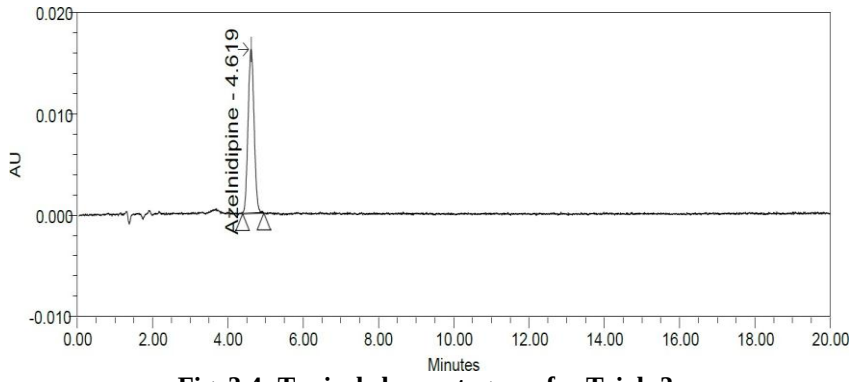


Fig. 3.4: Typical chromatogram for Trial- 3.

Conclusion:-The azelnidipine peak in chromatogram observed at 4.6 min. the peakresponse need to increase to optimum response. Hence method need to optimize.

Trial: 4

Chromatographic Condition

Mobile Phase	:	**Buffer pH 3.2: Methanol**

Mobile Phase ratio	:	30:70
Column	:	Inertsil ODS-3V, 250 x 4.6 mm, 5µ
Flow Rate	:	1.0 mL/min
Injection Volume	:	20 µL
Wavelength	:	255 nm
Column Temp	:	25°C
Sample Temp	:	25°C
Run time	:	25 min

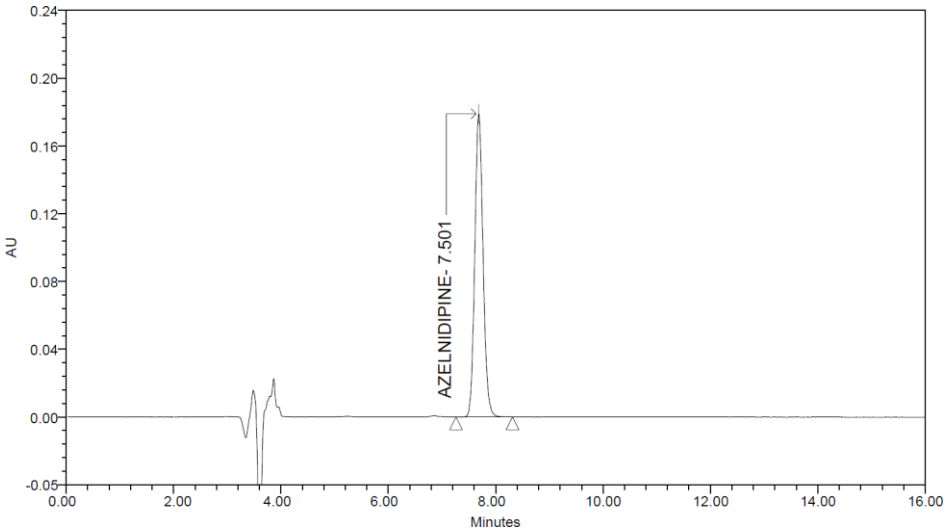


Fig. 3.5: Typical chromatogram for Trial- 4.

Conclusion:- the trial taken base to trial 3 to increase response injection volume increase column dimension change. The azelnidipine peak in chromatogram observed at 7.5 min. the retention time need to decrease. Hence method need to optimize.

