Research Article

Estimation of Benedipine by using HPLC

M. Sravani

Sri Vani School of Pharmacy, Chevuturu, Andhra Pradesh, India.

ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Benidipine, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Symmetry C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol and water (50:50% v/v) as the mobile phase at a flow rate of 0.8ml/min, the detection was carried out at 260nm. The retention time of the Benidipine was 2.379 ±0.02min. The method produce linear responses in the concentration range of 10-50ppm of Benidipine. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Benidipine, RP-HPLC, validation.

INTRODUCTION

Analytical techniques There are numerous chemical or physico-chemical processes that can be used to provide analytical information. The processes are related to a wide range of atomic and molecular properties and phenomena that enable elements and compounds to be detected and/or quantitatively measured under controlled conditions. The underlying processes define the various *analytical techniques*. The more important of these are listed in Table.No.1 together with their suitability for qualitative, quantitative or structural analysis and the levels of analyte(s) in a sample that can be measured. *Atomic, molecular spectrometry* and *chromatography*, which together comprise the largest and most widely used groups of techniques, can be further subdivided according to their physico-chemical basis. *Spectrometric techniques* may involve either the *emission or absorption* of *electromagnetic radiation* over a very wide range of energies, and can provide qualitative, quantitative and structural information for analytes from major components of a sample down to ultra-trace levels. The most important atomic and molecular spectrometric techniques and their principal applications are listed in Table.No.2. *Chromatographic techniques* provide the means of separating the components of mixtures and simultaneous qualitative and quantitative analysis, as required. The linking of chromatographic and spectrometric techniques, called *hyphenation*, provides a powerful means of separating and identifying unknown compounds. *Electrophoresis's* another separation technique with similarities to chromatography that is particularly useful for this parathion of charged species. The principal separation techniques and their applications are listed in Table.No.3.

Analytical methods

An analytical method consists of a detailed, stepwise list of instructions to be followed in the qualitative, quantitative or structural analysis of a sample for one or more analytes and using a specified technique. It will include a summary and lists of chemicals and reagents to be used, laboratory apparatus and glassware, and appropriate instrumentation. The quality and sources of chemicals, including solvents, and the required performance characteristics of instruments will also be specified as will the procedure for obtaining a representative sample of the material to be analyzed. This is of crucial importance in obtaining meaningful results. The preparation or pre-treatment of the sample will be followed by any necessary standardization of reagents and/or calibration of instruments under specified conditions. Qualitative tests for the analyte(s) or quantitative measurements under the same conditions as those used for standards complete the practical part of the method. The remaining steps will be concerned with data processing, computational methods for quantitative analysis and the formatting of the analytical report. The statistical assessment of quantitative data is vital in establishing the reliability and value of the data, and the use of various statistical parameters and tests is widespread. Many *standard analytical methods* have been published as papers in analytical journals and other scientific literature, and in textbook form. Collections by trades associations representing, for example, the cosmetics, food, iron and steel, pharmaceutical, polymer plastics and paint, and water industries are available standards

organizations and statutory authorities, instrument manufacturer's applications notes, the Royal Society of Chemistry and the US Environmental Protection Agency are also valuable sources of standard methods. Often, laboratories will develop their own *in-house methods* or adapt existing ones for specific purposes.

Method development forms a significant part of the work of most analytical laboratories, and *method validation and* periodic revalidation is a necessity. Selection of the most appropriate analytical method should take into account the following factors:

- The purpose of the analysis, the required time scale and any cost constraints;
- The level of Analyte(s) expected and the detection limit required;
- The nature of the sample, the amount available and the necessary sample preparation procedure;
- The accuracy required for a quantitative analysis;
- The availability of reference materials, standards, chemicals and solvents, instrumentation and any special facilities;
- Possible interference with the detection or quantitative measurement of the analyte(s) and the possible need for sample clean-up to avoid matrix interference;
- The degree of selectivity available − methods may be selective for a small number of analytes or specific for only one.
- Quality control and safety factors.

Table 1: Analytical techniques and principle applications

Technique	Property measured	Principle areas of application
Gravimetry	Weight of pure analyte or compound of known as stoichiometry	Quantitative for major or minor components
Titrimetry	Volume of standard reagent solution reacting with the analyte	Quantitative for major or minor Component
Atomic molecular spectrometry	Wavelength and intensity of electromagnetic radiation emitted/ absorbed by the analyte	Qualitative, quantitative or structural or for major down to trace level components
Mass spectrometry	Mass of analyte or fragments of it	Qualitative or structural for major down to trace level components isotope ratios
Chromatography and electrophoresis	Various physicochemical properties of separated analytes	Qualitative and quantitative separations of mixtures at major to trace levels
Thermal analysis	Chemical/physical changes in the analyte when heated or cooled	Characterization of single or mixed major/minor compounds
Electrochemical analysis	Electrical properties of the analyte in solution	Qualitative and quantitative for major to trace level components
Radiochemical analysis	Characteristic ionizing nuclear radiation emitted by the analyte	Qualitative and quantitative at major to trace levels

Table 2: Spectrometric Techniques and Principle Applications

Technique	Basis	Principle applications
Plasma emission spectrometry	Atomic emission after excitation in high temperature gas plasma	Determination of metals and some non-metals mainly at trace levels
Flame emission spectrometry	Atomic emission after flame excitation	Determination of alkali and alkaline earth metals
Atomic absorption spectrometry	Atomic absorption after atomization by flame or electro thermal means	Determination of trace metals and some non-metals
Atomic fluorescence spectrometry	Atomic fluorescence emission after flame excitation	Determination of mercury and hydrides of non-metals at trace levels
X-ray emission spectrometry	Atomic or atomic fluorescence emission after excitation by electrons or radiation	Determination of major and minor elemental components of metallurgical and geological samples
-spectrometry	-ray emission after nuclear excitation	Monitoring of radioactive elements in environmental samples
Ultraviolet/visible spectrometry	Electronic molecular absorption in solution	Quantitative determination of unsaturated organic
Infrared spectrometry	Vibrational molecular absorption	Identification of organic compounds
Nuclear magnetic resonance spectrometry	Nuclear absorption (change of spin states)	Identification and structural analysis of organic compounds
Mass spectrometry	Ionization and fragmentation of molecules	Identification and structural analysis of organic compounds

Table 3: Separation techniques and principle applications

Technique	Basis	Principle applications
Thin-layer chromatography	Differential rates of migration of analytes through a stationary phase by movement of a liquid or gaseous mobile phase	Qualitative analysis of mixtures
Gas chromatography	-Do-	Quantitative and qualitative determination of volatile compounds
High-performance liquid chromatography	-Do-	Quantitative and qualitative determination of non-volatile compounds
Electrophoresis	Differential rates of migration of analytes through a buffered medium	Quantitative and qualitative determination of ionic compounds

Chromatographic Process[4]

Chromatographic separations are based on a forced transport of the liquid (mobile phase) carrying the analyte mixture through the porous media and the differences in the interactions at analytes with the surface of this porous media resulting in different migration times for a mixture components. In the above definition the presence of two different phases is stated and consequently there is an interface between them. One of these phases provides the analyte transport and is usually referred to as the mobile phase, and the other phase is immobile and is typically referred to as the stationary phase. A mixture of components, usually called analytes, are dispersed in the mobile phase at the molecular level allowing for their uniform transport and interactions with the mobile and stationary phases. High surface area of the interface between mobile and stationary phases is essential for space discrimination of different components in the mixture. Analyte molecules undergo multiple phase transitions between mobile phase and adsorbent surface. Average residence time of the molecule on the stationary phase surface is dependent on the interaction energy. For different molecules with very small interaction energy difference the presence of significant surface is critical since the higher the number of phase transitions that analyte molecules undergo while moving through the chromatographic column, the higher the difference in their retention. The nature of the stationary and the mobile phases, together with the mode of the transport through the column, is the basis for the classification of chromatographic methods.

Types of Chromatography

The mobile phase could be either a liquid or a gas, and accordingly we can subdivide chromatography into Liquid Chromatography (LC) or Gas Chromatography (GC). Apart from these methods, there are two other modes that use a liquid mobile phase, but the nature of its transport through the porous stationary phase is in the form of either (a) capillary forces, as in planar chromatography (also called Thin-Layer Chromatography, TLC), or (b) electro osmotic flow, as in the case of Capillary Electro Chromatography (CEC).

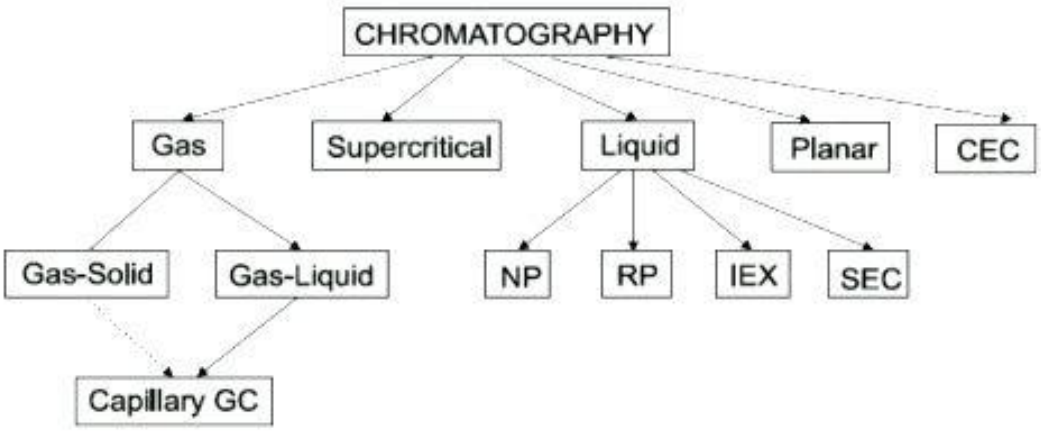


Fig.: Showing flow chart for classification of chromatography[4]

Table 4: Principles and classification of chromatography

Technique	Stationary Phase	Mobile Phase	Format	Principle sorption mechanism
Paper chromatography (PC)	Paper (cellulose)	Liquid	Planar	Partition (adsorption, ion-exchange, exclusion)
Thin-layer chromatography (TLC)	Silica, cellulose, ion-exchange, resin, controlled porosity solid	Liquid	Planar	Adsorption (partition, ion-exchange, exclusion)
Gas chromatography (GC)				
Gas-liquid chromatography (GLC)	Liquid	Gas	Column	Partition
Gas-solid chromatography (GSC)	Solid	Gas	Column	Adsorption
Liquid Chromatography (LC)				
High Performance Liquid Chromatography (HPLC)	Solid or bonded-phase	Liquid	Column	Modified partition (adsorption)
Size-Exclusion Chromatography (SEC)	Controlled porosity solid	Liquid	Column	Exclusion
Ion-Exchange Chromatography (IEC), Ion Chromatography (IC)	Ion-exchange resin or bonded-phase	Liquid	Column	Ion-exchange
Chiral Chromatography (CC)	Solid chiral Selector	Liquid	Column	Selective adsorption

ANALYTICAL METHOD VALIDATION

Method validation can be defined as per ICH "Establishing documented evidence which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics".

ICH Method validation parameters[18-19]

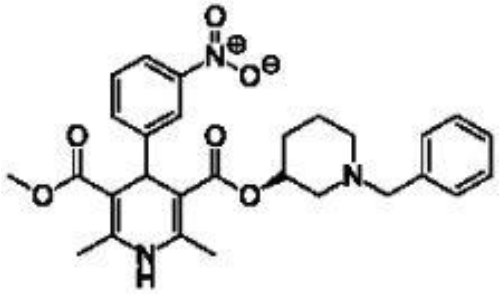
For chromatographic methods used in analytical applications there is more consistency in validation. Related substances are commonly present in the pharmaceutical products but those are always within the limits as specified in ICH (Q2B).

- Specificity
- Linearity
- Accuracy
- Precision
- Limit of Detection
- Limit of Quantitation
- Robustness
- System suitability

DRUG PROFILE

Drug : Benidipine
Drug category : Dihydropyridine calcium channel blocker

Structure :



Chemical name/ Nomenclature / IUPAC Name : O5-methyl O3-[(3R)-1-(phenylmethyl)piperidin-3-yl] 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate
Molecular Formula : $C_{28}H_{31}N_3O_6$
Molecular Weight : 505.57 g·mol−1

PHYSICOCHEMICAL PROPERTIES:
Description(Physical State): Solid, Crystalline powder
Solubility: Soluble in Ethanol, Methanol and Water
Storage Conditions: Store it at room temperature.
Melting point: 209-215 °C
PHARMACOKINETIC PROPERTIES:
Metabolism : Hepatic,
Metabolites : N-desbenzylbenidipine & dehydrobenidipine metabolites
Adverse effects/Side effects :

- Heart - Palpitations, facial flushing, hot flushes and chest pressure.
- Central Nervous System - Sensation, headache, dizziness, sleepiness and uneasiness.
  Gastrointestinal - Constipation, nausea and abdominal discomfort.
- ENT - Ringing in the ear.
- Musculoskeletal - Redness, warm feeling in the fingers and stiffness in shoulders.
- Genitourinary - Urinary frequency**.**
- Hypersensitive reactions - Rash and itching.
- Metabolic - Elevated liver enzyme.

PHARMACODYNAMICS:
Mechanism of action: Benidipine is a calcium channel blocker.
Benidipine has additionally been found to act as an antagonist of the mineralocorticoid receptor, or as an antimineralocorticoid.
Therapeutic efficacy/ Indications:
This medication is a calcium channel blocker, prescribed for high blood pressure and chest pain.

DRUG FORMULATION

S.No	Drug name	Label Claim	Brand name	Company	Formulation Type
1	Benidipine	8mg	Coniel	DEVA	tablet

METHODOLOGY
HPLC METHOD DEVELOPMENT
TRAILS
Preparation of standard solution:
Accurately weigh and transfer 10 mg of Benidipine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.
Further pipette 0.3ml of the above Benidipine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.
Procedure:
Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.
Mobile Phase Optimization:
Initially the mobile phase tried was methanol: Water and Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Methanol and Water in proportion 50:50 v/v respectively.
Optimization of Column:
The method was performed with various C18 columns like ODS column, Xterra, and X Bridge C18 column. Symmetry C18 (4.6 x 150mm, 5□m) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:
Instrument used : Waters HPLC with auto sampler and PDA detector 996 model.
Temperature : 40ºC
Column : Symmetry C18 (4.6 x 150mm, 5□m)
Mobile phase : Methanol: Water (50:50% v/v)
Flow rate : 0.8ml/min
Wavelength : 260nm
Injection volume : 10µl
Run time : 6minutes

VALIDATION

Preparation of mobile phase:

Accurately measured 500ml (50%) of HPLC Methanol and 500 ml of HPLC Water (50%) were mixed and degassed in a digital ultrasonicator for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

VALIDATION PARAMETERS

SYSTEM SUITABILITY

Accurately weigh and transfer 10 mg of Benidipine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3ml of the above Benidipine stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

SPECIFICITY STUDY OF DRUG:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Benidipine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3ml of the above Benidipine stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution:

Take average weight of the Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Benidipine sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.3ml of Benidipine above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

PREPARATION OF DRUG SOLUTIONS FOR LINEARITY:

Accurately weigh and transfer 10 mg of Benidipine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (10ppm of Benidipine):

Pipette out 0.1ml of stock solution in to a 10ml volumetric flask and make up the volume up to mark by using diluent.

