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# DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF BENIDIPINE IN BULK AND TABLET DOSAGE FORM

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*Abstract:* In this study, we present a new and unique RP-HPLC method for the accurate and cost-effective estimation of benidipine (BEN) in both bulk and tablet dosage forms. The separation of the drug was achieved using an AgilentC18 reverse phase column (100mm×4.6 mm id) packed with 2.5µm particle size. A solvent system comprising methanol: water in the ratio of 40:60%v/v with pH 5.0 was used, and the flow rate was set at 1.0 ml/min. Detection was performed at a wavelength of 237 nm. The retention time for benidipine was found to be 3.33 minutes.The linearity of the method was established over a concentration range of 10µg/ml - 50µg/ml, yielding an impressive correlation coefficient (r^2) value of 0.9997. To assess accuracy, recovery studies were conducted, and the method exhibited a recovery percentage of 100.03%, confirming its accuracy. Additionally, the method demonstrated high sensitivity, with a limit of detection (LOD) and limit of quantitation (LOQ) of 0.03935µg/ml and 0.1192µg/ml, respectively, for Benidipine Hydrochloride.The precision of the method was evaluated by determining the %RSD (relative standard deviation) from multiple studies, which consistently fell within acceptable limits. Furthermore, the developed method was statistically validated according to the guidelines outlined by the International Council for Harmonisation (ICH), ensuring its precision, accuracy, robustness, LOD, LOQ, and recovery parameters met the required standards.In conclusion, this newly developed RP-HPLC method offers a simple, precise, accurate, and cost-effective approach for the estimation of benidipine in bulk and tablet dosage form. Its successful validation, in accordance with ICH guidelines, establishes its reliability and suitability for further analysis and routine use.

***Index Terms*** **- RP-HPLC, Agilent, Benidipine, ICH, LOD, and LOQ.**

## 1. INTRODUCTION:

Hypertension, commonly known as high blood pressure, is a prevalent and significant medical condition affecting a substantial portion of the global population. It is characterized by persistently elevated blood pressure levels, which can put a strain on the cardiovascular system and increase the risk of serious health complications. Hypertension often develops silently, with few noticeable symptoms, making regular blood pressure monitoring essential for early detection and management. With its association to various cardiovascular diseases, such as heart attacks, strokes, and kidney problems, hypertension has become a major public health concern worldwide.

Benidipine is a calcium channel blocker that is used for the treatment of high blood pressure (hypertension). It works by blocking the entry of calcium into smooth muscle cells in the blood vessels, which leads to relaxation and widening of the blood vessels, thereby reducing blood pressure.

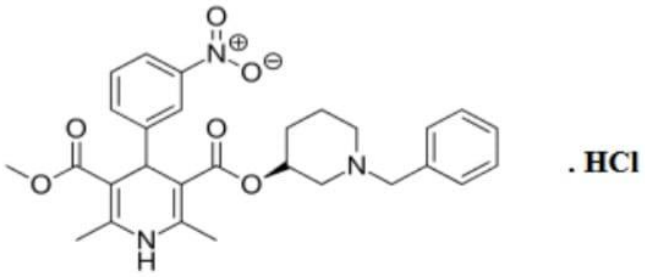


Figure II : Structure of Benidipine Hydrochloride

**Fig. No. 1; Chemical Structure of benidipine**

**IUPAC name:** O5-methyl O3-[(3R)-1-(phenylmethyl)piperidin-3-yl] 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate**.**

**1.1 Analytical chemistry**

Analytical chemistry is used to determine the qualitative and quantitative composition of material under the study. Both these aspects are necessary to understand simple material. Analytical chemistry is divided into two branches quantitative and qualitative. A qualitative analysis gives us the information about nature of sample by knowing about the presence or absence of certain components. A quantitative analysis provides numerical information as to the relative amount of one or more components. For analyzing the drugs sample in bulk pharmaceutical formulation and biological fluids different analytical methods are routinely begins used.

In non-instrumental the conventional and physiological property are uses to analyte the sample. The instrument methods of analysis are based upon the measurement of some physical property of substance using instruments to determine its chemical composition. The instrumental methods are simple, precise and reproducible as compared to classical method. Therefore analytical methods are developed using sophisticated instruments such as UV spectroscopic method. HPLC, HPTLC and TLC, have wide applications in assuring quality and quantity of raw materials and finished.

**1.2 Chromatography**

Chromatography is a technique used for separation of the competent of mixture by continuous distribution of components between two phases (stationary phase) in a continuous manner. Chromatography according to USP can be defined as a procedure by which solute are separated by a differential migration process in a system consisting of two or more phase one of which move continuously in a given direction.

*1.2.1 Principle of chromatography*

I. **Adsorption chromatography:** when the stationary phase is a solid and mobile phase is liquid or gaseous phase. It is called Adsorption chromatography example thin layer chromatography, column chromatography, gas-solid chromatography

II. **Partition chromatography:** When the stationary phase and mobile phase are liquid, it is called partition chromatography example paper partition chromatography, gas-liquid chromatography

III. *Ion exchange chromatography:* ***The stationary phase contains ionic groups like $NR_3$+,$SO_3$, which interact with the ionic groups of the sample molecule***

IV. **Ion pair chromatography:** This technique is also referred to as reversed phase Ion pair chromatography

V. **Affinity chromatography:** This technique used highly specific biochemical interactions, for separation. The stationary phase contains specific groups of molecules which can absorb the sample if certain steric and charge related condition are satisfied

VI. **Size exclusion chromatography:** It separate molecules according their molecular mass. Largest molecules are eluted first and the smallest molecules last. This is method is generally used when a mixture contains compounds with a molecular mass difference of at least 10%. This mode can be further subdivided into gel permissions chromatography. (With organic solvent)And gel filtration chromatography (with aqueous solvent)

*1.2.2 Phase of chromatography*

I. **Normal phase chromatography:** In Normal phase mode the stationary phase is polar and mobile phase is non polar in nature. In this technique, non polar compounds travel faster and are eluted first. This is because of lower affinity between the non-polar compound and the stationary phase. Polar compound are retained for longer times because of their higher affinity with the stationary phase. These compounds, therefore take more times to elute. Normal phase mode of separation is therefore not generally used for pharmaceutical application. Because most of the drugs molecules are polar takes longer time to elute.

II. **Reversed phase chromatography:** It is the most popular mode for analytical and preparative separation of compounds of internet in chemical, biological, pharmaceutical food, and biomedical science in this mode the stationary phase is non polar hydrophobic packing with octal or octal decal functional group bonded to silica gel. And the mobile phase is polar solvent. The polar compounds gets eluted first in this mode and non polar compounds are retained for longer time. As most of the drugs and pharmaceutical are polar in nature.