Trial: 5

Chromatographic Condition

Column	**:**	**Inertsil ODS-3V, 150 x 4.6 mm, 5µ**
Flow Rate	:	1.0 mL/min
Injection Volume	:	20 µL
Wavelength	:	255 nm
Column Temp	:	35°C
Sample Temp	:	10°C
Run Time	:	8.0 minute
Retention Time	:	About 3.8 minutes

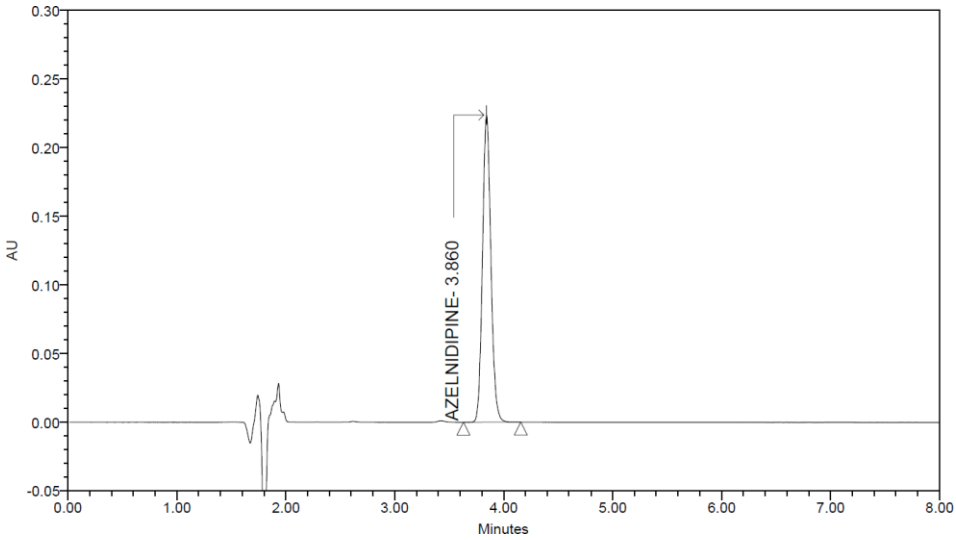


Fig. 3.6: Typical chromatogram for Trial- 5.

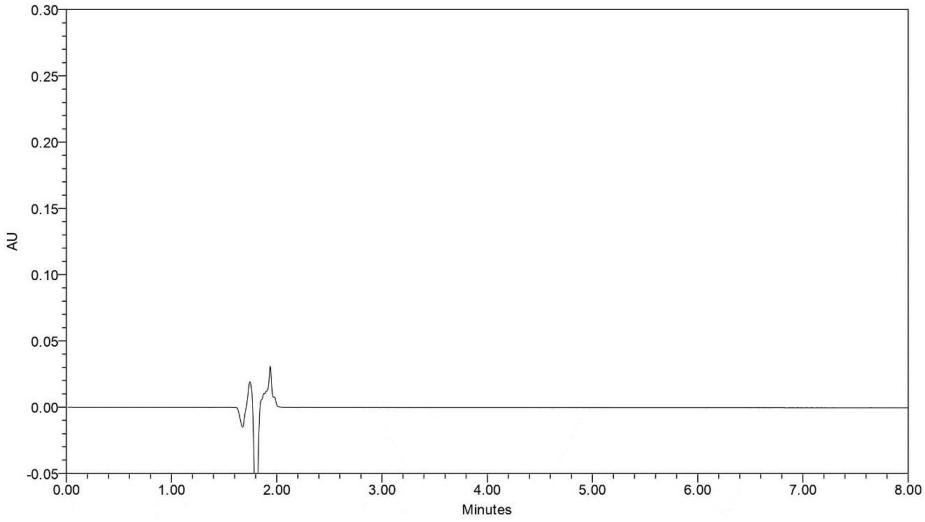
Conclusion:-On the base of all development trial by optimizing mobile phase ratio and column dimension. azelnidipine peak observed at retention time of 3.8 the system suitability of analyte like theoretical plates, tailing factor and resolution between two analyte meeting criteria. Hence development trial 5 considered as final analytical method.

Specificity: (Identification, Interference & Peak Purity)

Inject Blank (Diluent), standard solution, placebo solution and sample solution. The data obtained is summarized in Table.