Preparation of Level – II (20ppm of Benidipine):

Pipette out 0.2ml of stock solution in to a 10ml volumetric flask and make up the volume up to mark by using diluent.

Preparation of Level – III (30ppm of Benidipine):

Pipette out 0.3ml of stock solution in to a 10ml volumetric flask and make up the volume up to mark by using diluent.

Preparation of Level – IV (40ppm of Benidipine):

Pipette out 0.4ml of stock solution in to a 10ml volumetric flask and make up the volume up to mark by using diluent.

Preparation of Level – V (50ppm of Benidipine):
Pipette out 0.5ml of stock solution in to a 10ml volumetric flask and make up the volume up to mark by using diluent.
Procedure:
Inject each level into the chromatographic system and measure the peak area.
Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

PRECISION
REPEATABILITY
Preparation of Benidipine Product Solution for Precision:
Accurately weigh and transfer 10 mg of Benidipine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.3ml of the above Benidipine stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.
The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

INTERMEDIATE PRECISION:
To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.
Procedure:
Analyst 1:
The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.
Analyst 2:
The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.
Accuracy:
For preparation of 50% Standard stock solution:
Accurately weigh and transfer 10 mg of Benidipine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.15ml of the above Benidipine stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.
For preparation of 100% Standard stock solution:
Accurately weigh and transfer 10 mg of Benidipine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.3ml of the above Benidipine stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.
For preparation of 150% Standard stock solution:
Accurately weigh and transfer 10 mg of Benidipine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.45ml of the above Benidipine stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.
Procedure:
Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Benidipine and calculate the individual recovery and mean recovery values.

ROBUSTNESS:
The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

For preparation of Standard solution:
Accurately weigh and transfer 10 mg of Benidipine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.3ml of the above Benidipine stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.
Effect of Variation of flow conditions:
The sample was analyzed at 0.7 ml/min and 0.9 ml/min instead of 0.8ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded
Effect of Variation of mobile phase organic composition:
The sample was analyzed by variation of mobile phase i.e. Methanol: Water was taken in the ratio and 45:55, 55:45 instead of 50:50, remaining conditions are same.10µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION

Trails

Trail 1:
Column : SymmetryC18 (4.6 x 250mm, 5µm)
Column temperature : 35ºC
Wavelength : 226 nm
Mobile phase ratio : Methanol: water (90:10% V/V)
Flow rate : 1mL/min
Injection volume : 10 µl
Run time : 10 minutes

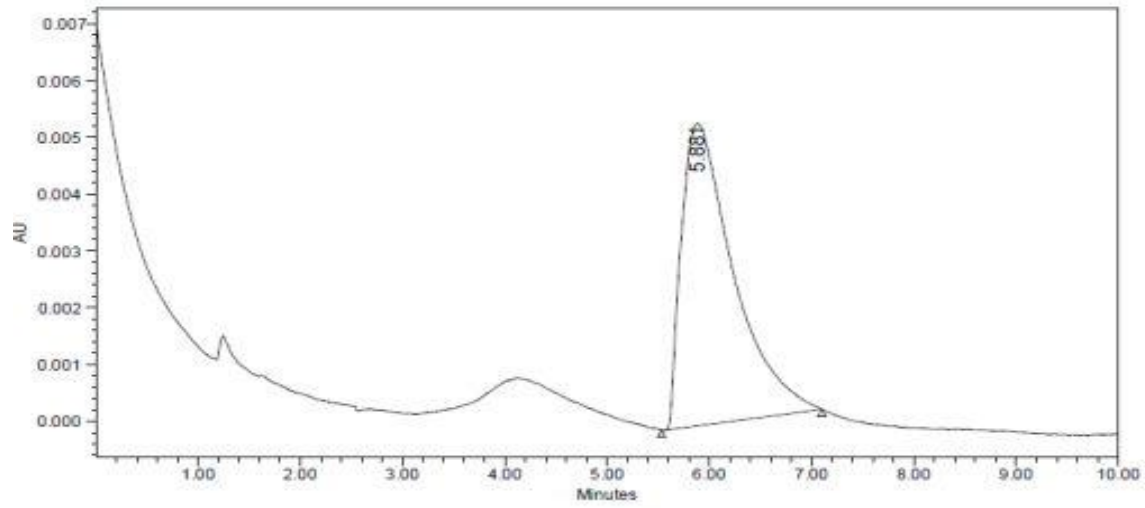


Fig.: chromatogram for trail 1

Table: peak results for trail 1

S.No	Peak Name	R_t	Area	Height	USP Tailing	USP Plate count
1	Benidipine	5.881	940480	26622		591

Observation:
This trial doesn't show proper base line and plate count in the chromatogram, so more trials were required for obtaining good peaks.

Trail 2:
Column : SymmetryC18 (4.6 x 150mm, 5µm)
Column temperature : 35ºC
Wavelength : 226 nm
Mobile phase ratio : Methanol: Water (80:20% v/v)
Flow rate : 1 mL/min
Injection volume : 10 µl
Run time : 9 minutes

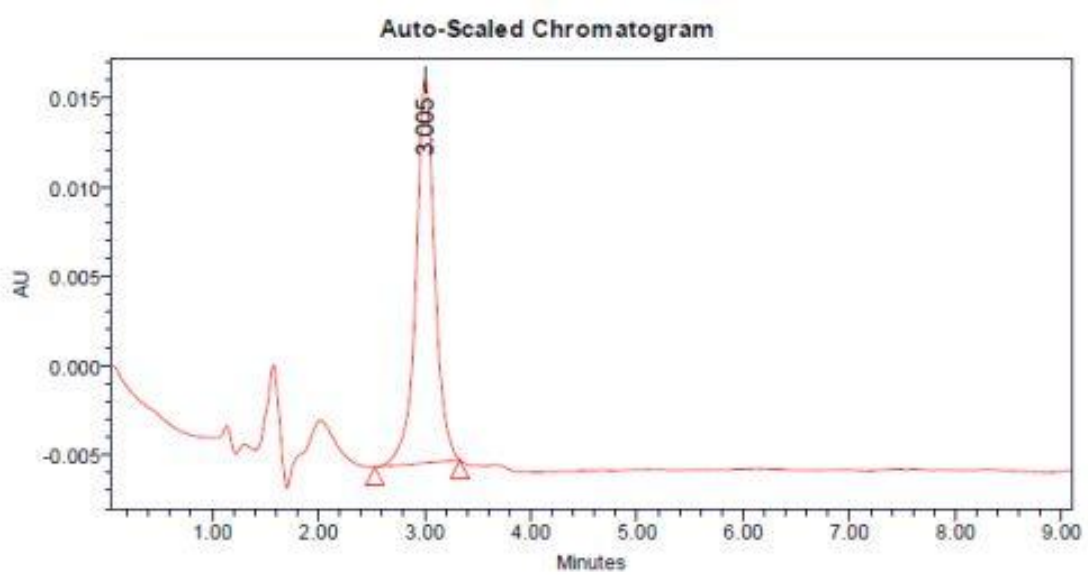


Fig.: chromatogram for trail 2

Table: peak results for trail 2

S. No	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Benidipine	3.005	265008	21567	0.97	638

Observation: This trial show more void peaks in the chromatogram, so more trials were required for obtaining peaks.

Trail 3:

Column : X-Bridge C18 (4.6 x 150mm, 5µm)
Column temperature : 35ºC
Wavelength : 226 nm
Mobile phase ratio : Methanol: Water (70:30% v/v)
Flow rate : 1 mL/min
Injection volume : 10 µl
Run time : 7 minutes

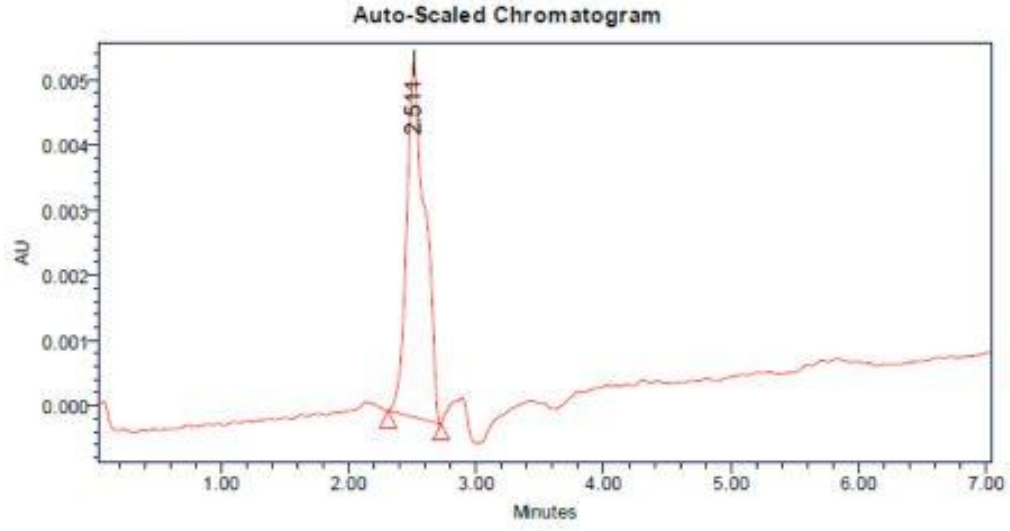


Fig.: chromatogram for trail 3

Table: - peak results for trail 3

S. No	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Benidipine	2.511	54628	5421	1.16	1090

Observation: This trial show very less plate count, and not maintain proper base line in the chromatogram, so more trials were required for obtaining good peaks.

Trail 4:
Column : SymmetryC18 (4.6 x 150mm, 5µm)
Column temperature : 35ºC
Wavelength : 226 nm
Mobile phase ratio : Methanol: Water (60:40% v/v)
Flow rate : 1 mL/min
Injection volume : 10 µl
Run time : 6 minutes

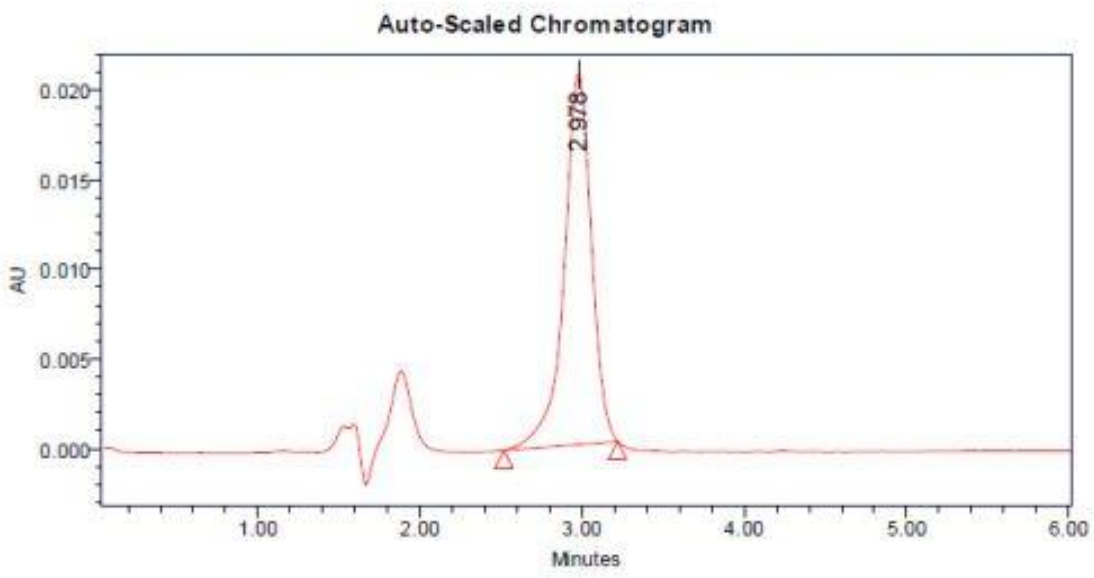


Fig.: chromatogram for trail 4

Table: peak results for trail 4

S.No	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Benidipine	2.978	242286	20725	0.85	2644

Observation: This trial does not show Proper base line. So go for further trails.

Optimized Chromatogram (Standard)
Column : HypersilC18 (4.6 x 150mm, 5µm)
Column temperature : 35ºC
Wavelength : 226 nm
Mobile phase ratio : Methanol: Water (50:50% v/v)
Flow rate : 1mL/min
Injection volume : 10 µl
Run time : 5 minutes

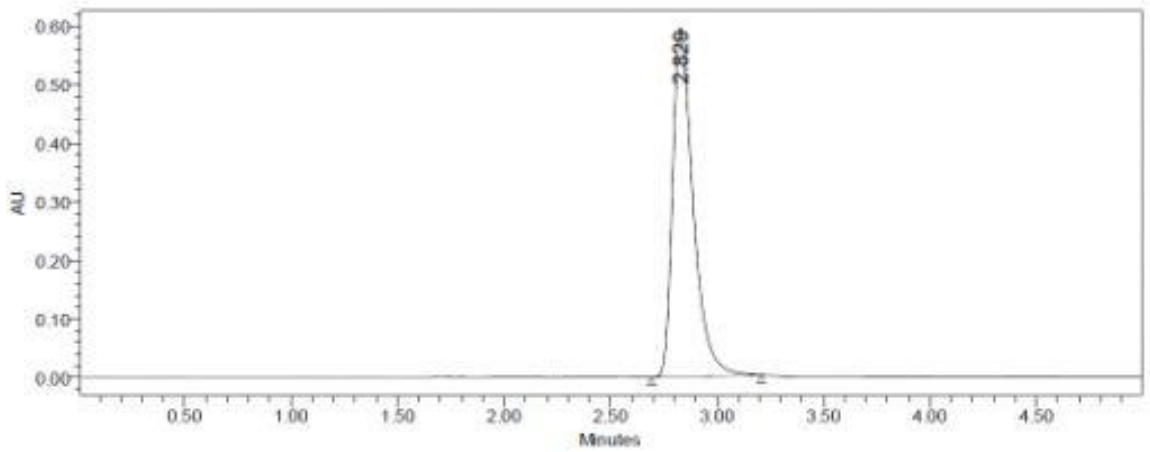


Figure: Optimized Chromatogram (Standard)

Table: Optimized Chromatogram (Standard)

S.no	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Benidipine	2.820	1812535	548932	1.2	6853.0

Observation: This trial shows good peak, proper plate count and less tailing in the chromatogram. And it's Passes the all system suitability parameters. So it's optimized chromatogram.