They are not retained for longer times and hence elute faster the different column used are octal dactyl saline (ODS )or$C_{18}$,$C_8$,$C_4$,CN in the order of increasing polarity of the stationary phase. An aqueous mobile phase allows the use of stationary solute chemical equilibrium such as ionization control, ionization suppression ion pairing and complexation, to control retention and selectivity

**1.3 High performance liquid chromatography ( HPLC )**

High performance liquid chromatography is now one of the most powerful tools in anytical chemistry. It has the ability to separate, identify, and quantify. The compounds those are present in any sample that can be dissolved in a liquid. High performance liquid chromatography (HPLC) is the most accurate anytical method widely used for the quantitative as well as qualitative analysis of drug products. The principle is that a solution of the sample is injected into a column of a porous ma [stationary phase) liquid (mobile phase) is pumped at high pressure through the column. The separation of sample is based on the difference in the rates of migration through the column. A rising from different partition of the sample between the stationary and mobile phase, depending upon the partition behavior of different components, elation at different time takes place.

The sample compound with greater affinity to the stationary layer will travel slower and for a shorter distance in comparison to compound with less affinity which travel faster and for longer distance. HPLC is more versatile than gas chromatography since it is not limited to volatile and thermally stable samples and choice of mobile phase and stationary phase is water

HPLC has numerous advantages

- Simultaneous analysis
- High Resolution

- High sensitivity
- Good repeatability
- Small sample size
- Moderate analysis condition
- Easy to fractionate the sample and purity

**1.3.1 Principle of separation in HPLC:**

The principle of separation in normal phase mode and reverse phase mode is adsorption. When mixtures of components are introduced in to a HPLC column, they travel according to their relative affinities towards the stationary phase. The component which has more affinity towards the adsorbent travels slower. The component which has less affinity towards the stationary phase travels faster. Since no two components have the same affinity towards the stationary phase, the components are separated. Today, HPLC is the most widely used analytical separation method. The method is popular because non-destructive and may be applied to thermally labile compounds (unlike GC); it is also very sensitive technique since it incorporates a wide choice of detection methods. The wide applicability of HPLC as separation methods makes it a valuable separation tool in scientific fields.

**1.3.2 Instrumentation:**

HPLC requires very special apparatus, which includes: -

- Extremely precise gradient mixers.
- HPLC high pressure pumps with very constant flow.
- Unique high accuracy, low dispersion, HPLC sample valves.
- Very high efficiency HPLC columns with inert packing materials.
- High sensitivity low dispersion HPLC detectors.
- High-speed data acquisition systems.
- Low dispersion connecting tubes for valve to column and column to detector.

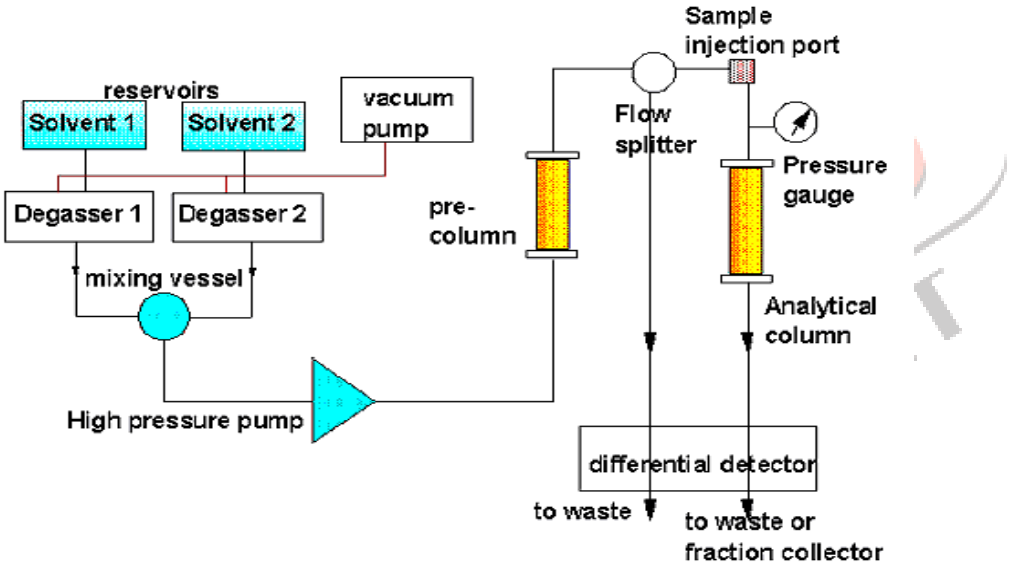


**Fig. No 2; HPLC system**

**A. HPLC Gradient mixers**

HPLC gradient mixers provide a very precise control of solvent composition to maintain a reproducible gradient profile. This can be complicated in HPLC due to the small elution volumes required by many systems. It is much more difficult to produce constant gradient when mixing small volumes than mixing large volumes. For low pressure systems this requires great precision in the operation of the miniature mixing valves used and low dispersion flows throughout the mixer. For multi-pump high pressure systems it requires a very precise control of the flow rate while making very small changes of the flow rate.

**B. HPLC Pumps**

Because of the small particles used in modern HPLC column packing, modern LC pumps need to operate reliably and precisely at pressures of 10,000 p.s.i. or at least 6,000 p.s.i. To operate at these pressures and remain sensibly inert to the wide variety of solvents used, HPLC pumps usually have sapphire pistons, stainless steel cylinders and return valves fitted with sapphire balls and stainless steel seats. For analytical proposes, HPLC pumps should have flow rates that range from 0 to 10 mL/min., but for preparative HPLC, flow rates in excess of 100 mL/min may be required. It is extremely difficult to provide a very constant flow rate at very low flow rate.

**C. HPLC Columns**

Column is often referred to as the heart of the HPLC separation process. HPLC columns are packed with very fine particles (usually a few microns in diameter) to attain the low dispersion that give the high plate counts expected of modern HPLC. LC columns, in general, achieve their separation by exploiting the different intermolecular forces between the solute and the stationary phase and those between the solute and the mobile phase. The column will retain those substances that interact more strongly with the stationary phase than those that interact more strongly with the mobile phase. C-18 and C-8 HPLC Column

- Classic reversed-phases for all general-purpose applications.
- Excellent peak shape and efficiency compared to competitive columns.
- Classic reversed-phase retention and selectivity.
- C-18 is generally more retentive than the C-8.

**C. 1. Internal diameter:**

The internal diameter (ID) of an HPLC column is a critical aspect that determines quantity of analyte that can be loaded onto the column and also influences sensitivity. Larger columns are usually seen in industrial applications such as the purification of a drug product for later use. Low ID columns have improved sensitivity and lower solvent consumption at the expense of loading capacity.

- ✓ LargeD columns (over 10 mm) are used to purify usable amounts of material because of their large loading capacity.
- ✓ Analytical scale columns (4.6 mm) have been the most common type of columns, though smaller columns are rapidly gaining popularity. They are used in traditional quantitative analysis of samples and often use a UV-Vis
- ✓ Narrow-bore columns (1-2 mm) are used for applications when more sensitivity is desired either with special UV-Vis detectors, fluorescence detection or with other detection methods like liquid chromatography-mass spectrometry.
- ✓ Capillary columns (under 0.3 mm) which are used almost exclusively with alternative detection means such as mass spectrometry. They are usually made from fused silica capillaries, rather than the stainless steel tubing that larger columns employ.