Table 7.4 Specificity (Identification and Interference)

Component	Retention time (min)	Theoretical Plates	Tailing Factor	Purity angle	Purity threshold
Blank (Diluent)	-	-	-	-	-
Placebo Solution	-	-	-	-	-
Standard solution	3.847	15600	1.0	0.8	1.6
Sample solution	3.842	15308	1.0	0.9	1.9



Chromatogram of Blank

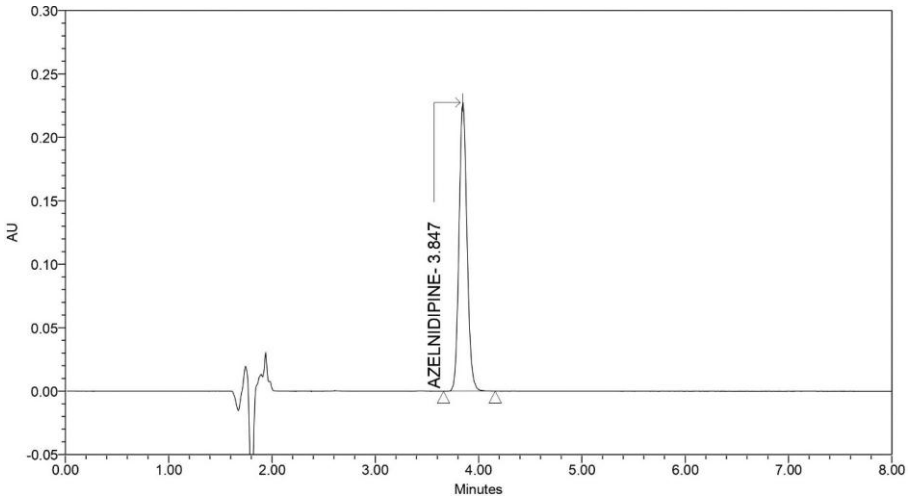


Fig 7.9 Chromatogram of Standard.

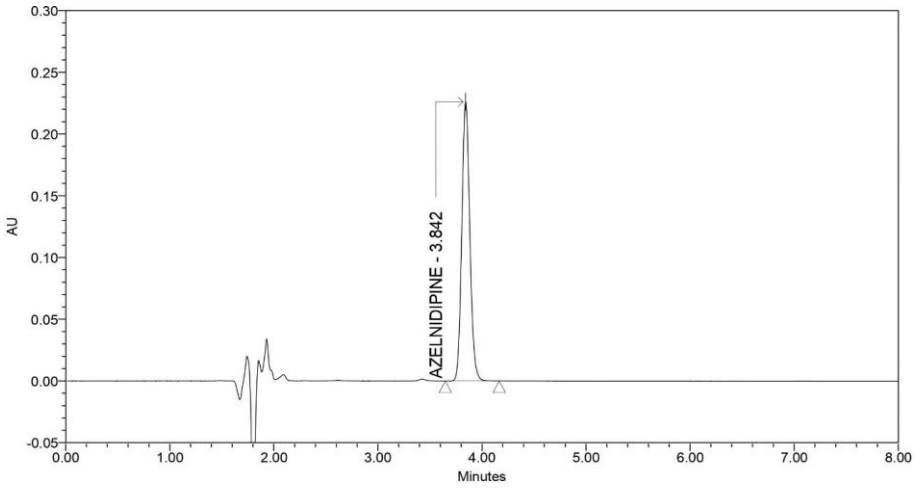


Fig 3.7: Chromatogram of Sample.