Optimized Chromatogram (Sample)

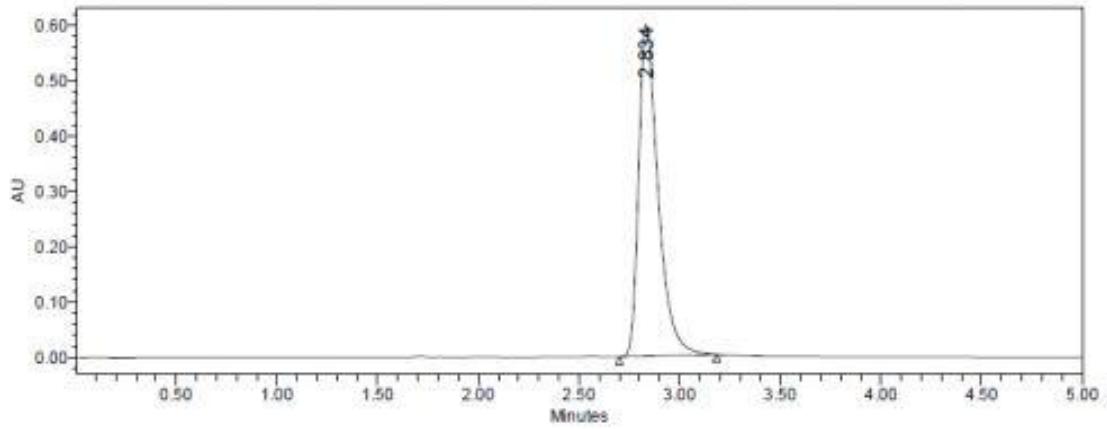


Fig.: Optimized Chromatogram (Sample)

Table: Optimized Chromatogram (Sample)

S.no	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Benidipine	2.834	1819555	598932	1.2	6723.0

Acceptance criteria:

- Theoretical plates must be not less than 2000
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

VALIDATION

Blank:

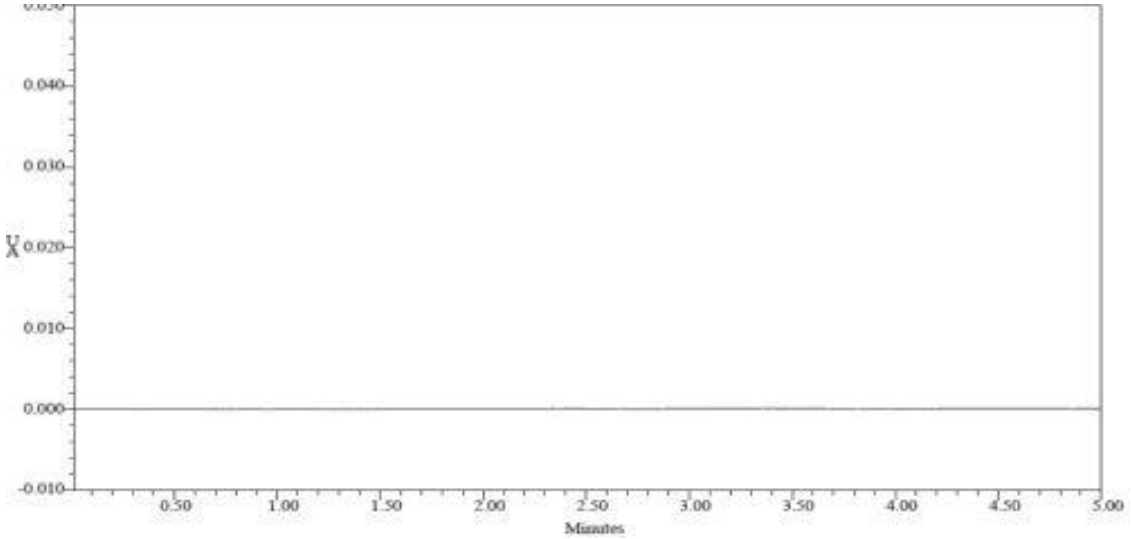


Fig: Chromatogram showing blank (mobile phase preparation)

System suitability:

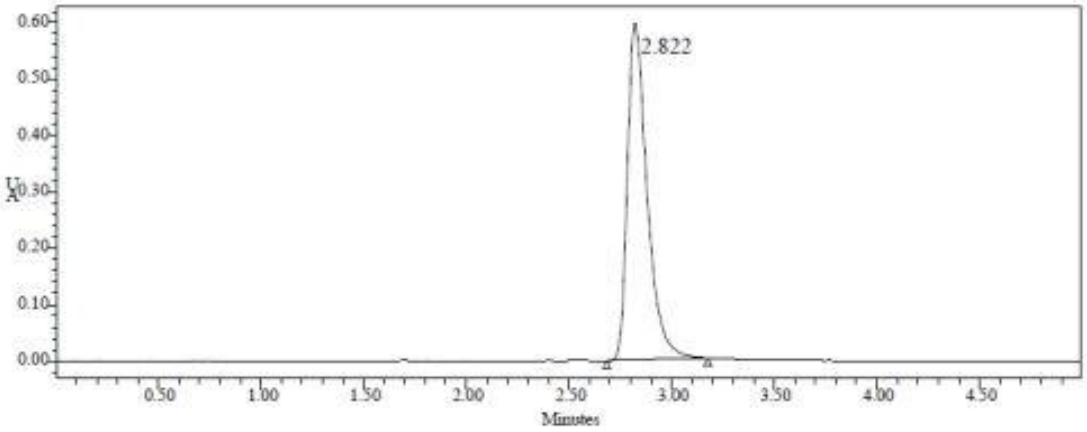


Fig: Chromatogram showing injection -1

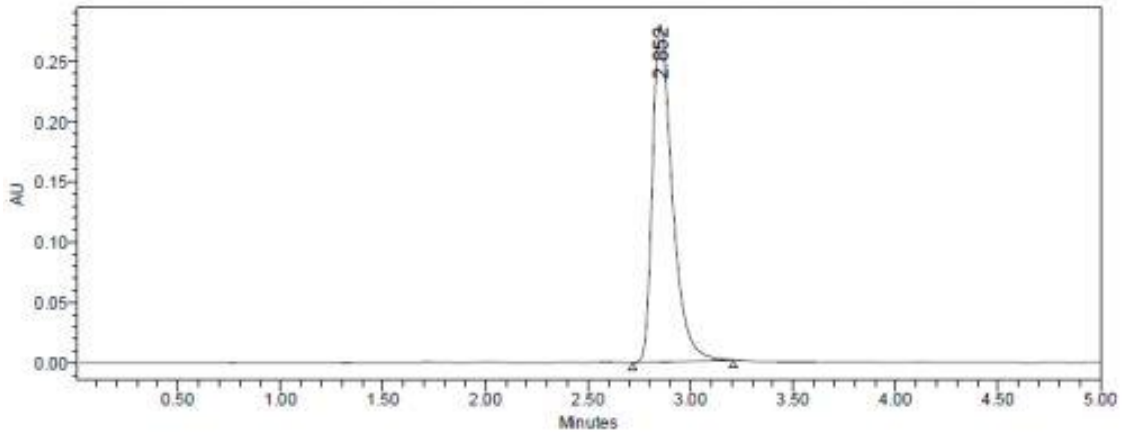


Fig: Chromatogram showing injection -2

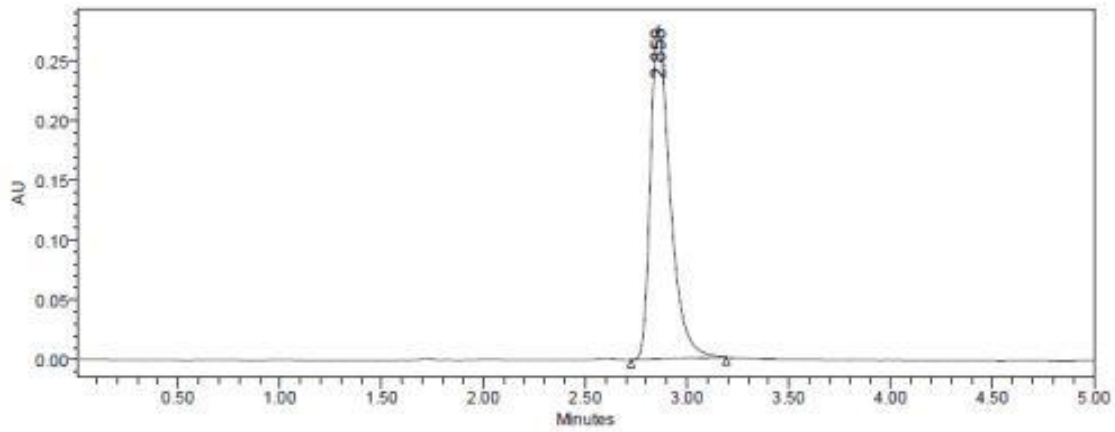


Fig: Chromatogram showing injection -3

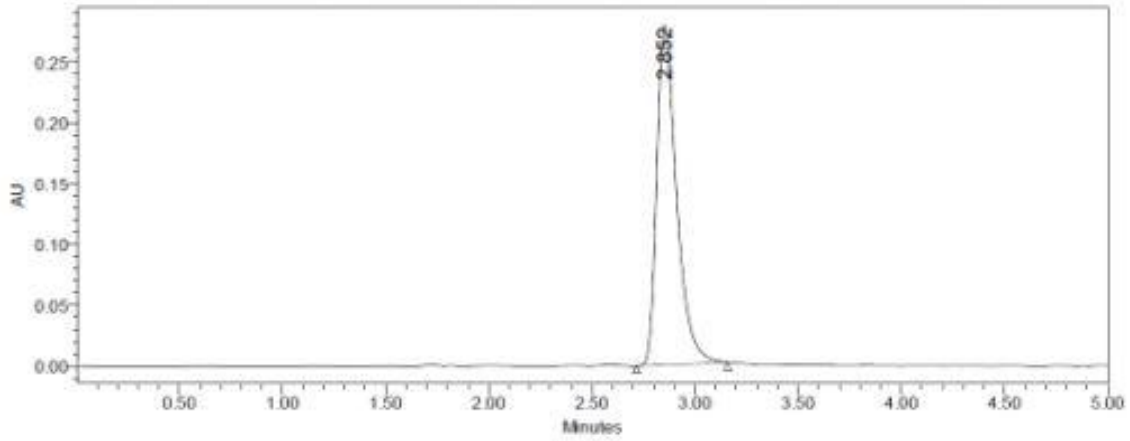


Fig: Chromatogram showing injection -4

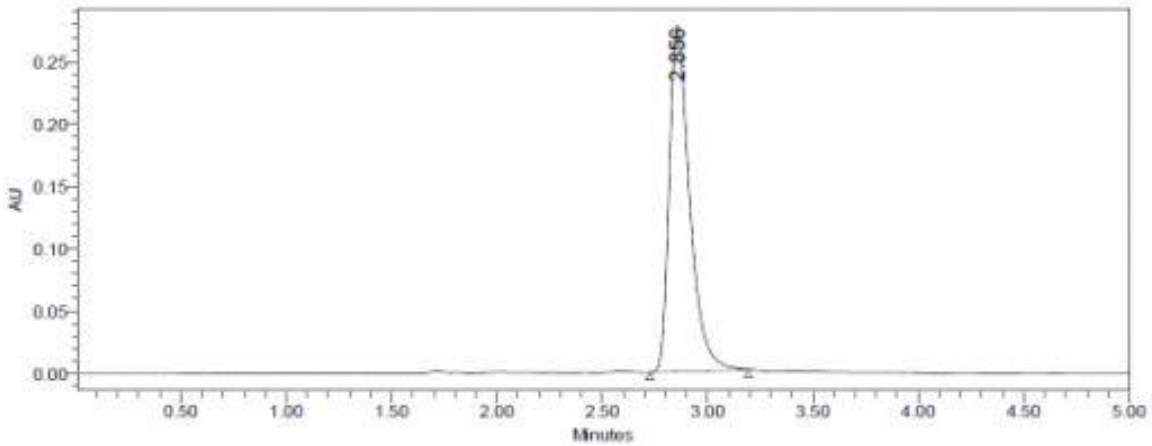


Fig: Chromatogram showing injection -5

Table: Results of system suitability for Benidipine Hydrochloride

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Benidipine Hcl	2.822	1801235	600073	6315	1.5
2	Benidipine Hcl	2.856	1871564	278283	6341	1.5
3	Benidipine Hcl	2.858	1874317	278560	6339	1.5
4	Benidipine Hcl	2.856	1868866	278230	6312	1.5
5	Benidipine Hcl	2.852	1866212	278106	6214	1.5
Mean			1856439			
Std. Dev.			31007.31			
% RSD			1.6			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

SPECIFICITY

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantitate Benidipine Hydrochloride in drug product.

Assay (Standard):

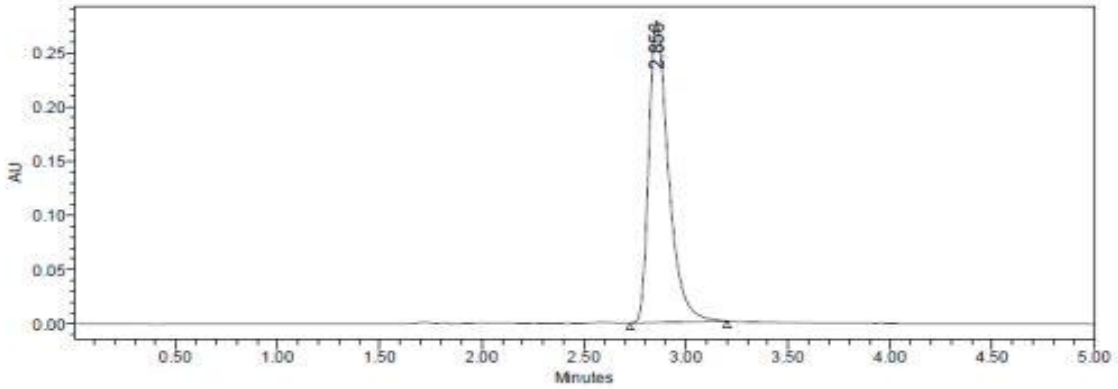


Fig. Chromatogram showing assay of standard injection -1

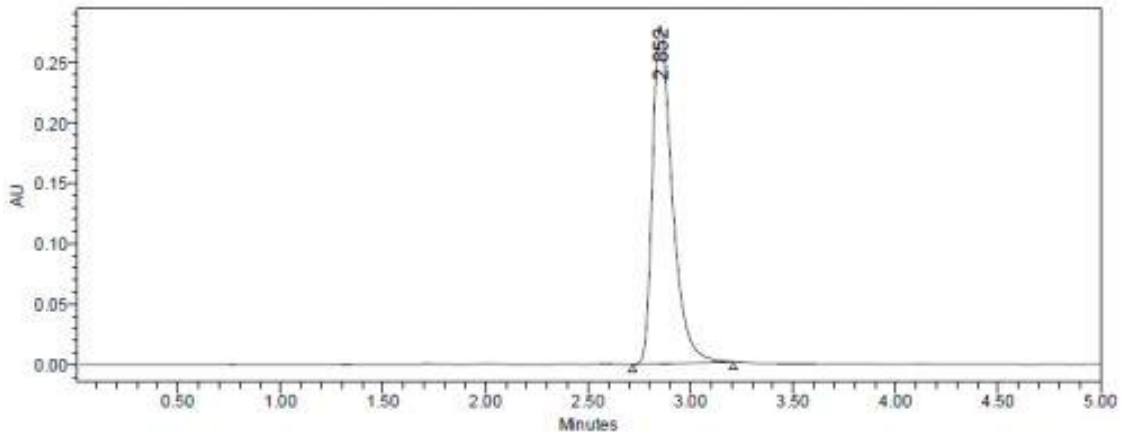


Fig. Chromatogram showing assay of standard injection -2

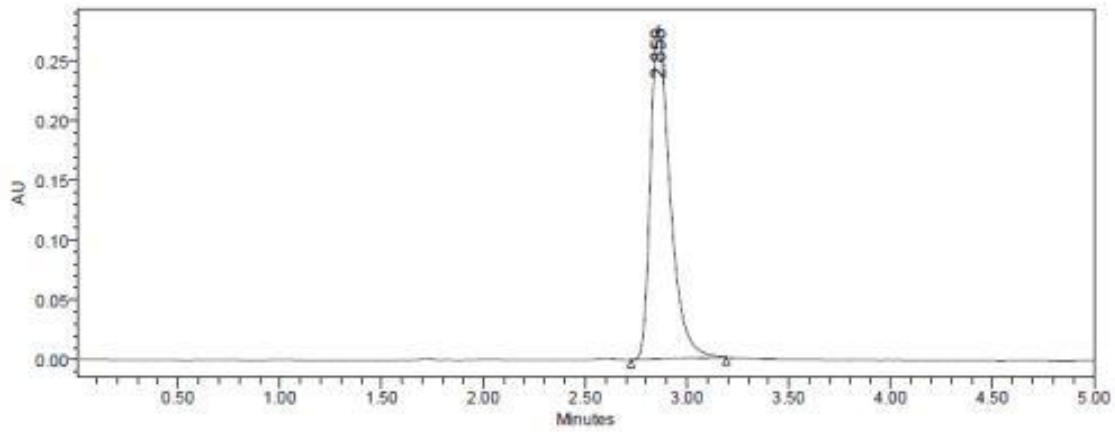


Fig. Chromatogram showing assay of standard injection -3

Table: Peak results for assay standard

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Benidipine Hcl	2.852	1879662	279992	1.5	6293	1
2	Benidipine Hcl	2.856	1871564	278283	1.5	6341	2
3	Benidipine Hcl	2.858	1874317	278560	1.5	6339	3