**C. 2. Particle size:**

Most traditional HPLC is performed with the stationary phase attached to the outside of small spherical silica particles (very small beads). These particles come in a variety of sizes with 5μm beads being the most common. Smaller particles generally provide more surface area and better separations, but the pressure required for optimum linear velocity increases by the inverse of the particle diameter cubed. This means that changing to particles that are half as big in the same size of column will double the performance, but increases the required pressure by a factor of eight. Larger particles are more often used in non-HPLC applications such as solid-phase extraction.

**C. 3. Pore size:**

Many stationary phases are porous to provide greater surface area. Small pores provide greater surface area while larger pore size has better kinetics especially for larger analytes. For example a protein which is only slightly smaller than a pore might enter the pore but not easily leave once inside.

**D. HPLC Detectors:**

The function of the detector in HPLC is to monitor the mobile phase as it emerges from the column. HPLC detectors use the same detection principles with extra care being given to the small solute elution volumes that result from the combination of high column efficiencies with small volumes. In order to give an accurate chromatographic profile the detector sampling (cell) volume must be a small fraction of the solute elution volume. If the detector volume were larger than the elution volume then peaks that appeared are with flat tops as the whole peak would be resident in the detector at the same time. This means that as column volumes decrease and system efficiencies increase the volume of the detector cell must also decrease. This is of course at odds for the requirement of detector to maintain high sensitivity, as this is usually dependant on having a larger cell volume. Again, this requires careful designing of modern detectors.

**D. 1. Types of Detectors**:

**a) Ultraviolet light detector (UV)** Most widely used and suitable for gradient elution.

- ✓ **Principle:** Absorption of UV visible light as the eluent from the column is passed through a small flow cell held in radiation beam.

**b) Refractive Index detector (RI)**

- Universal analyte detector.
- ✓ **Principle**: Change of refractive index of the eluent from the column with respective to pure mobile phase.

**c) Fluorescence Detector (FD)**

- ✓ **Principle:** Enable fluorescent compounds present in mobile phase to be detected by passing the column eluent through a cell irradiated with ultraviolet light and measuring any resultant fluorescent radiation.

**E. Recorders**

The signals from a detector are recorded as deviation from a baseline .two pen recorders are used with instruments having two detectors .the peak postion along the curve relative to the starting point denotes the particular component .with proper calibration ,the peak is a measure of amount of component in a sample .

**1.4 ICH Guidelines:**

Tests Analytical methods are a critical component of any QA/QC system in pharmaceuticals. A good efficient analytical method is based on sound scientific judgment of analysis and utilizes the most suitable instrumentation and technique available. Two steps are required to evaluate an analytical method. First determine the classification of the method. According to International Conference on Harmonization (ICH) analytical methods are classified in the following groups.

- Identification
- Quantitative measurement for impurity content
- Quantitative tests for active moiety

For analytical method validation, FDA (USA) gives some guidelines in USP and are referred as “Eight steps of analytical method validation”

**1.4.1 Linearity and range**
**1.4.2 Precision**
**1.4.3 Accuracy**

**1.4.4 Robustness**
**1.4.5 Limit of detection (LOD)**
**1.4.6 Limit of quantification (LOQ)**
**1.4.7 Repeatability**
**1.4.8 Ruggedness**

**1.4.1 Linearity and range:**

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in sample within a given range. It should be established across the range of the analytical procedure. Linearity is generally reported as the correlation coefficients, the slope of regression line, etc. r > 0.999. It may be demonstrated directly on the drug substance (by dilution of a standard stock solution) and/or separate weighing of synthetic mixtures of the drug product components, using the proposed procedure. The latter aspect can be studied during investigation of the range. Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. If there is a linear relationship, test results should be evaluated by appropriate statistical methods, for example, by calculation of a regression line by the method of least squares. In some cases, to obtain linearity between assays and sample concentrations, the test data may need to be subjected to a mathematical transformation prior to the regression analysis. Data from the regression line itself may be helpful to provide mathematical estimates of the degree of linearity.

**1.4.2 Precision:**

The precision of an analytical method is determined by assaying a sufficient number of aliquots of a homogeneous sample to be able to calculate statistically valid estimates of standard deviation or relative standard deviation. In the precision results of all samples should have RSD< 2%. Repeatability RSD< 2%, Intermediate precision RSD < 2%.

**1.4.3 Accuracy:**

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of an analytical method should be established across its range. In the case of the assay of a drug in a formulated product, accuracy may be determined by application of the analytical method to synthetic mixtures of the drug product components to which known amount of analyte have been added within the range of the method. Minimum of five test concentrations from 80% 100% to 120% are normally used, for establishment of accuracy in assay of drug substance (or a finished product).

**1.4.4 Robustness:**

The robustness of analytical method 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. The evaluation of robustness should be considered during the development phase and depends on the type of procedure understudy. If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure. One consequence of the evaluation of robustness should be that a series of system suitability parameters (resolution test) is established to ensure that the validity of the analytical procedure is maintained whenever used. Experiments are performed by varying conditions such as:

- influence of variations of pH (±1) in a mobile phase;
- influence of variations in mobile phase composition;
- different columns (different lots and/or suppliers);
- temperature(±20C);
- flow rate (±2)

The method must be robust enough to withstand slight changes and allow routine analysis.

**1.4.5 Limit of detection:**

The limit of detection (LOD) is the lowest amount of analyte in a sample that can be detected, but not necessarily quantities under the stated experimental conditions. Thus, limit test rarely substantiates that amount of analyte is above or below a certain level. The detection limit is usually expressed as the concentration of analyte (e.g. percentage, parts per billion) in the sample. Several approaches for determining the detection limit are possible, depending on whether the procedure is a non-instrumental or instrumental. Approaches other than those listed below may be acceptable

**1.4.6 Limit of quantification:**

The limit of quantification (LOQ) is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products. It is expressed as the concentration of analyte (e.g. percentage, parts per billion) in the sample. Several approaches for determining the quantitation limit possible, depending on whether the procedure is a non-instrumental or instrumental. Approaches other than those listed below may be accept.

**MATERIAL AND METHODS**

**Chemicals;**

Methanol and Distilled water was purchase from K. R. Chemicals, India, Research Lab Fine Chem Industry Mumbai 400 002.