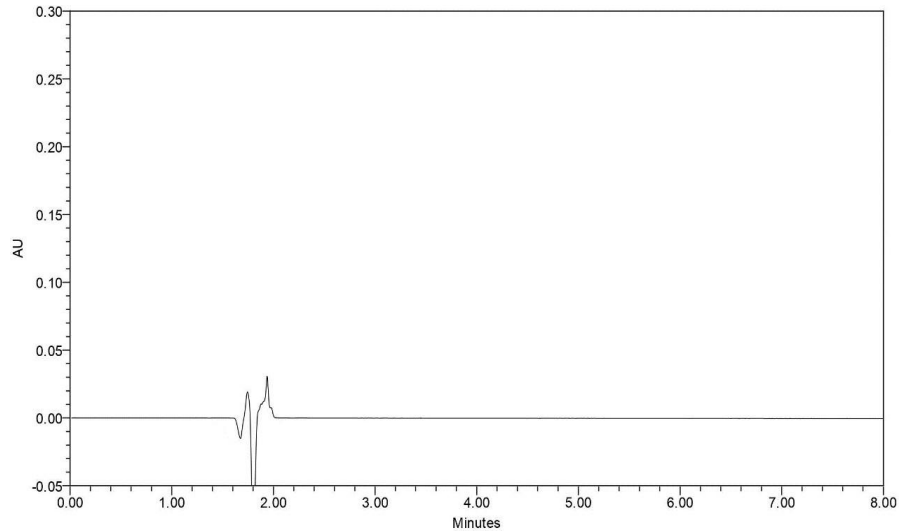


Fig 3.8: Chromatogram of Placebo.

Conclusion

The data demonstrates that retention time in standard and sample is same for azelnidipine peak. The data demonstrates that there is no interference in Blank and Placebo at theretention time of azelnidipine peak. Peak Purity match in both chromatograms obtained from Standard and Samplesolution.

Linearity

Linearity was evaluated in the range of 50% to 150% of

Azelnidipine for working concentration. The working concentration of Azelnidipine is 40 ppm.

Table 3.3: Linearity of Azelnidipine.

Level (%)	Concentration w.r.t sample (mg/mL)	Peak Area Injection - 1	Peak Area Injection - 2	Mean Peak Area
50	20	369854	371399	370627
75	30	558241	554928	556585
100	40	742160	743629	742895
125	50	930312	928634	929473
150	60	1123694	1099936	1111815
Correlation Co-coefficient (R)				0.9993
Slope of regression line				18423.674
%Y- intercept				0.02

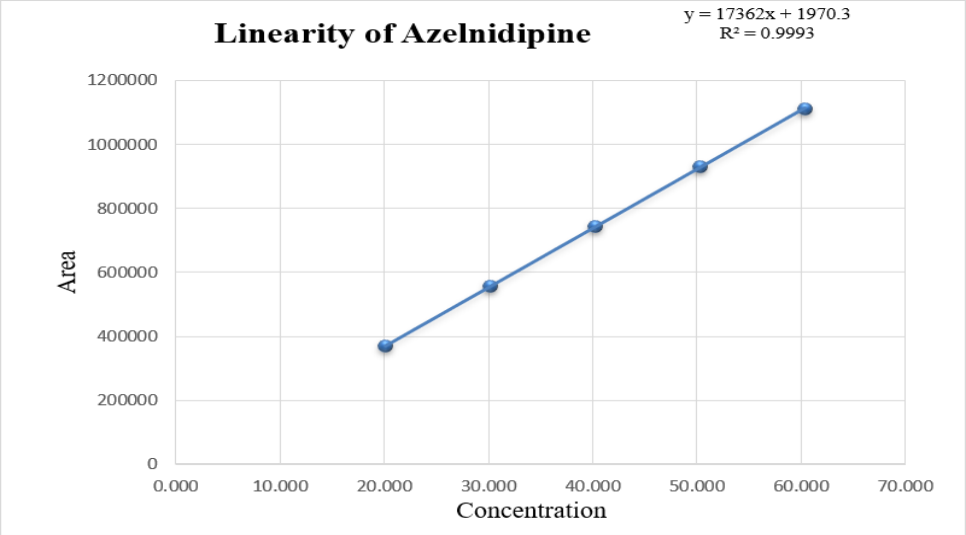


Fig 3.9: Linearity plot of Azelnidipine.

Conclusion

The data shows that system suitability is fulfilled. The data shows that the response is found to be linear.

% Limit of Y- Intercept is within ± 2.0% of the corresponding Y-co-ordinate of theworking level.

Forced Degradation

Table 3.4: Force Degradation for Azelnidipine.