Assay (Sample):

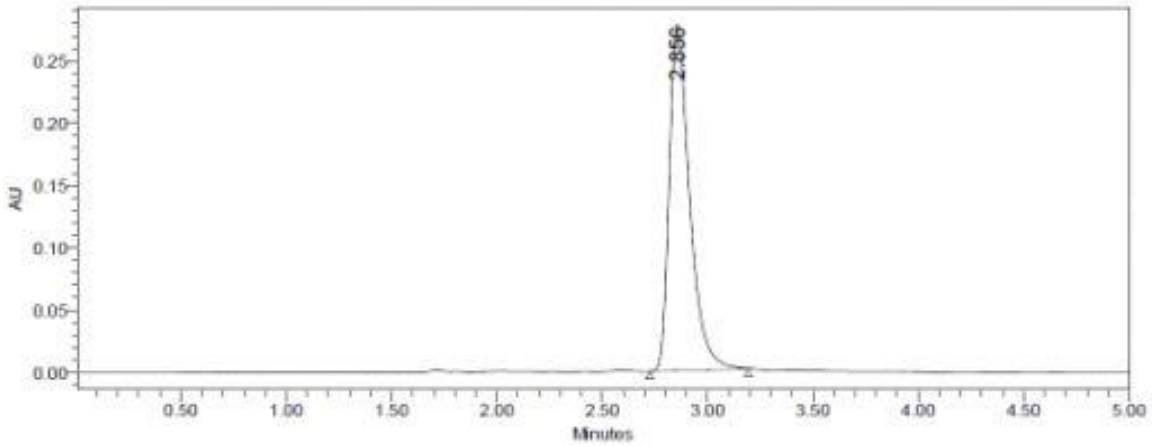


Fig: Chromatogram showing assay of sample injection-1

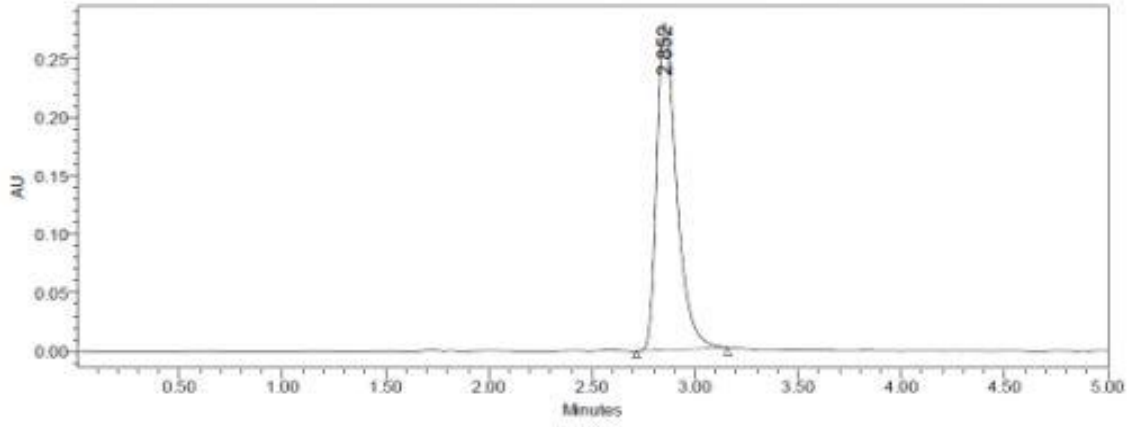


Fig. Chromatogram showing assay of sample injection-2

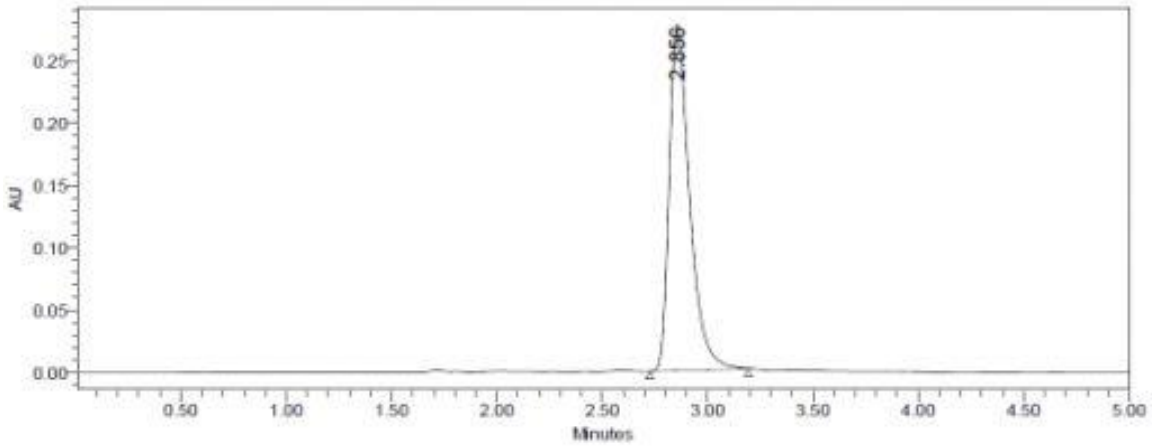


Fig: Chromatogram showing assay of sample injection-3

Table: Peak results for Assay sample

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Benidipine Hcl	2.852	1865942	279706	1.5	6236	1
2	Benidipine Hcl	2.856	1868866	278230	1.5	6312	2
3	Benidipine Hcl	2.852	1866212	278106	1.5	6214	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

=1867007/1875181×10/15×15/0.0130×99.7/100×0.0521/40×100
= 99.4%
The % purity of Benidipine Hydrochloride in pharmaceutical dosage form was found to be 99.4%.

LINEARITY

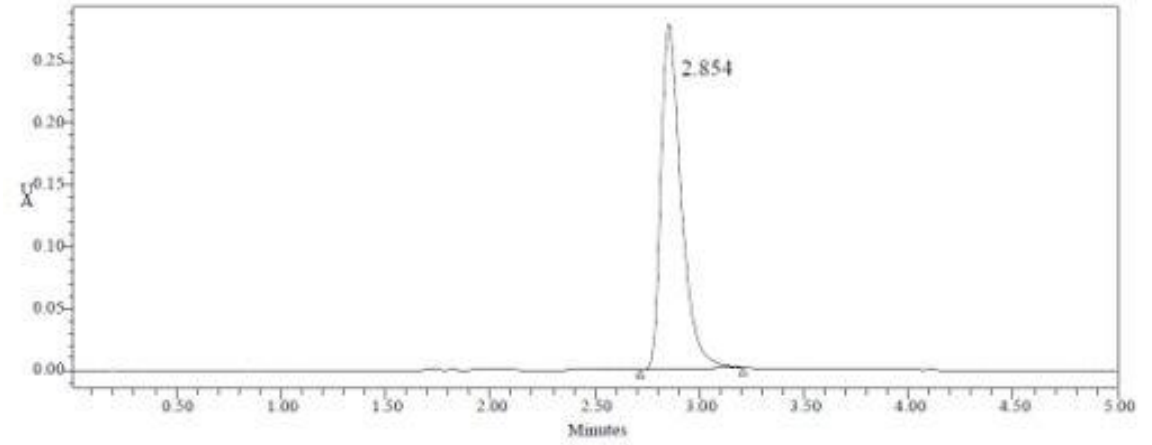


Fig. Chromatogram showing linearity level-1

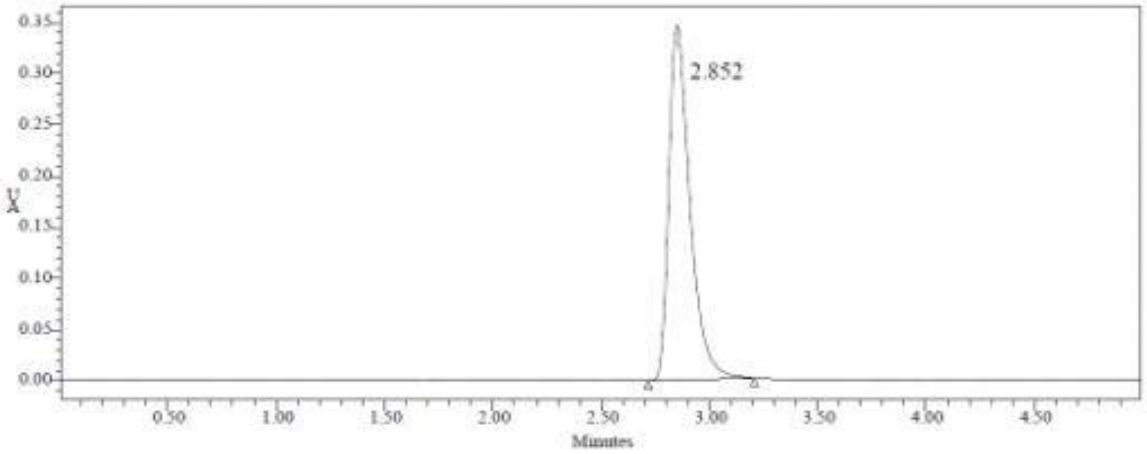


Fig. Chromatogram showing linearity level-2

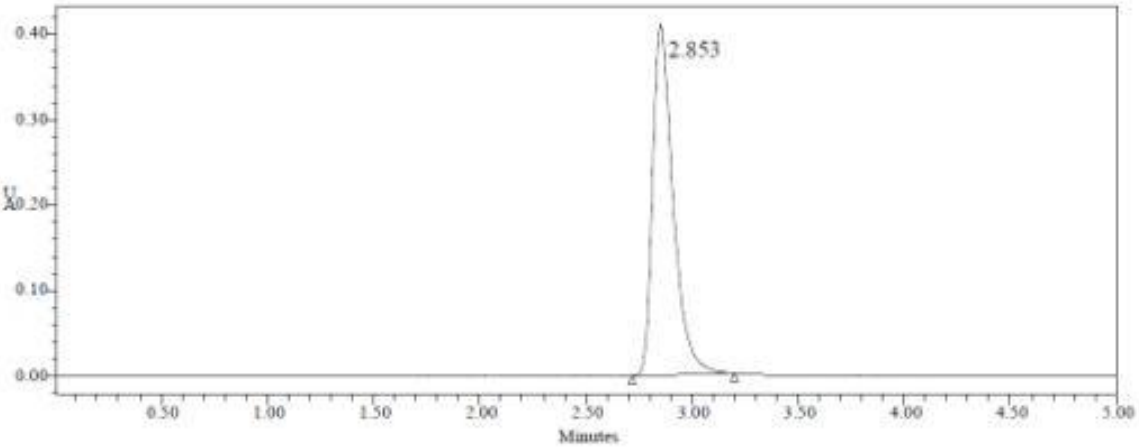


Fig. Chromatogram showing linearity level-3

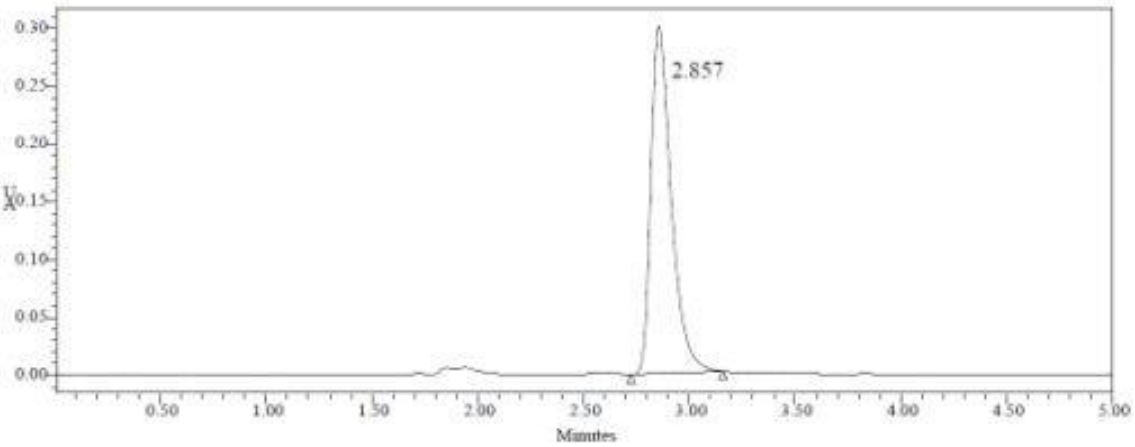


Fig. Chromatogram showing linearity level-4

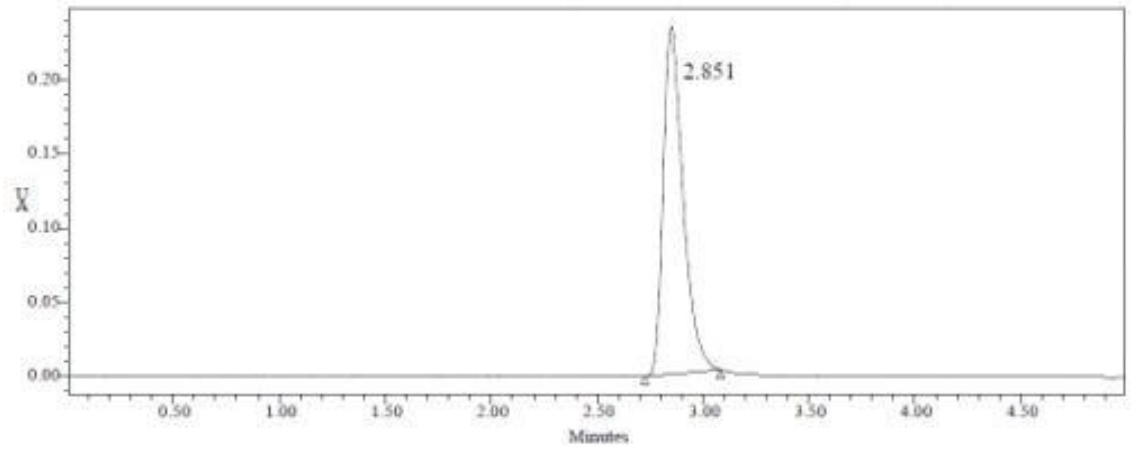


Fig. Chromatogram showing linearity level-5

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:

Concentration Level (%)	Concentration µg/ml	Average Peak Area
	µg/ml	Peak Area
33	10	791554
66	20	1647073
100	30	2283804
133	40	3058339
166	50	3839630

LINEARITY PLOT:

The plot of Concentration (x) versus the Average Peak Area (y) data of Benidipine is a straight line.