**Glassware's;**

i. Round bottom flask – borosilicate glass
ii. Test tube. – borosilicate glass
iii. Breaker. – borosilicate glass
iv. Conical flask -- borosilicate glass

**Instrumentation**

The HPLC system (Agilent HPLC) consisted of a pump with manual injection facility. The capacity of loop was 20µl. The detector consisted of a Photodiode Array UV-VIS spectrophotometer operated at a wavelength range is 190nm-900 nm. The software used was Chemstation. The column used was Agilent C-18 100 mm × 4.6 mm, partical size.2.5µm). Absorbance measurements were made on PDA detector. The pH meter used was of VSI pH meter (VSI1-B) All the weights were taken on an electronic Contech balance and sonication of mobile phase was done using sonicator ultrasonic's electronic instrument.

## 3. RESULT AND DISCUSSION:

### 3.1Linearity

Linearity of the method was studied by injecting five concentrations of the drug prepared in the mobile phase in the range of 10-50µg/ml for benidipine in triplicate into the HPLC system keeping the injection volume constant. The peak areas were plotted against the corresponding concentrations to obtain the calibration curves.

**Table No. 1 Linearity table for Benidipine**

| Concentration (µg/ml) | Peak area |
|---|---|
| 10 | 1012.4309 |
| 20 | 1932.1981 |
| 30 | 2920.7610 |
| 40 | 3758.9250 |
| 50 | 4766.7017 |