Reagents	Conditions	% Assay	% difference degradation	Purity angle	Purity threshold
Control	As Such	100.2	NA	1.0	1.8
Acid	5 mL, 0.1 N HCl for 5 Hrs atbench top	92.8	7.3	1.2	1.9
Base	5 mL, 0.1 N NaOH for 5 Hrsat bench top	57.7	42.4	1.3	2.4
Thermal	80 ºC for 24 Hrs	83.3	16.8	1.1	1.9
Peroxide	5 mL 5% H_2O_2 for 3 Hrs.	89.6	10.5	1.2	2.0
Photo-Open	1.2 million luxHrs	87.4	12.7	0.9	1.9

Photo Degradation

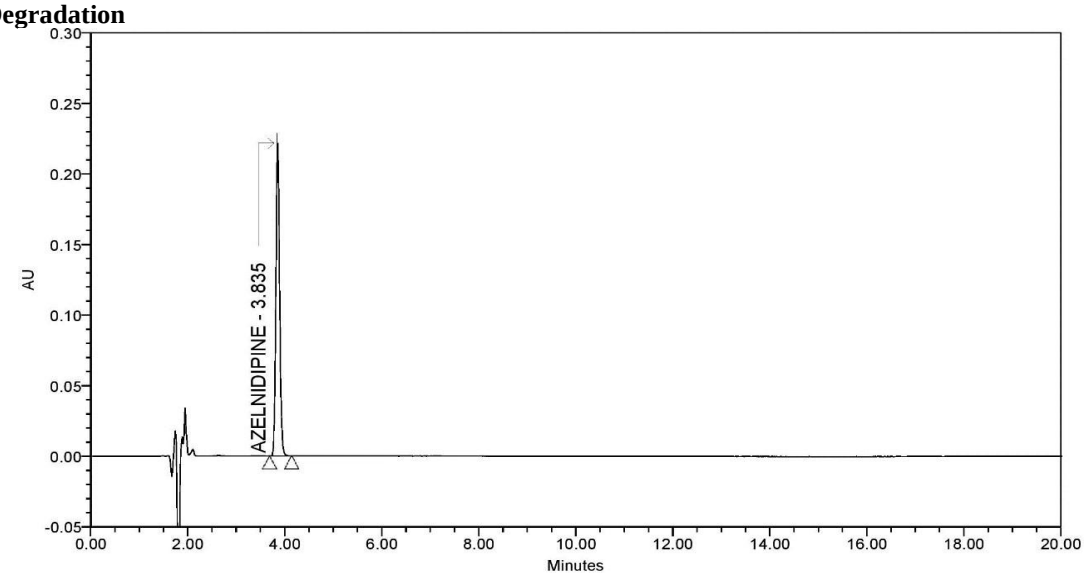


Fig.3.10: Photo Degradation of Azelnidipinetablet (open).