Y = mx + c

Slope (m) = 76101

Intercept (c) = 34216

Correlation Coefficient (r) = 0.998

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is 34216. These values meet the validation criteria.

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.

REPEATABILITY

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

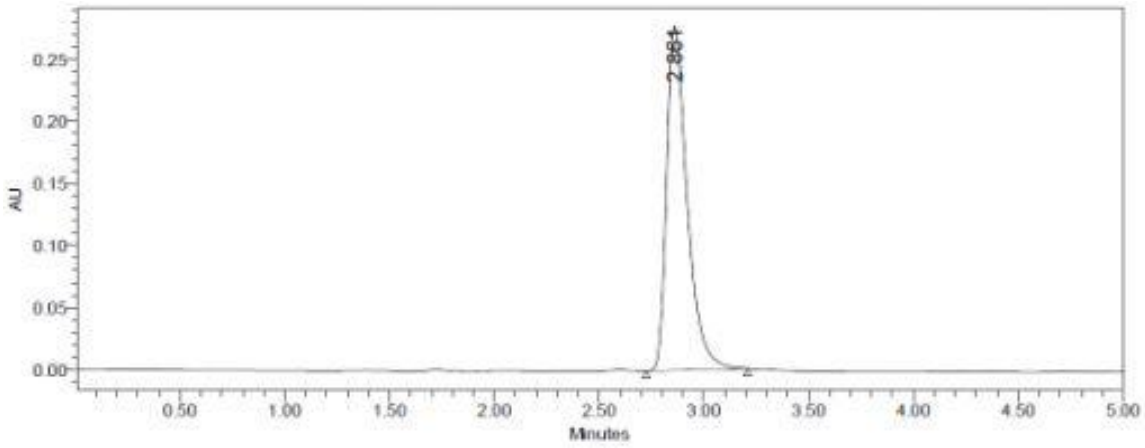


Fig. Chromatogram showing precision injection -1

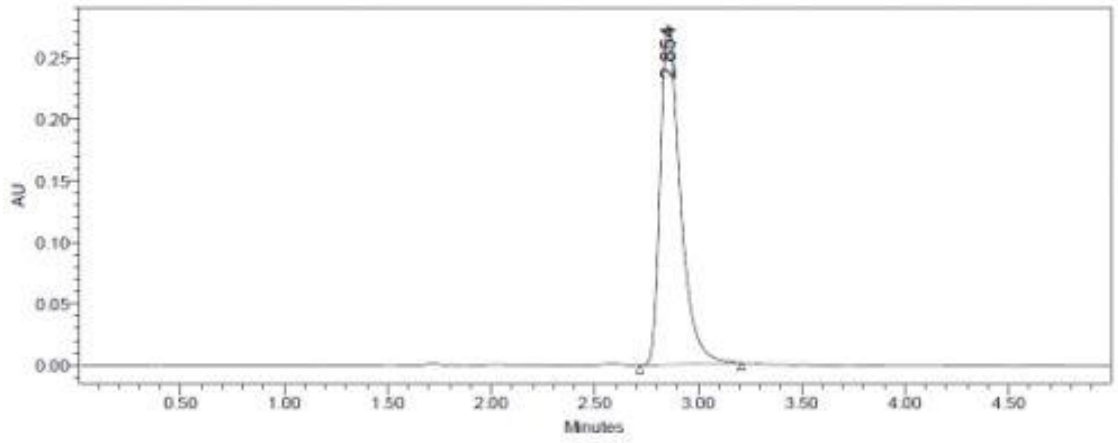


Fig. Chromatogram showing precision injection -2

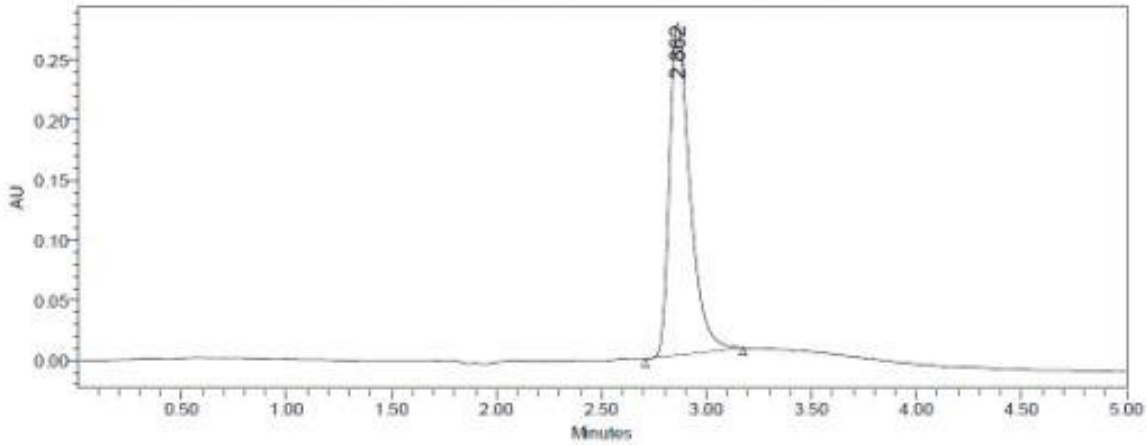


Fig. Chromatogram showing precision injection -3

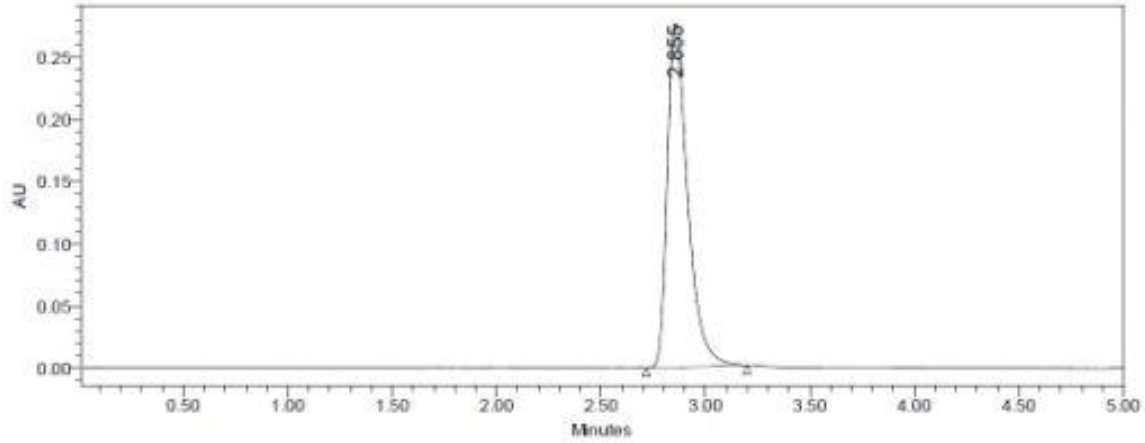


Fig. Chromatogram showing precision injection -4

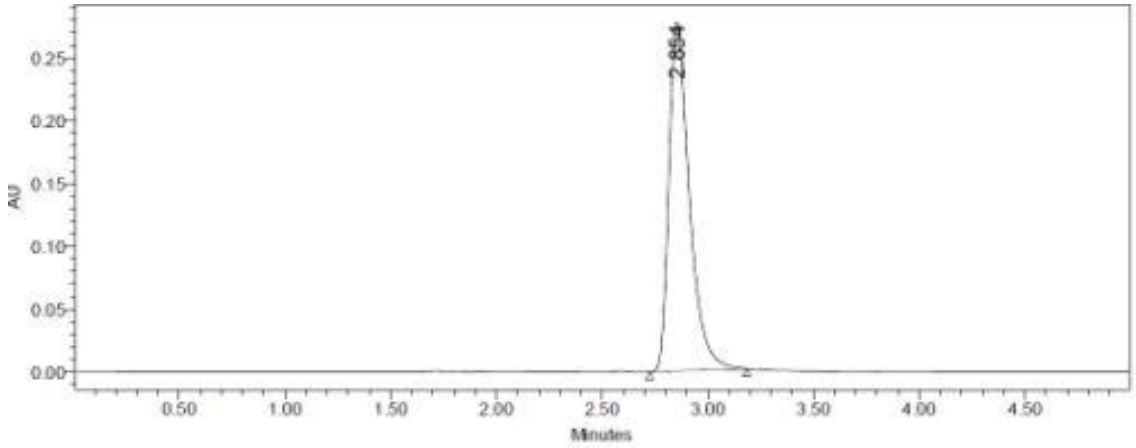


Fig. Chromatogram showing precision injection -5

Table: Results of repeatability for Benidipine HCl

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
		time		(µV)	Count	Tailing
1	Benidipine Hcl	2.861	1871423	278602	6802	1.5
2	Benidipine Hcl	2.854	1876279	277598	6546	1.5
3	Benidipine Hcl	2.882	1874529	276855	6633	1.5
4	Benidipine Hcl	2.855	1879273	277491	6812	1.5
5	Benidipine Hcl	2.854	1873436	277959	6802	1.5
Mean			1874988			
Std.dev			2973.067			
%RSD			0.15			

Acceptance criteria:

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision:
Analyst 1:

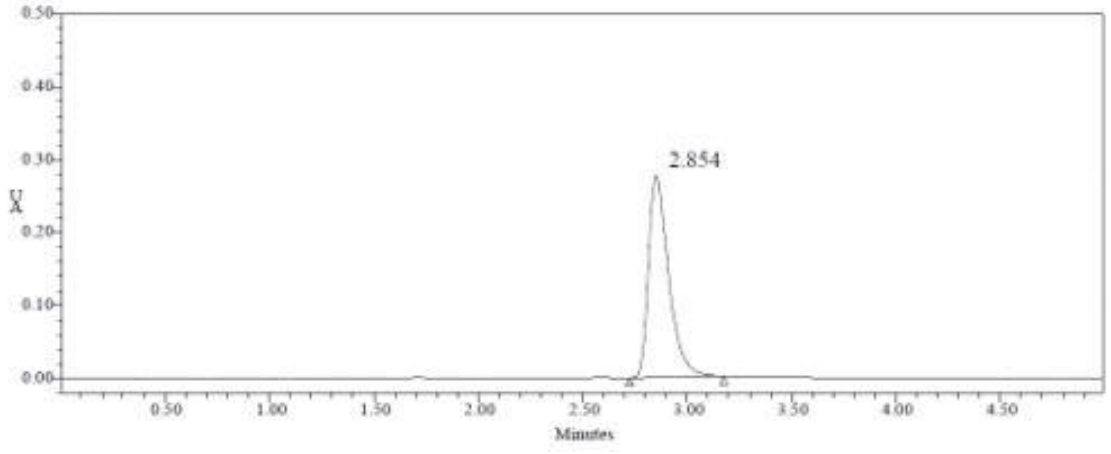


Fig: Chromatogram showing Analyst 1 injection -1

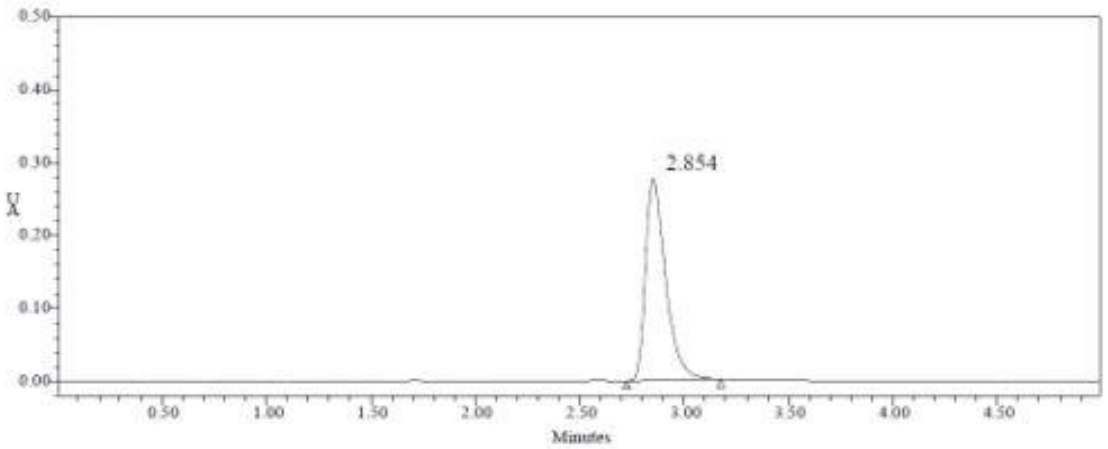


Fig: Chromatogram showing Analyst 1 injection -2

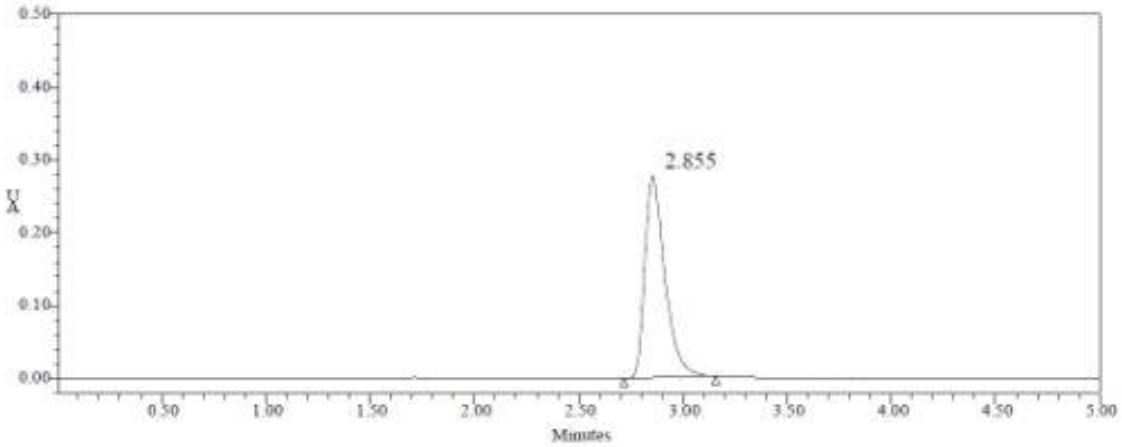


Fig: Chromatogram showing Analyst 1 injection -3

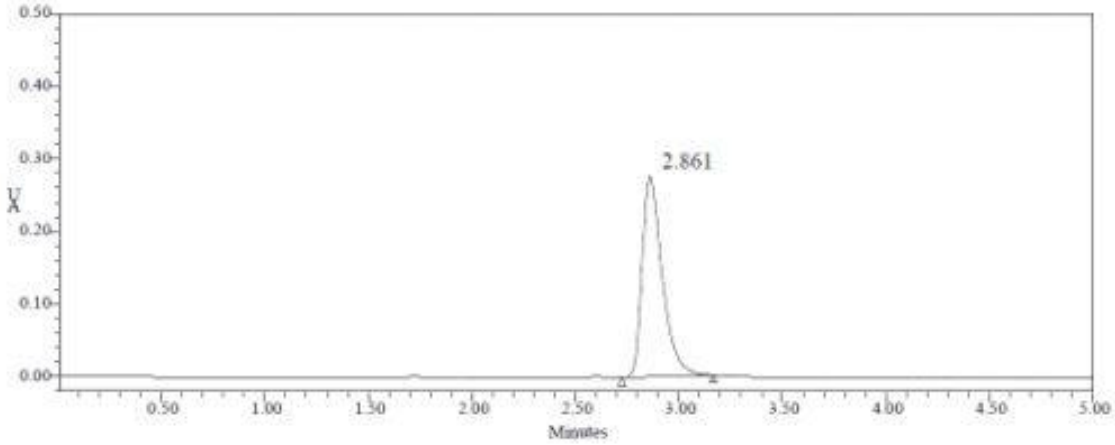


Fig: Chromatogram showing Analyst 1 injection -4

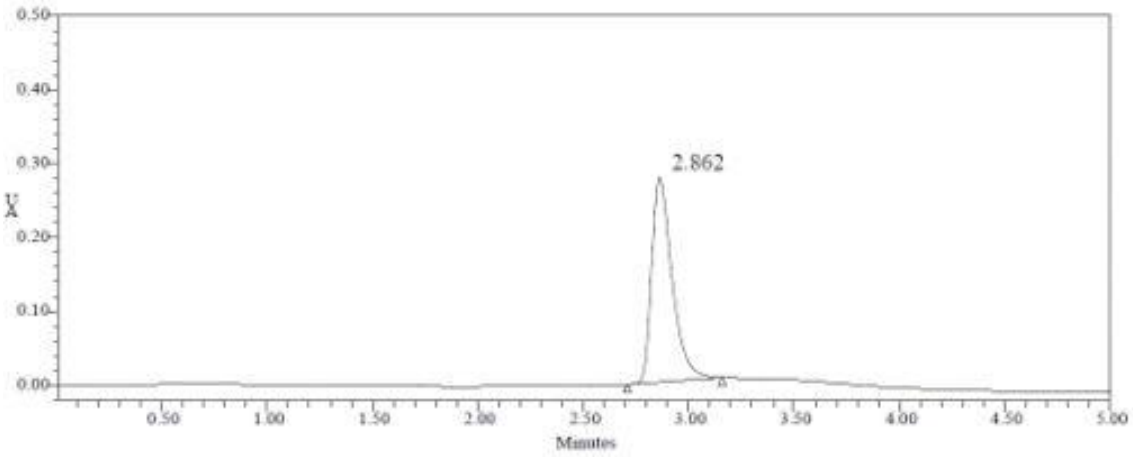


Fig: Chromatogram showing Analyst 1 injection -5

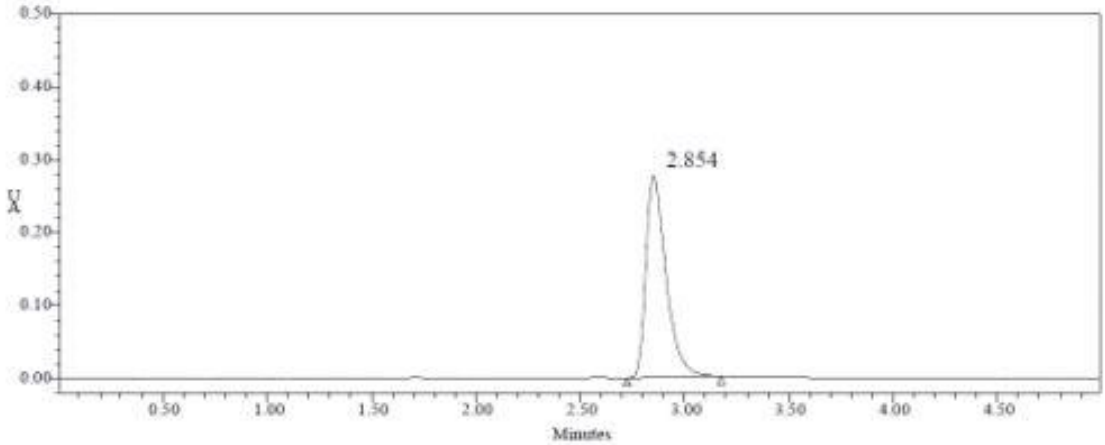


Fig: Chromatogram showing Analyst 1 injection -6

Table: Results of Intermediate precision for Benidipine HCl

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Benidipine Hcl	2.854	1869365	278559	6478	1.5
2	Benidipine Hcl	2.854	1868938	277455	6894	1.5
3	Benidipine Hcl	2.855	1861814	276579	6908	1.5
4	Benidipine Hcl	2.861	1867522	277241	6998	1.5
5	Benidipine Hcl	2.862	1866552	277789	6284	1.5
6	Benidipine Hcl	2.854	1868218	276105	6928	1.5
Mean			1867068			
Std. Dev.			2763.06			
% RSD			0.14			

Acceptance criteria:

%RSD of five different sample solutions should not more than 2

Analyst 2:

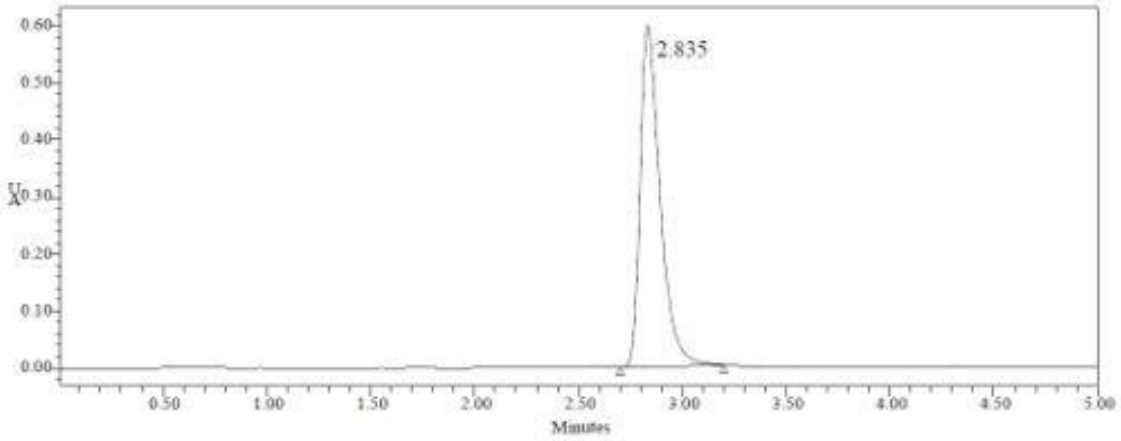


Fig: Chromatogram showing Analyst 2 injection -1

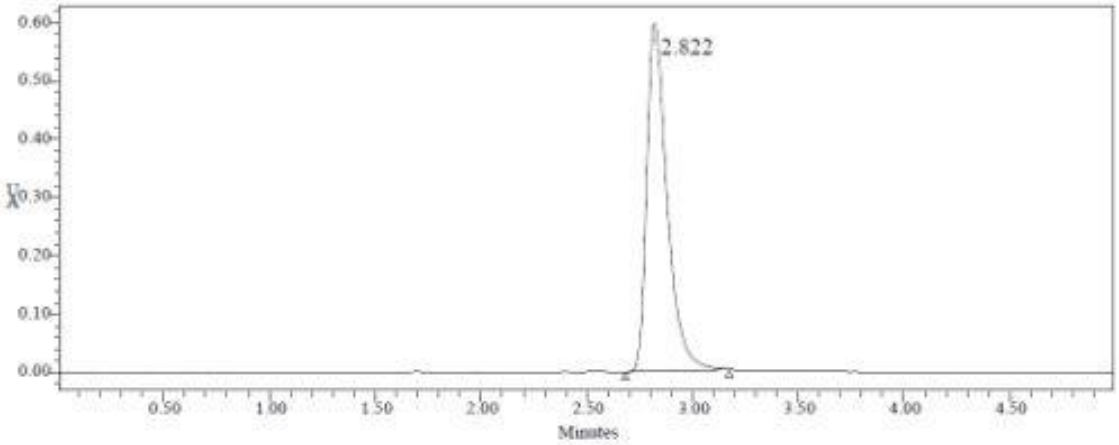


Fig: Chromatogram showing Analyst 2 injection -2

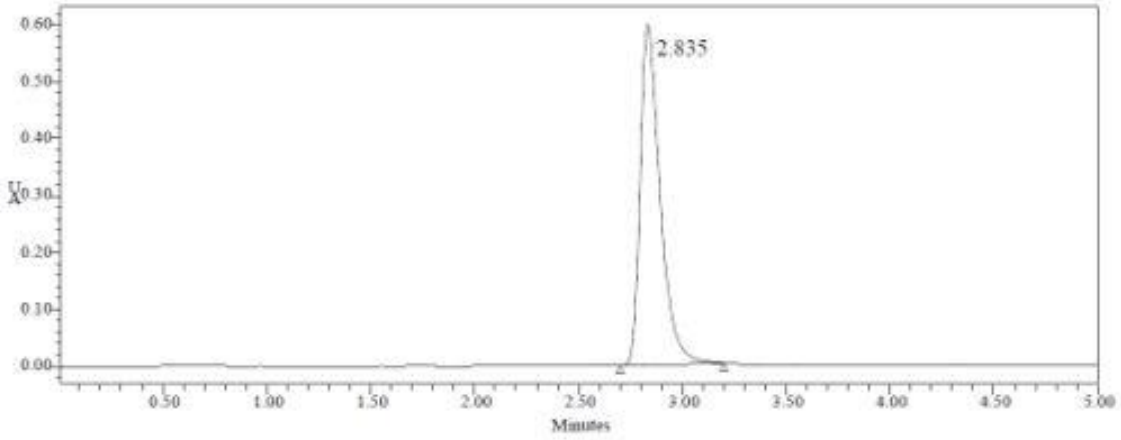


Fig: Chromatogram showing Analyst 2 injection -3

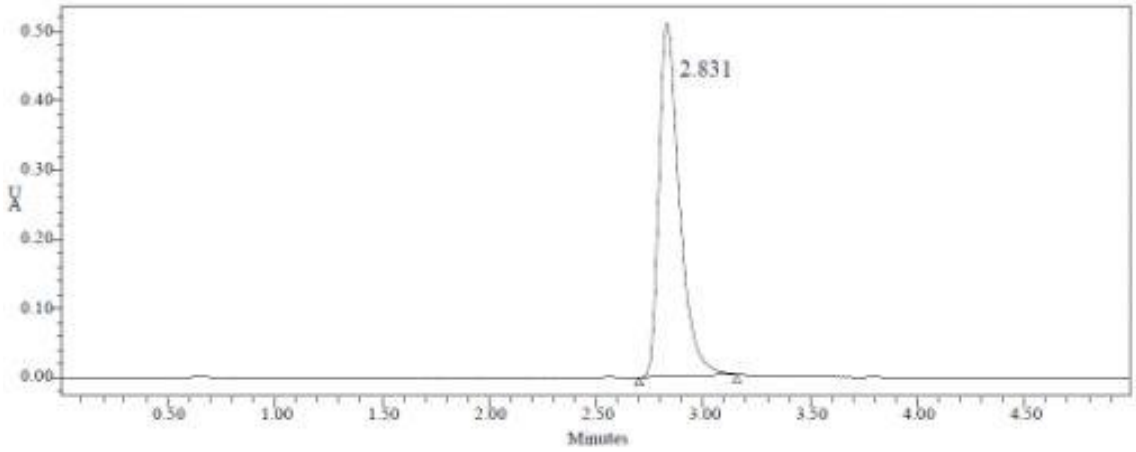


Fig: Chromatogram showing Analyst 2 injection -4

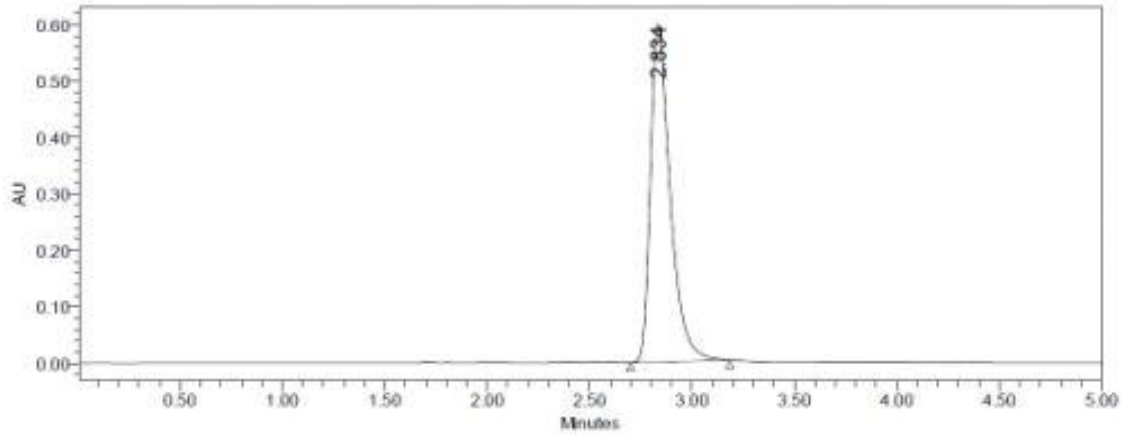


Fig: Chromatogram showing Analyst 2 injection -5

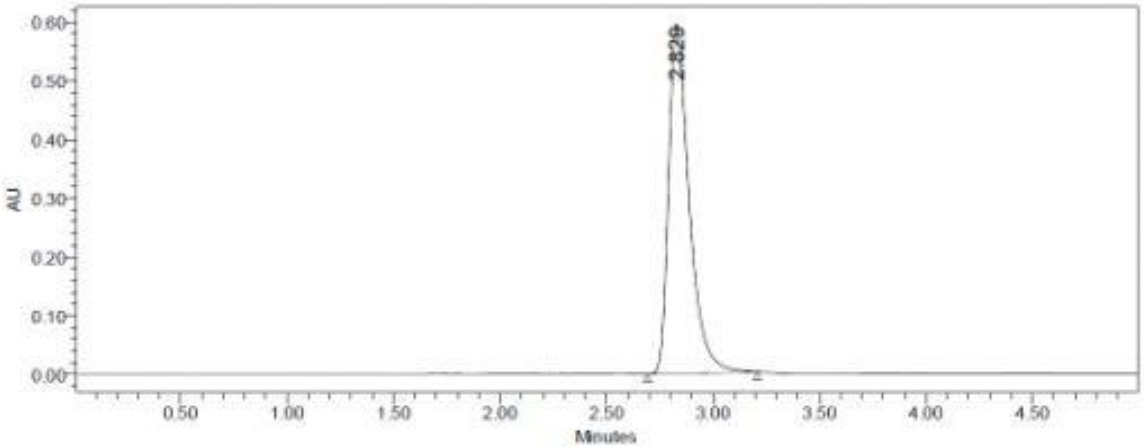


Fig: Chromatogram showing Analyst 2 injection -6

Table: Results of Intermediate precision Analyst 2 for Benidipine Hydrochloride

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Benidipine	2.835	4001377	598296	1.5	6297
2	Benidipine	2.835	4004114	600073	1.5	6315
3	Benidipine	2.835	4031077	597291	1.5	6277