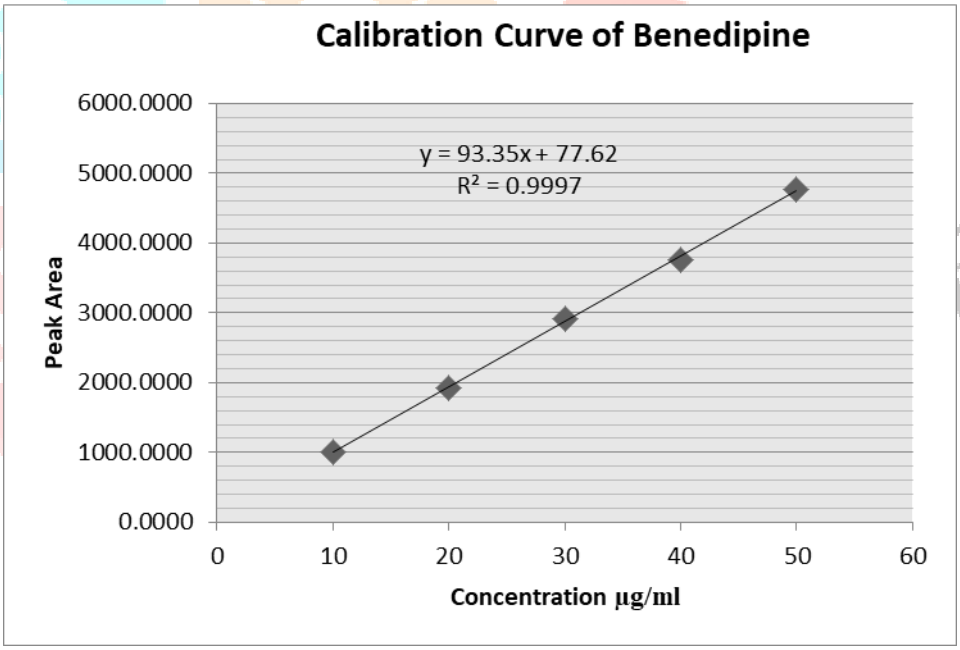


**Fig. No. 3 Calibration curve of benidipine**

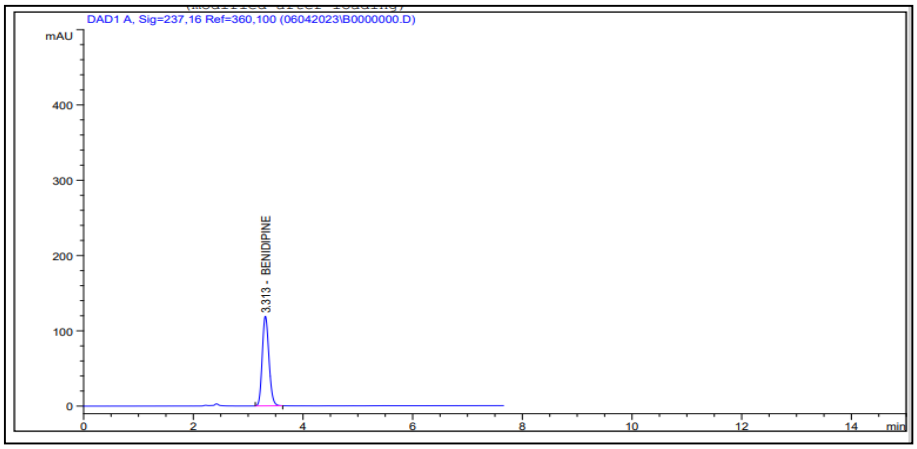


**Fig. No.4 Chromatogram of Benidipine 10ug/ml conc**

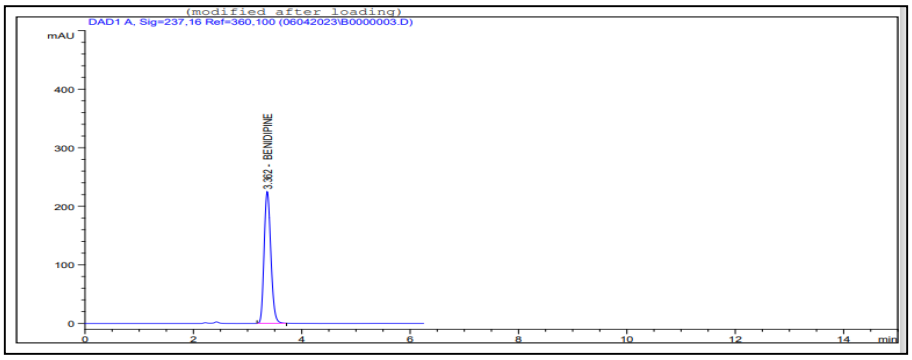


**Fig. No.5Chromatogram of Benidipine 20 ug/ml conc**

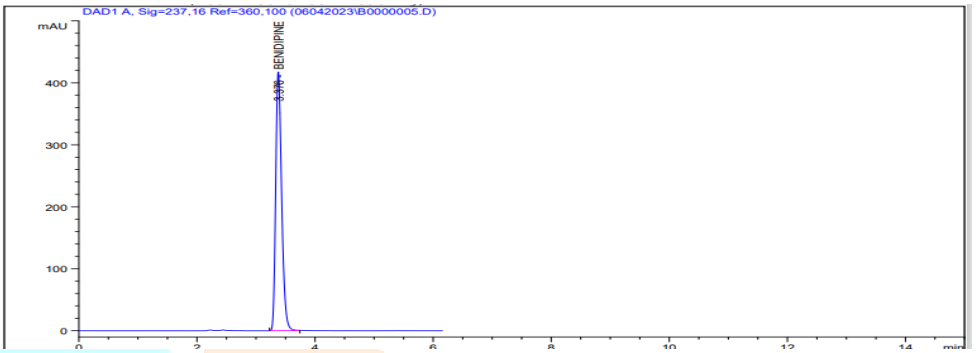


**Fig. No.6 Chromatogram of Benidipine 30 ug/ml conc**

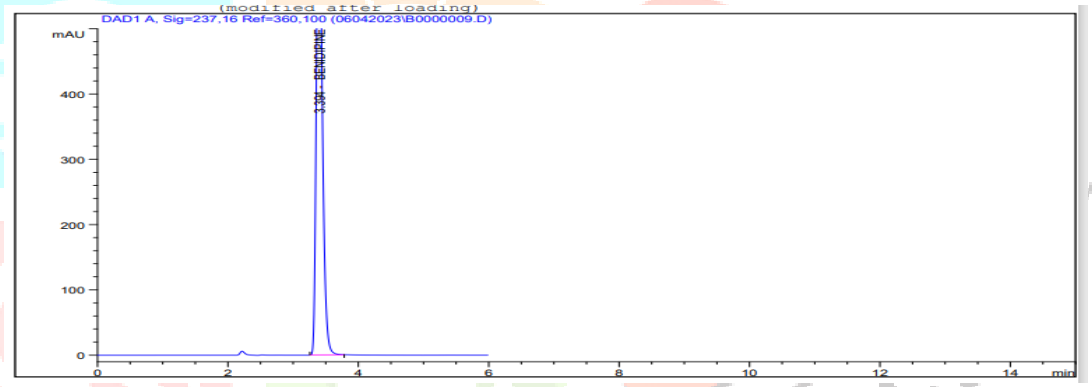


**Fig. No.7 Chromatogram of Benidipine 40 ug/ml conc**

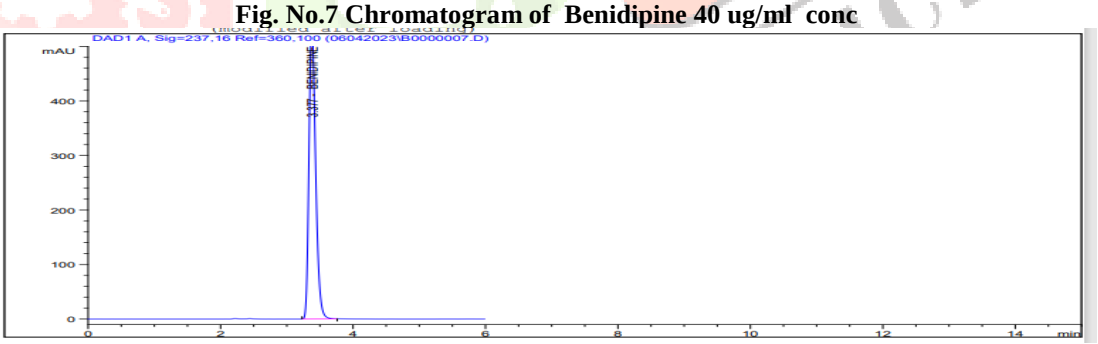


**Fig. No.8 Chromatogram of Benidipine 50 ug/ml conc**

**3.2 Precision;**

Precision of the method was verified by repeatability and intermediate precision studies. The repeatability of sample application and measurement of peak area for active compounds were expressed in terms of %RSD (relative standard deviation). Repeatability studies were performed by analyses of concentrations 30µg/ml of benidipine for HPLC on the same day. Intermediate precision of the method was checked by repeating these studies on two different days.

**Table No. 2 Intraday and Interday data for Precision Studies for benidipine**

| Intraday | | | | | | |
|---|---|---|---|---|---|---|
| **Sr. No.** | **Conc.** | **Area** | **Amount found** | **% amount found** | **SD** | **%RSD** |
| 1 | 20 | 1931.09 | 19.86 | 99.30 | 0.53 | 0.03 |
| 2 | 30 | 2922.24 | 30.48 | 101.59 | 0.42 | 0.01 |
| 3 | 40 | 3760.10 | 39.46 | 98.66 | 2.05 | 0.05 |
| **Interday** | | | | | | |
| 1 | 20 | 1945.30 | 19.59 | 99.35 | 3.62 | 0.52 |
| 2 | 30 | 2919.93 | 29.94 | 99.13 | 0.70 | 0.07 |
| 3 | 40 | 3878.38 | 40.06 | 100.22 | 0.65 | 0.05 |

