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**Research**

# Reverse Phase High Performance Liquid Chromatography Method Development and Validation for Estimation of Benazepril and Hydrochlorothiazide HCL in Pure and Pharmaceutical Dosage Form

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## Abstract

A novel, accurate, and reproducible reverse phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Benazepril and Hydrochlorothiazide HCl in pure form and pharmaceutical dosage formulations. Chromatographic separation was achieved using a Waters HPLC system equipped with an autosampler and PDA detector (996 model), on an Altima C18 column (4.6 × 150 mm, 5 µm) maintained at 35°C. The optimized mobile phase consisted of Water: Methanol: Acetonitrile (25:65:10 v/v/v), delivered at a flow rate of 1 mL/min. Detection was carried out at 265 nm, with an injection volume of 10 µL and a total run time of 14 minutes. The method was validated as per ICH guidelines, showing excellent linearity, accuracy, precision, specificity, and robustness. The results confirmed that the proposed method is suitable for routine quality control analysis of Benazepril and Hydrochlorothiazide HCl in bulk and combined dosage forms.

**Keywords:** RP-HPLC, Hydrochlorothiazide HCl, Benazepril, Altima C18 column (4.6 × 150 mm, 5 µm), simultaneous estimation, validation, Waters HPLC system.

## 1. INTRODUCTION

HPLC is an analytical technique in which solutes are resolved by differential rates of elution as they pass through a chromatographic column. The method of separation by this instrument is governed by distribution between the mobile phase and stationary phase. The instrumentation is made-up of eight basic components, mobile phase reservoir, solvent delivery system, sample introduction device, column, detector, waste reservoir, connective tubing and computer, integrator or recorder. The successful use of HPLC for the possible problem requires the

right combination of variety of operating conditions such as the type of column packing and mobile phase, column length and diameter, mobile phase flow rate, column temperature and sample size [1].

Now a day reversed-phase chromatography is the most commonly used separation technique in HPLC due to its broad application range. It is estimated that over 65% (possibly up to 90%) of all HPLC separations are carried out in the reversed phase mode. The reasons for this include the simplicity, versatility and scope of the reversed-phase method as it is able to handle compounds of a diverse polarity and molecular mass [2-4].

**PRINCIPLE**

In isocratic HPLC the analyte is forced through a column of the stationary phase (usually a tube packed with small round particles with a certain surface chemistry) by pumping a liquid (mobile phase) at high pressure through the column. The sample to be analyzed is introduced in a small volume to the stream of mobile phase and is retarded by specific chemical or physical interactions with the stationary phase as it traverses the length of the column. The amount of retardation depends on the nature of the analyte, stationary phase and mobile phase composition. The time at which a specific analyte elutes (comes out of the end of the column) is called the retention time and is considered a reasonably unique identifying characteristic of a given analyte. The use of pressure increases the linear velocity (speed) giving the components less time to diffuse within the column, leading to improved resolution in the resulting chromatogram. Common solvents used include any miscible combinations of water or various organic liquids (the most common are methanol and acetonitrile). Water may contain buffers or salts to assist in the separation of the analyte components. A further refinement to HPLC has been to vary the mobile phase composition during the analysis, this is known as gradient elution. A normal gradient for reverse phase chromatography might start at 5% methanol and progress linearly to 50% methanol over 25 minutes, depending on how hydrophobic the analyte is. The gradient separates the analyte mixtures as a function of the affinity of the analyte for the current mobile phase composition relative to the stationary phase. This partitioning process is similar to that which occurs during a liquid-liquid extraction but is continuous, not step-wise. In this example, using a water/methanol gradient, the more hydrophobic components will elute (come off the column) under conditions of relatively high methanol; whereas the more hydrophilic compounds will elute under conditions of relatively low methanol. The choice of solvents, additives and gradient depend on the nature of the stationary phase and the analyte. Often a series of tests are performed on the analyte and a number of generic runs may be processed in order to find the optimum HPLC method for the analyte - the method which gives the best separation of peaks.