4	Benidipine	2.831	4056124	589641	1.5	6123
5	Benidipine	2.834	4102584	592134	1.5	6896
6	Benidipine	2.820	4021542	589642	1.5	6412
Mean			4036136			
Std. Dev.			38175.99			
% RSD			0.9			

Acceptance criteria:

%RSD of five different sample solutions should not more than 2

ACCURACY:

Accuracy at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

Accuracy50%:

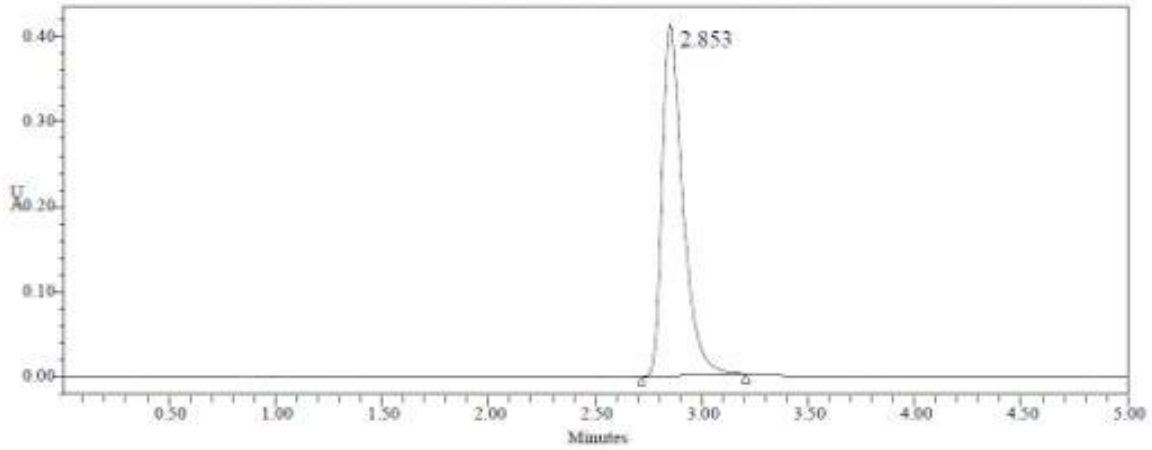


Fig. Chromatogram showing accuracy-50% injection-1

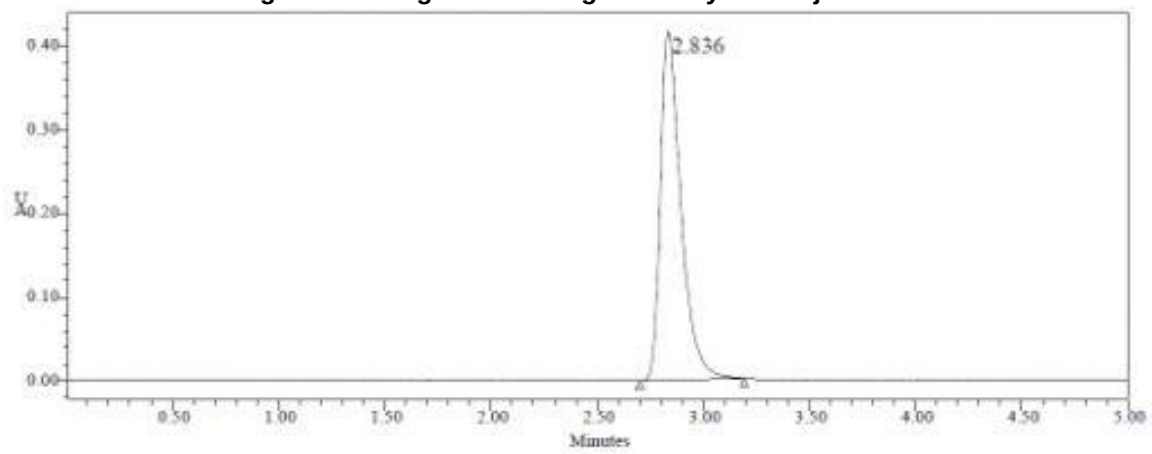


Fig. Chromatogram showing accuracy-50% injection-2

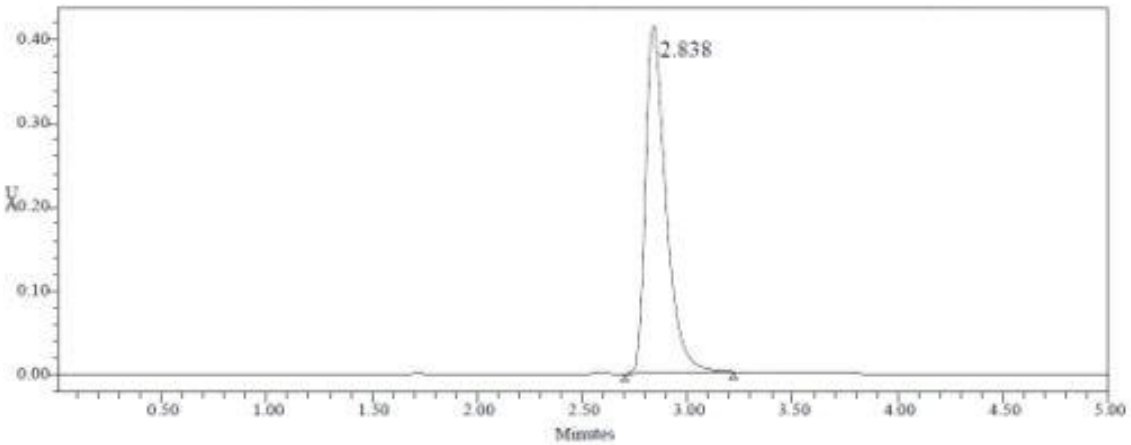


Fig. Chromatogram showing accuracy-50% injection-3

Table: Results of Accuracy for concentration-50%

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Benidipine Hcl	2.836	553410	417631	1.5	6597	1
2	Benidipine Hcl	2.838	558083	417717	1.5	6715	2
3	Benidipine Hcl	2.853	549124	414831	1.5	6677	3

Accuracy100%:

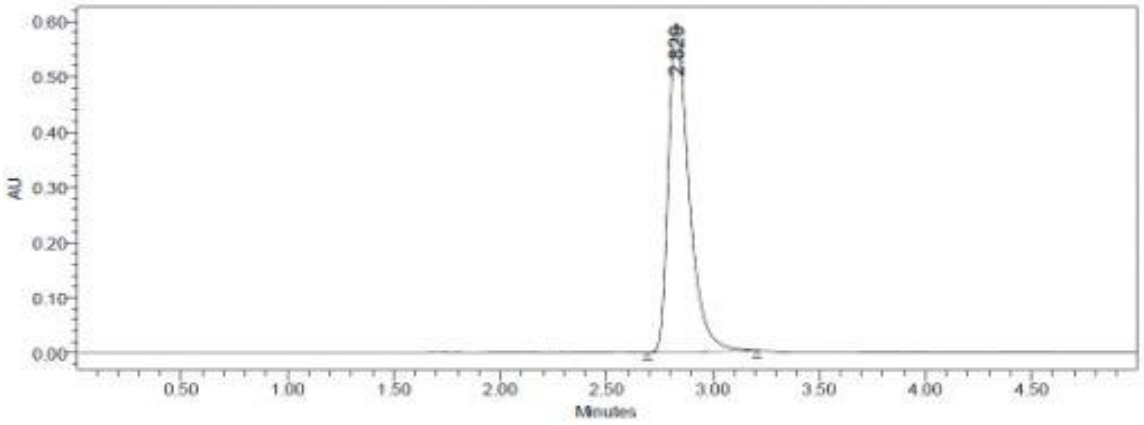


Fig Chromatogram showing accuracy-100% injection-1

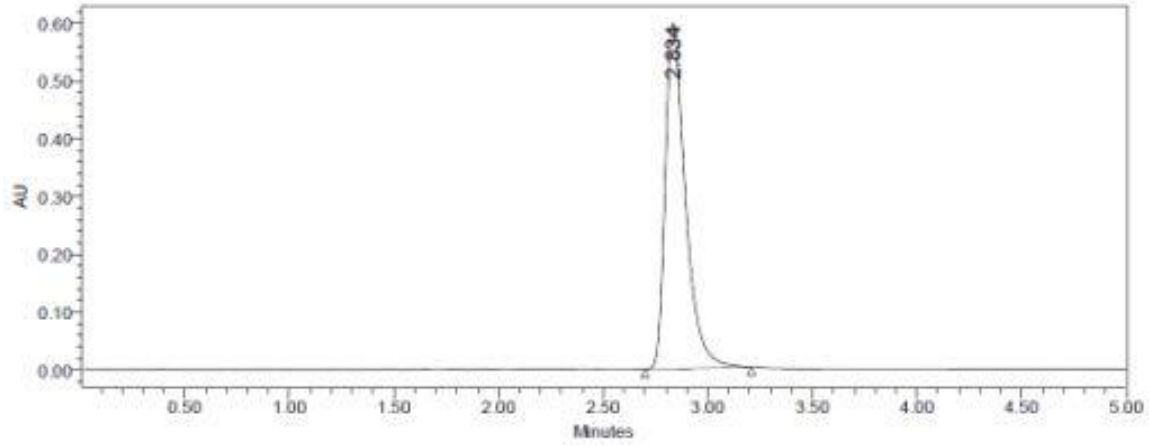


Fig: Chromatogram showing accuracy-100% injection-2

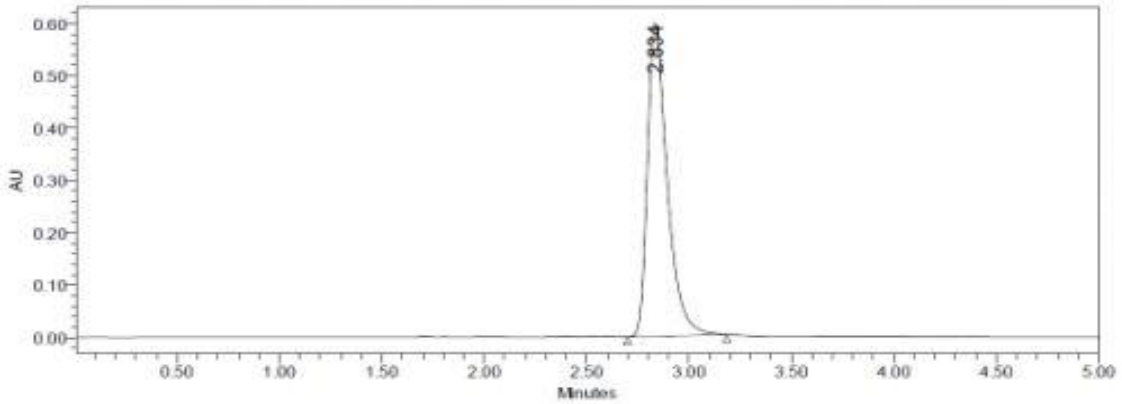


Fig: Chromatogram showing accuracy-100% injection-3

Table Results of Accuracy for concentration-100%

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Benidipine Hcl	2.829	1110378	598016	1.5	6937.9	1
2	Benidipine Hcl	2.834	1219555	598932	1.5	6067.2	2
3	Benidipine Hcl	2.834	1012409	598318	1.5	6949.2	3

Accuracy150%:

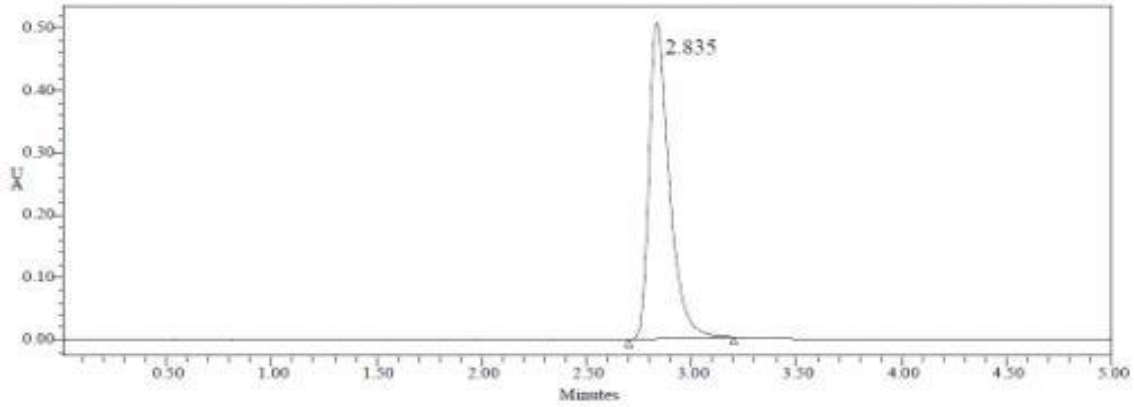


Fig. Chromatogram showing accuracy-150% injection-1

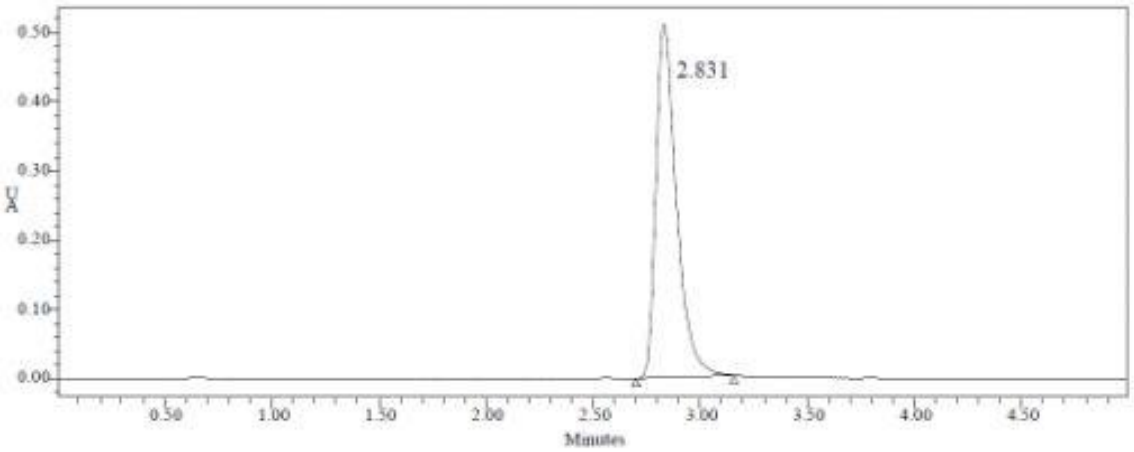


Fig. Chromatogram showing accuracy-150% injection-2

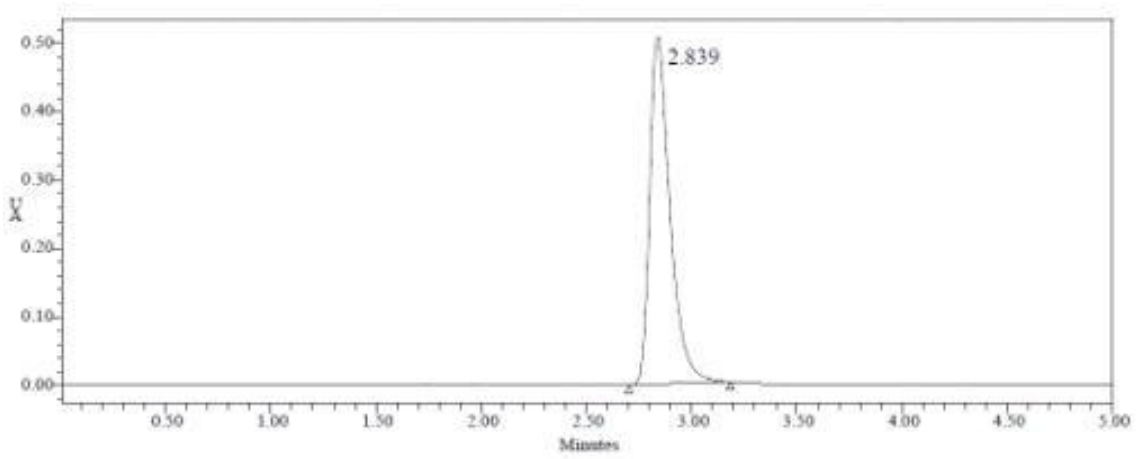


Fig. Chromatogram showing accuracy-150% injection-3

Table Results of Accuracy for concentration-150%

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Benidipine Hcl	2.831	1106776	509832	1.5	6567.9	1
2	Benidipine Hcl	2.835	1102165	508398	1.5	6432.8	2
3	Benidipine Hcl	2.839	1105472	508216	1.5	6653.1	3

The accuracy results for Benidipine Hydrochloride

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	553539	7.5	7.2	96%	99.9%
100%	1114114	15	14.96	99.7%	
150%	1658138	22.5	22.4	99.5%	

Acceptance Criteria:

The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

LIMIT OF DETECTION FOR BENIDIPINE HCl

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Where,

σ = Standard deviation of the response

S = Slope of the calibration curve

Result: =3.3×7447.93/36202

=0.67µg/ml

QUANTITATION LIMIT

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

Where,

σ = Standard deviation of the response

S = Slope of the calibration curve

Result: =10×7447.93/36202

= 2.05µg/ml

ROBUSTNESS

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Benidipine Hydrochloride. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Benidipine Hydrochloride were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Variation in flow

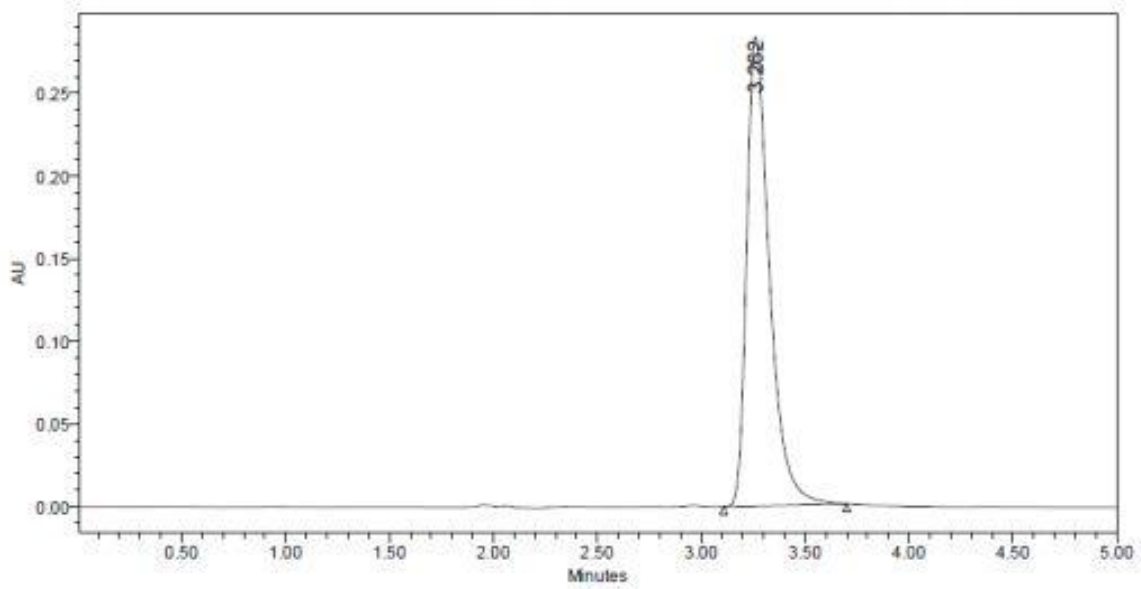


Figure: chromatogram showing less flow of 0.9ml/min

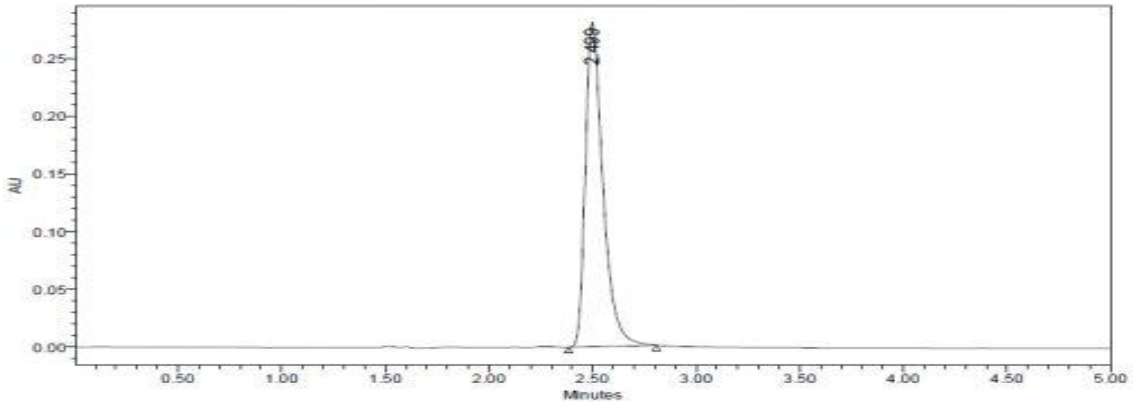


Figure: chromatogram showing more flow of 1.1 ml/min

Variation of mobile phase organic composition

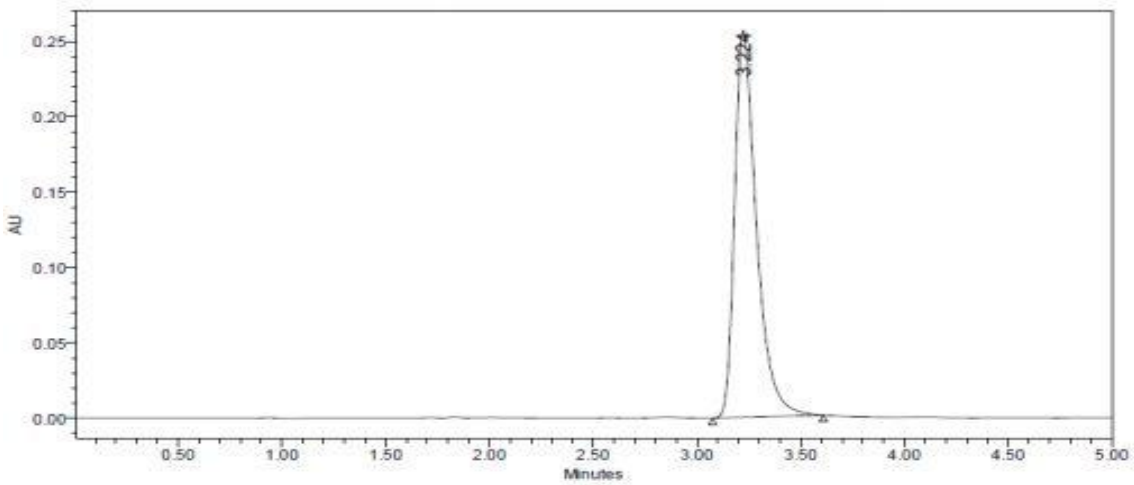


Figure: chromatogram showing less organic composition

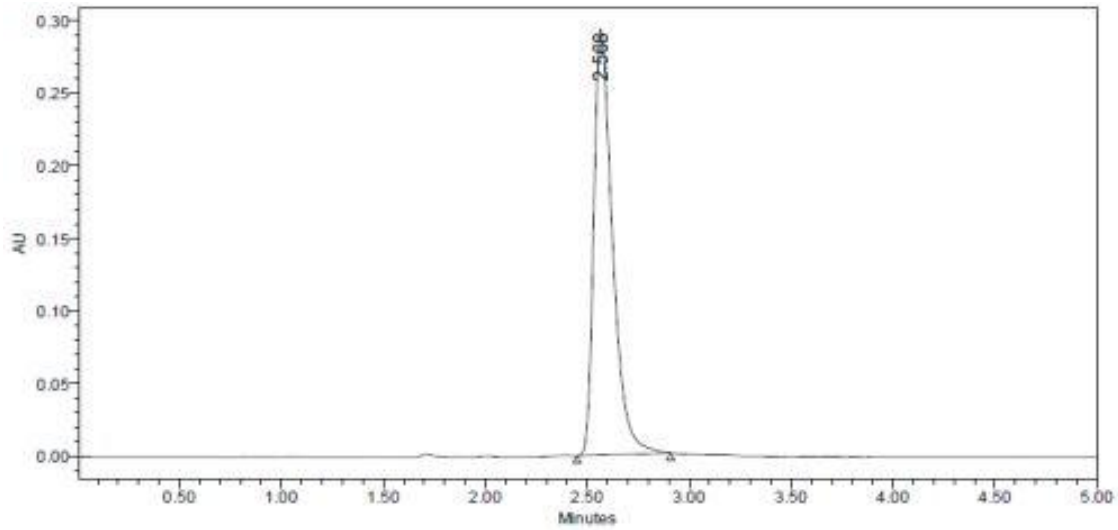


Figure: chromatogram showing more organic composition

Table: Results for Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Less Flow rate of 1.0 mL/min	2157820	3.262	6521	1.5
Actual Flow rate of 0.9 mL/min	1812535	2.820	6853	1.5
More Flow rate of 1.1 mL/min	1658013	2.499	6920	1.4
Less Organic phase	1871157	3.224	6458	1.4
More Organic phase	1871882	2.568	6807	1.5

Acceptance criteria:
The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

SUMMARY
The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 260nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area.
The column used for study was Symmetry C_{18} because it was giving good peak. 40ºC temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 0.8ml/min because of good peak area and satisfactory retention time.

Mobile phase is Methanol: water was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Methanol: water was selected because of maximum extraction sonication time was fixed to be 10min at which all the drug particles were completely soluble and showed good recovery. Run time was selected to be 6min because analyze gave peak around 2.3 and also to reduce the total run time.

The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range.The analytical method was found linearity over the range of 10-50ppm of the Benidipine target concentration.

The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Benidipine in bulk drug and pharmaceutical dosage forms.

This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps.

Benidipine was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: water was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Benidipine in bulk drug and in Pharmaceutical dosage forms.

REFERENCES

1. Dr. Kealey and P.J Haines, Analytical Chemistry, 1st edition, Bios Publisher, (2002), PP 1-7.
2. A.BraithWait and F.J.Smith, Chromatographic Methods, 5th edition, Kluwer Academic Publisher, (1996), PP 1-2.
3. Andrea Weston and Phyllisr. Brown, HPLC Principle and Practice, 1st edition,Academic press, (1997), PP 24-37.
4. Yuri Kazakevich and Rosario Lobrutto, HPLC for Pharmaceutical Scientists, 1st edition, Wiley Interscience A JohnWiley & Sons, Inc., Publication, (2007), PP 15-23.
5. Chromatography, (online). URL:http://en.wikipedia.org/wiki/Chromatography.
6. Meyer V.R. Practical High-Performance Liquid Chromatography, 4th Ed. England, John Wiley & Sons Ltd, (2004), PP 7-8.
7. Sahajwalla CG a new drug development, vol 141, Marcel Dekker Inc., New York, (2004), PP 421–426.
8. Introduction to Column. (Online),URL:http://amitpatel745.topcities.com/index_files/study/column care.pdf
9. Detectors used in HPLC (online)URL:http://wiki.answers.com/Q/What_detectors_are_used_in_HPLC
10. Detectors (online) ,URL:http://hplc.chem.shu.edu/NEW/HPLC_Book/Detectors/det_uvda.html
11. Detectors (online) ,URL:http://www.dionex.com/enus/webdocs/64842-31644-02_PDA-100.pdf
12. Detectors (online),URL:http://www.ncbi.nlm.nih.gov/pubmed/8867705
13. Detectors (online),URL:http://www.chem.agilent.com/Library/applications/59643559.pdf
14. Detectors (online),URL:http://hplc.chem.shu.edu/new/hplcbook/detector
15. Draft ICH Guidelines on Validation of Analytical Procedures Definitions and terminology. Federal Register, vol 60. IFPMA, Switzerland, (1995), PP 1126.
16. Code Q2B, Validation of Analytical Procedures; Methodology. ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, (1996), PP 1- 8.
17. Introduction to analytical method validation (online), available from: URL: http://www.standardbase.hu/tech/HPLC%20validation%20PE.pdf.
18. Data elements required for assay validation, (online) available from: URL: http://www.labcompliance.com/tutorial/methods/default.aspx.
19. Snyder LR practical HPLC method development, 2nd edition. John Wiley and sons, New York, (1997), PP 180-182.
20. Skoog D A, West D M, Holler FJ: Introduction of analytical chemistry. Sounder college of publishing, Harcourt Brace college publishers. (1994), PP 1-5.
21. Sharma B K, Instrumental method of chemical analysis Meerut. (1999), PP 175-203.

22. Breaux J and Jones K: Understanding and implementing efficient analytical method development and validation. Journal of Pharmaceutical Technology (2003), 5, PP 110-114.
23. Willard, H. y. Merritt L.L, Dean J.A and Settle F.A “Instrumental methods of analysis” 7th edition CBS publisher and distributors, New Delhi, (1991), PP 436-439.
24. ICH Q2A, “validation of analytical methods, definitions and terminology”, ICH Harmonized tripartite guideline, (1999).
25. http://www.medindia.net/doctors/drug_information/benidipine.htm