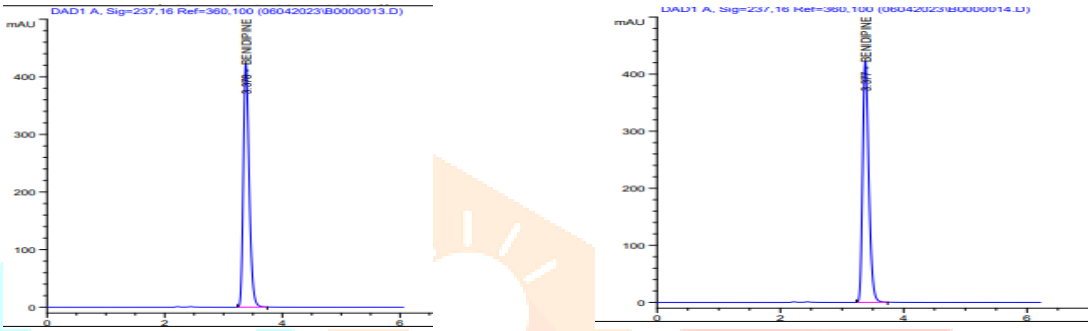


**Fig. No. 9 Chromatogram of Benidipine (20ug/ml) concentration for precision study**

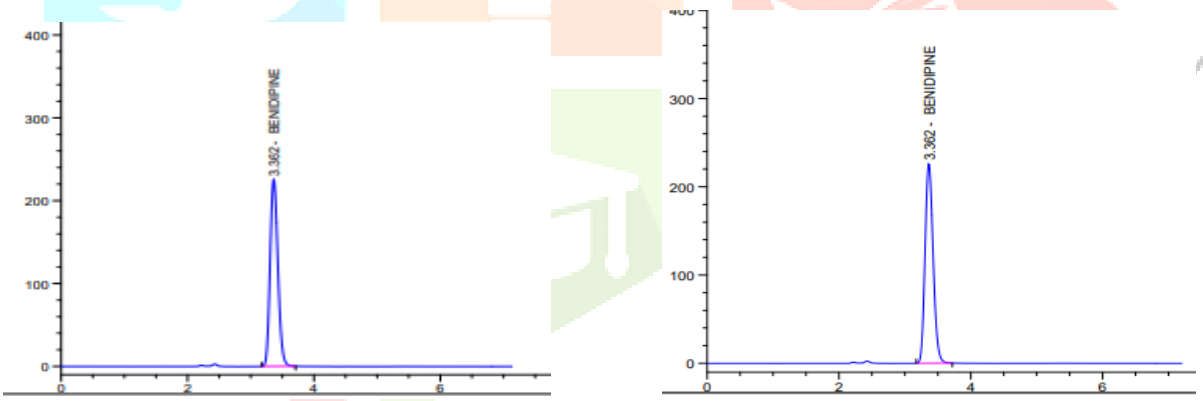


**Fig. No.10 Chromatogram of Benidipine (30ug/ml) concentration for precision study**

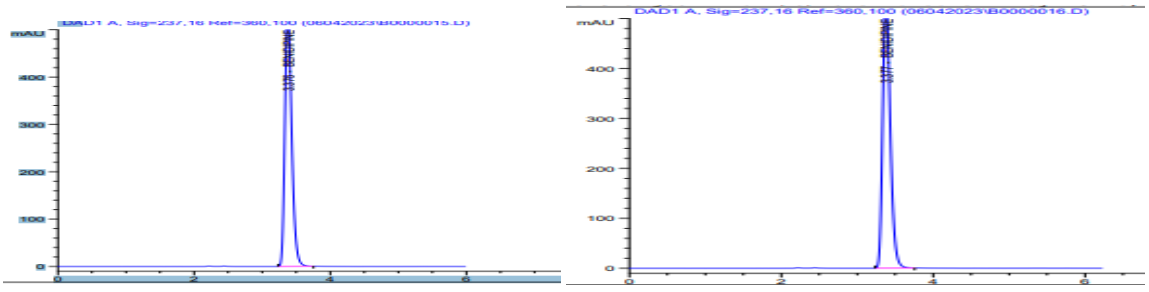


**Fig. No. 11 Chromatogram of Benidipine (40ug/ml)concentration for precision study**

### 3.3 Accuracy

For RP-HPLC method recovery studies was carried out by spiking known amount of standard drug corresponding to 80%, 100% and 120% w/w of label claim had been added to marketed drug sample (Standard addition method). At each level of the amount three determinations were performed and the results obtained were compared with expected results.

**Table No.3 Accuracy data for benidipine**

| 80% | | | | | | |
|---|---|---|---|---|---|---|
| **Drug taken** | **Amount drugs added** | **Area** | **Amount found** | **%amount found** | **SD** | **RSD** |
| 10 | 8 | 1750.13 | 17.91 | 99.5 | 0.051 | 0.28 |
| 10 | 8 | 1756.89 | 17.98 | 99.8 | 0.051 | 0.64 |
| 10 | 8 | 1761.32 | 18.04 | 100.22 | 0.034 | 0.53 |
| **100%** | | | | | | |
| 10 | 10 | 1950.09 | 20.05 | 100.25 | 0.010 | 0.52 |
| 10 | 10 | 1951.47 | 20.07 | 100.35 | 0.10 | 0.14 |
| 10 | 10 | 1945.86 | 19.98 | 99.90 | 0.011 | 0.42 |
| **120%** | | | | | | |
| 10 | 12 | 2133.93 | 22.02 | 100.09 | 0.026 | 0.17 |
| 10 | 12 | 2130.52 | 21.99 | 99.95 | 0.016 | 0.11 |
| 10 | 12 | 2135.65 | 22.05 | 100.24 | 0.024 | 0.27 |

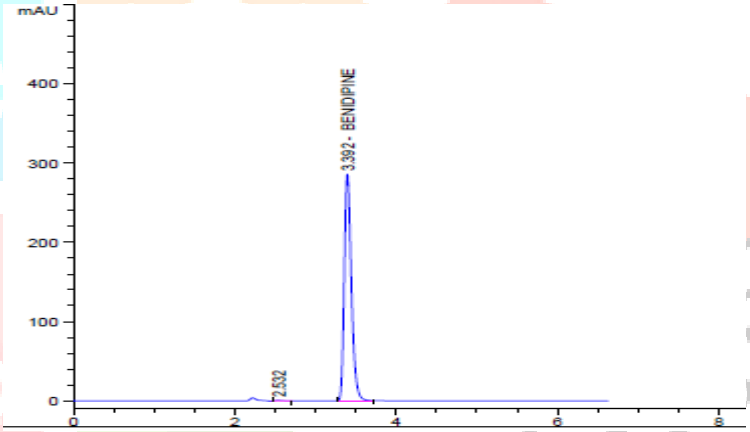


**Fig. No.12 Chromatogram of benidipine 80 % Conc. of accuracy study**

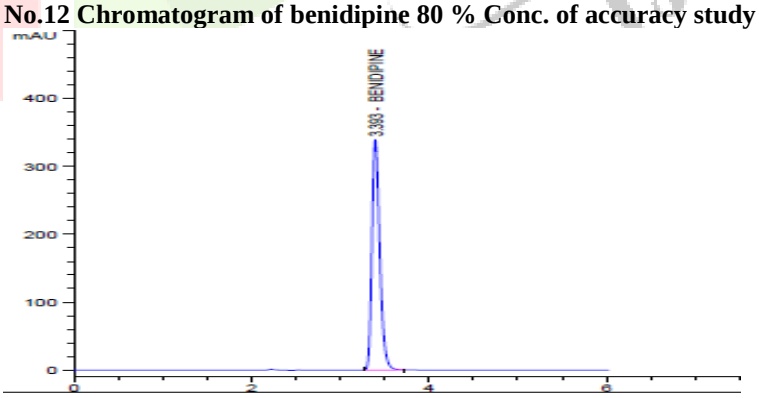


**Fig. No.13 Chromatogram of benidipine 100 % Conc. of accuracy study**

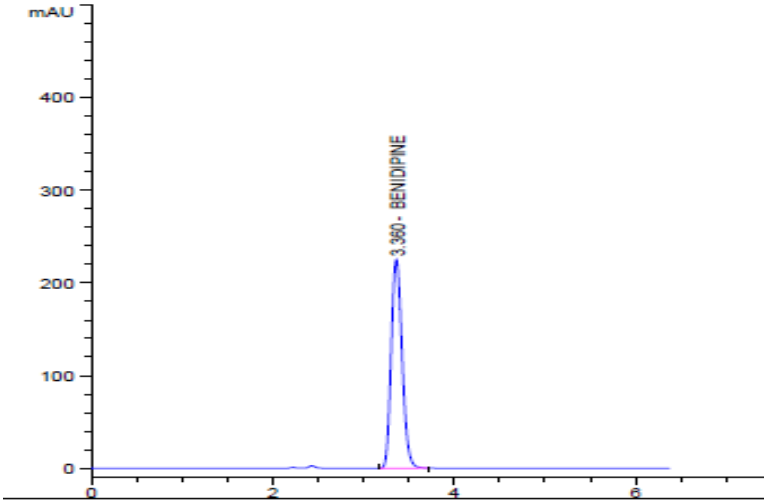


**Fig. No.14 Chromatogram of benidipine 120 % Conc. of accuracy study**

### 3.4 Repeatability

Implementing the procedure under chromatographic condition of experimental section, the homologous mixture of 20µg/ml of each selected analytes was injected six times with similar procedure within a same day. The% RSD was calculated and found it is less than 2%.