Thermal degradation

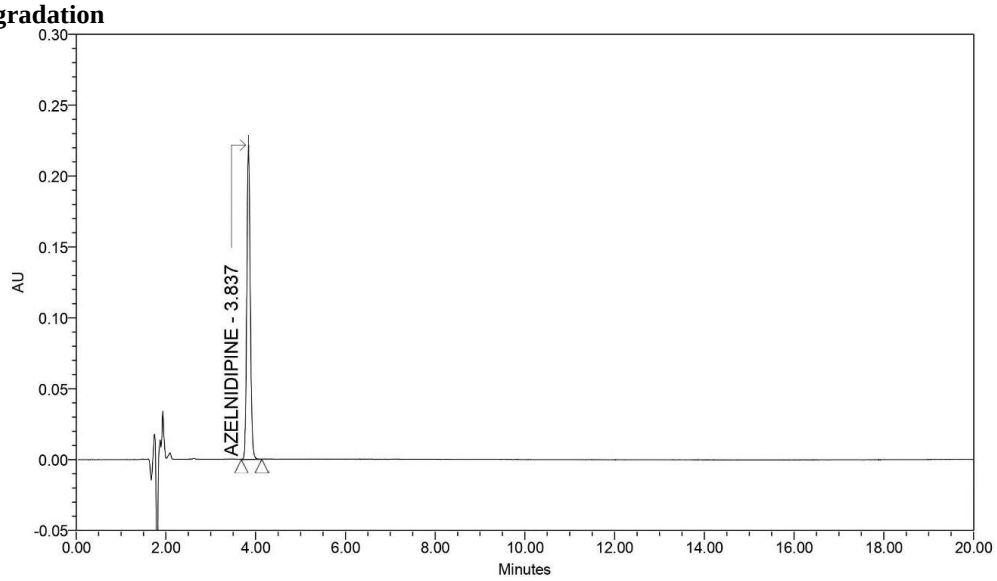


Fig.3.11: Thermal degradation of Azelnidipine tablet.

Acid Degradation

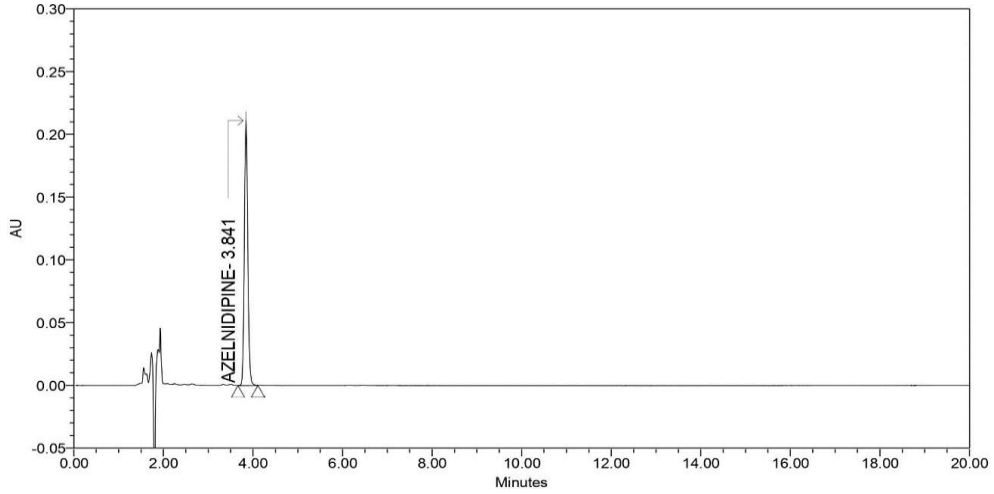


Fig. 3.12: Acidic Degradation of Azelnidipine tablet.

4. CONCLUSION

The stability indicating RP-HPLC method was found to be simple, rapid, sensitive, specific, precise, accurate and cost effective for estimation of azelnidipine in tablet dosageform and bulkdrugs substance. This technique was employed in the present investigation for estimation of Azelnidipine using HPLC with Inertsil ODS-3V, 150 x 4.6 mm, 5µ column, UV/PDA detector at 255 nm wavelength, flow rate was 1 ml/min, injection volume with empower Software was used for the study. The standard and sample solution of azelnidipine were prepared in diluent. the column make injection volume and different pure solvents of varying polarity in different proportions were tried as mobile phase for development of the chromatogram. The mobile phase that was found to be most suitable was Buffer pH 3.2 and Methanol, the wavelength 255 nm were selected for the evaluation of the chromatogram of azelnidipine respectively. The selection of the wavelength was based on the λ max obtained by scanning of standard laboratory mixture in water: methanol. This selected chromatographic condition gave good resolutionand optimum retention time with appropriate theoretical plates, tailing factor. The force degradation was performed by using acid, base, thermal, peroxide and photo-open stress condition the drug molecule degraded in stress condition to establish specificity of developed method. it was observed that Azelnidipine found stable under Acid Thermal, oxidation, photolytic condition and degradation observed in alkali stress condition. Hence fromthe obtained result it can be concluded that the developed stability indicating RP-HPLC method is employed successfully for the estimation of azelnidipine in tablet dosage form and bulk drugs substance.

The practically taken development trails results and validation of developed RP-HPLC results with performed forced degradation result from table clearly indicate that the RP- HPLC technique can be successfully applied for the estimation of azelnidipine in tablet dosage form and bulk drugs substance.

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