**APPLICATIONS**

Preparative HPLC refers to the process of isolation and purification of compounds. Important is the degree of solute purity and the throughput, which is the amount of compound produced per unit time. This differs from analytical HPLC, where the focus is to obtain information about the sample compound. The information that can be obtained includes identification, quantification, and resolution of a compound. Chemical Separations can be accomplished using HPLC by utilizing the fact that certain compounds have different migration rates given a particular column and mobile phase. Thus, the chromatographer can separate compounds (more on chiral separations) from each other using HPLC; the extent or degree of separation is mostly determined by the choice of stationary phase and mobile phase.

Identification of compounds by HPLC is a crucial part of any HPLC assay. In order to identify any compound by HPLC a detector must first be selected. Once the detector is selected and is set to optimal detection settings, a separation assay must be developed. The parameters of this assay should be such that a clean peak of the known sample is observed from the chromatograph. The identifying peak should have a reasonable retention time and should be well separated from extraneous peaks at the detection levels which the assay will be performed. To alter the retention time of a compound, several parameters can be manipulated. The first is the choice of column, another is the choice of mobile phase, and last is the choice in flow rate. All of these topics are reviewed in detail in this document. Identifying a compound by HPLC is accomplished by researching the literature and by trial and error. A sample of a known compound must be utilized in order to assure identification of the unknown compound. Identification of compounds can be assured by combining two or more detection methods.

**1.1. Types of HPLC methods**

1. Reverse Phase HPLC Reversed phase chromatography has found both analytical and preparative applications in the area of biochemical separation and purification. Molecules that possess some degree of hydrophobic character can be separated by reversed phase chromatography with excellent recovery and resolution [5]. Uses water-organic as mobile phase, columns may be C18 (ODS), C8, phenyl, Trimethyl Silane (TMS), cyano as a stationary phase. It is first choice for most samples especially neutral or non-ionized compounds, that dissolve in water organic mixtures.

2. Normal Phase HPLC In normal-phase chromatography, the stationary phase is polar and the mobile phase is nonpolar. In reversed phase we have just the opposite; the stationary phase is nonpolar and the mobile phase is

polar. Typical stationary phases for normal-phase chromatography are silica or organic moieties with cyano and amino functional groups. In this the mixtures of organic solvents for mobile phase and columns i.e. cyano, diol and amino silica can be used as stationary phase. It is first choice for mixtures of isomers and for preparative scale HPLC and second choice for lipophilic samples that cannot dissolve well in water organic mixtures [6].

**Selectivity and Specificity**

Selectivity of the analytical method is defined as the degree to which a method can quantify the analyte in the presence of interferents [11]. The other components which may be present include impurities, degradants, matrix, etc. The term specificity and selectivity is often used interchangeably. The term specific generally refers to a method that produces a response for a single analyte only, while the term selective refers to a method that provides responses for a number of chemical entities that may or may not be distinguished from each other. The International Union of Pure and Applied Chemistry (IUPAC) have expressed the view that "Specificity is the ultimate of Selectivity'. The IUPAC discourages use of the term specificity and instead encourages the use of the term selectivity. Specificity study of the chromatographic method is performed by the separation of the analyte from the other potential components such as impurities, degradants or excipients etc. In addition, forced degradation studies are carried out to challenge the method. The forced degradation studies are of particular importance when the impurities are not available. During forced degradation studies, the sample is subjected to the stressed conditions of light, heat, humidity, acid/base hydrolysis and oxidation. The scheme which is generally used for forced degradation studies for drug substances and drug products are summarized in table 1 below [12]. The selectivity of chromatographic methods may be assessed by examination of peak homogeneity or peak purity test. Peak purity test shows that there is no co-elution of any sample component. For this, peak purity assessment is done by using PDA or MS detectors. Representative chromatograms with peaks labelled should be included with resolution, plate count and tailing factor reported in the validation report.

**Linearity**

Linearity of a method is its ability to obtain test results that are directly proportional to the sample concentration over a given range. For HPLC methods, the linear relationship between detector response (peak area and height) and sample concentration is determined. The relationship can be demonstrated directly on drug substance by dilution of standard stock or by separate weighing of the sample components, using the proposed procedures. Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. If there is linear relationship, test results should be evaluated by appropriate statistical methods, for example, by regression analysis. Data from the regression line is helpful to provide mathematical estimates of the degree of linearity. It is generally expressed in terms of variance around the slope of regression line. In some cases, the analytical responses should be described by the appropriate function of the analyte concentration. The widely used linearity ranges and acceptance criteria for various pharmaceutical methods are listed in the table 2 [13].