**Table No.4 Repeatability data for Benidipine**

| Sr. No. | Concentration in µg/ml | Area | Amount found | Amount mean | SD | %RSD |
|---|---|---|---|---|---|---|
| 1 | 20 | 2797.856 | 20.23 | 101.15 | 0.62 | 0.023 |
| 2 | 20 | 2788.32 | 20.12 | 100.6 | | |
| 3 | 20 | 2792.65 | 19.95 | 99.75 | | |
| 4 | 20 | 2794.83 | 20.05 | 100.25 | | |
| 5 | 20 | 2796.74 | 20.08 | 100.4 | | |

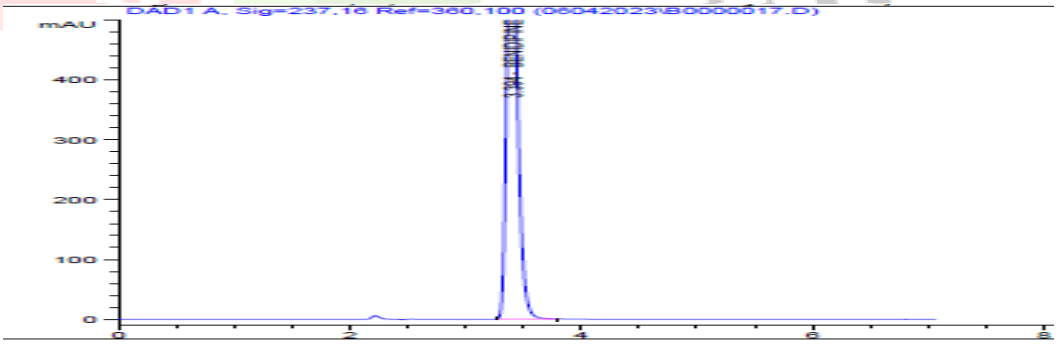


**Fig. No.15 Chromatogram of benidipine 20 µg/ml concentration**

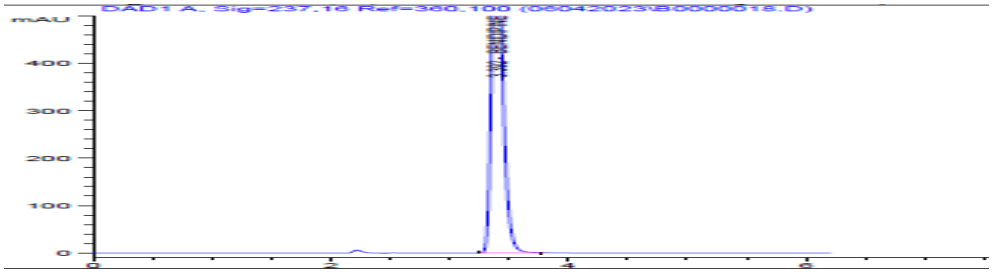


**Fig. No.16 Chromatogram of benidipine 20 µg/ml concentration**

### 3.5 Robustness

To evaluate robustness of RP-HPLC method, few parameters were deliberately varied. The parameters included variation of flow rate, pH, percentage ratio of Solvent used in the mobile phase and wavelength changes.

**Table No.5 Robustness data for Benidipine**

| Factor | Concentration in µg/ml | ( Rt min ) |
|---|---|---|
| **A. Flow Rate (ml/min)** | | |
| 0.9 | 20 µg/ml | 3.749 |
| 1.1 | 20 µg/ml | 3.069 |
| Mean+SD | | 3.409+3.75 |
| **B. Mobile Phase** | | |
| 39+61 | 20 µg/ml | 3.436 |
| 41+59 | 20 µg/ml | 3.376 |
| Mean+SD | | 3.406+9.58 |
| **C. Wavelength Change** | | |
| 237nm | 20 µg/ml | 3.376 |
| 238nm | 20 µg/ml | 3.376 |
| Mean+SD | | 3.376+3.55 |

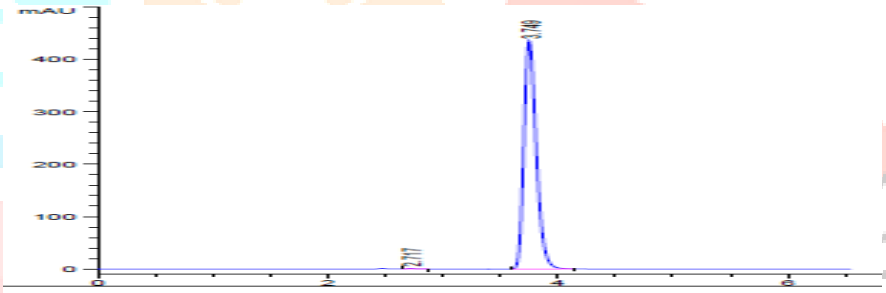


**Fig. No.17 Chromatogram of benidipine Flow rate 0.9ml/min**

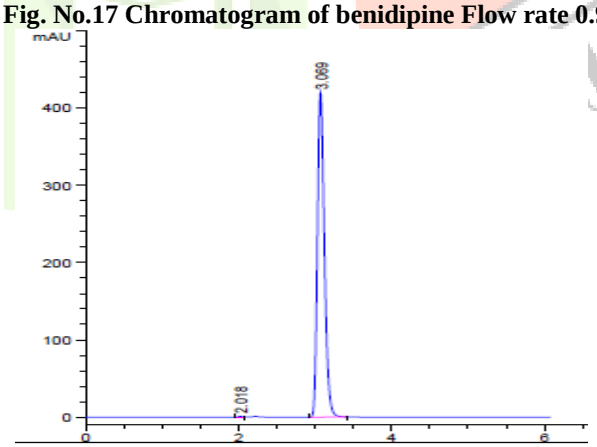


**Fig. No.18 Chromatogram of benidipine Flow rate 1.1 ml/min**

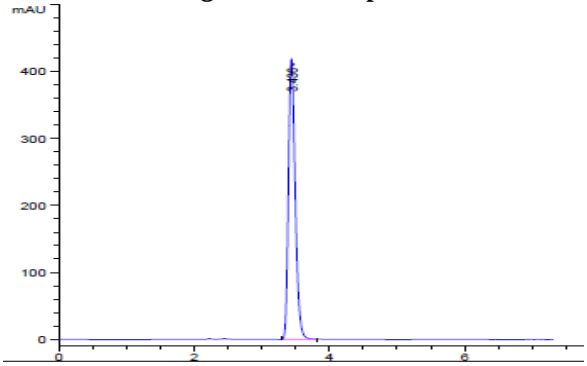


**Fig. No.19 Chromatogram of benidipine Mobile phase change 39:61% v/v**

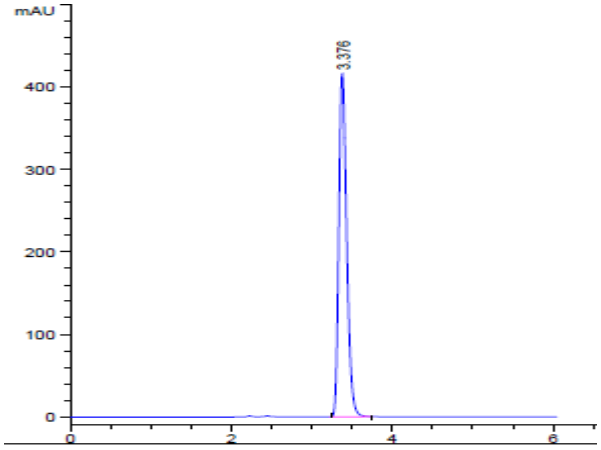


**Fig. No.20 Chromatogram of benidipine Mobile phase change 41:59% v/v**

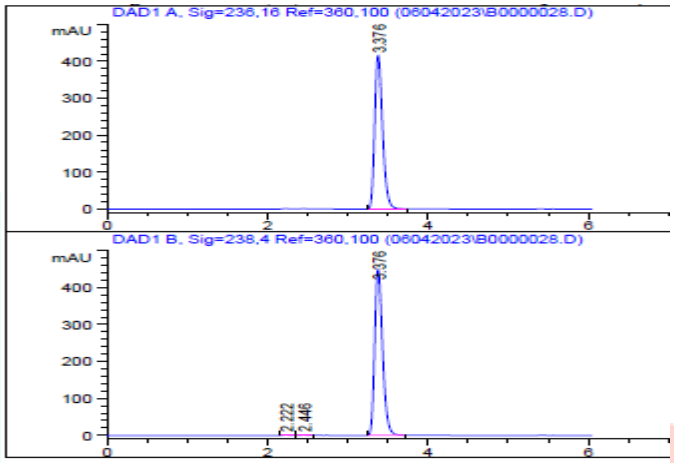


**Fig. No. 21 Chromatogram of benidipine wavelength changes 236nm and 238nm**

### 3.6 HPLC Assay of Tablet formulation

Twenty tablet of (Benidipine Hydrochloride mankind pharma LTD) were weighed accurately and powdered weigh equivalent to 50 mg of benidipine hydrochloride was weighed accurately and transferred to 100.0 ml volumetric flask to get 500µg/ml. The solution was filtered through filter paper no. 41; 10 ml of this filtrate was further diluted to 50 ml Distilled Water. From this solution, 3ml pipette out and diluted to 10ml to get the final concentration of 30µg/ml of benidipine hydrochloride. The solution was scanned in the range of 200-400 nm against blank. Absorbance's were recorded at wavelength 237 nm. The concentration of drug was then calculated.

**Table No.6 Assay of Tablet Benidipine Hydrochloride 4mg tablet**

| Sr. No. | Name of drug | Label claim (4mg) | Peak area | Amount of drugs found | %Drug content Found | %RSD |
|---|---|---|---|---|---|---|
| 1 | Benidipine | 30 µg/ml | 2905.12 | 30.09 | 100.33 | 0.59 |
| 2 | Benidipine | 30 µg/ml | 2988.23 | 30.29 | 100.76 | 0.27 |

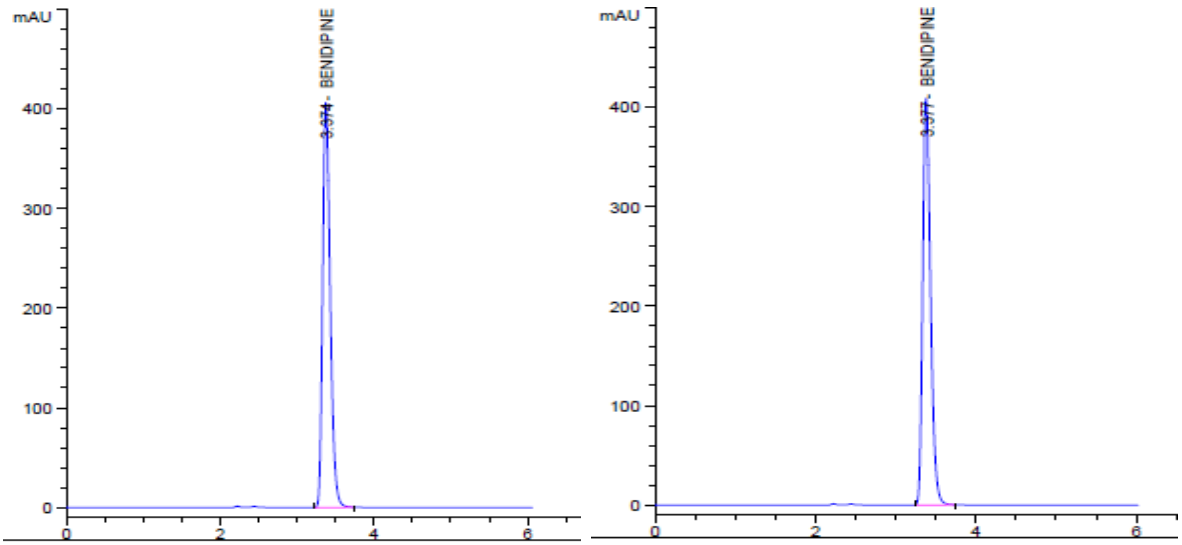


**Fig. No. 21 Assay chromatogram of benidipine 30 µg/ml concentration**

### 3.7 LOD and LOQ

The LOD and LOQ were found to be 0.03935µg/ml and 0.1192µg/ml for Benidipine Hydrochloride these low values of LOD and LOQ confirmed that the method is sensitive.

| Parameters | Observation |
|---|---|
| LOD (µg/ml) | 0.03935 |
| LOQ (µg/ml) | 0.1192 |

## 4. CONCULUSIONS

In conclusion, the present study focused on the development and validation of RP-HPLC methods for the estimation of benidipine in bulk and tablet dosage form, following the guidelines set forth by the International Council for Harmonisation (ICH). The developed methods were found to be accurate, linear, precise, and cost-effective, making them suitable for the simultaneous estimation of benidipine.The validation process confirmed that the methods complied with the ICH guidelines in terms of accuracy, precision, linearity, and other required parameters. The results obtained for the estimation of benidipine in both bulk and tablet dosage forms were within the acceptable range. This demonstrates the reliability and suitability of the developed RP-HPLC methods for further analysis.Based on these findings, it can be concluded that the developed methods offer a robust and efficient approach for the estimation of benidipine. Researchers and analysts can utilize these methods confidently for routine analysis of benidipine in bulk and tablet dosage form, enabling accurate and reliable results. The cost-effectiveness of the methods further enhances their practical utility in pharmaceutical research and quality control laboratories.

## 5. AKNOWLEGEMENT

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