**Precision**

Precision of an analytical method expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Repeatability is the precision under the same operating conditions over a short interval of time. It is also termed as intra-assay precision. It is assessed by making six sample determinations at 100% concentration or by preparing three samples at three concentrations in triplicates covering the specified range for the procedure. It involves repeated determination of same sample. Intermediate precision expresses within laboratories variation: different days, different analyst, different equipments, etc. It is the term synonymous with the term 'ruggedness', defined by USP. The extent to which intermediate precision should be established depends on the circumstances under which the procedure is intended to be used. To study intermediate precision, use of an experimental design is encouraged. The intermediate precision is generally studied by multiple preparations of sample and standard solution. Reproducibility is the precision obtained by analysis between laboratories. It is generally assessed during collaborative studies at the time of technology or method transfer. It is assessed by means of an interlaboratory trial. The precision data is generally expressed in the form of standard deviation, relative standard deviation and confidence intervals. To ensure precision of method for major analytes, RSD should be ≤ 2 %. For low level impurities, RSD of 5-10 % is usually acceptable [14].

**EXPERIMENTAL METHODS**

**INSTRUMENTS USED**

HPLC WATERS, software: Empower 2, Alliance 2695 separation module. 996 PDA detector.
pH meter Lab India
Weighing machine Sartorius

Volumetric flasks Borosil
Pipettes and Burettes Borosil

**CHEMICALS USED**
Benazepril Provided by Sura Pharma labs
Hydrochlorothiazide Provided by Sura Pharma labs
Water and Methanol for HPLC LICHROSOLV (MERCK)
Acetonitrile for HPLC Merck

**HPLC METHOD DEVELOPMENT**
**TRAILS**
**Preparation of standard solution**
Accurately weigh and transfer 10 mg of Benazepril and Hydrochlorothiazide 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 0.1 ml of the above Benazepril and Hydrochlorothiazide 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.

**RESULTS AND DISCUSSION**
**(Optimized chromatogram)**

| | | |
|---|---|---|
| Column | : | Altima C18 (4.6×150mm, 5µ) |
| Temperature | : | 35ºc |
| Mobile phase | : | Water: Methanol: ACN (25:65:10v/v/v) |
| Flow rate | : | 1ml/min |
| Wavelength | : | 265 nm |
| Injection volume | : | 10 µl |
| Run time | : | 14 min |

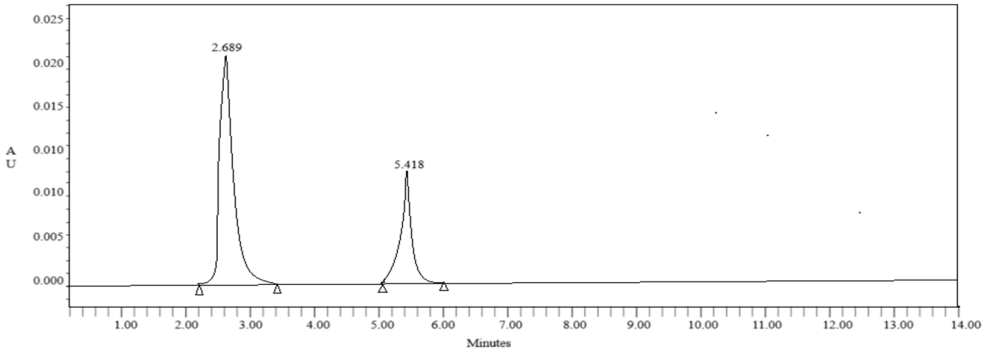



**Fig 1: Optimized Chromatogram (Standard)**

**Table 1: Optimized Chromatogram (Standard)**

| S.No | Name | RT | Area | Height | USP Tailing | USP Plate Count | USP Resolution |
|---|---|---|---|---|---|---|---|
| 1 | Benazepril | 2.689 | 2391746 | 39726 | 1.2 | 9028 | |
| 2 | Hydrochlorothiazid | 5.418 | 194627 | 8497 | 1.1 | 7398 | 7.4 |

**Observation:**
This trial shows improper separation sample peaks, baseline and show very less plate count in the chromatogram. So it's required more trials to obtain good peaks.
From the above chromatogram it was observed that the Benazepril and Hydrochlorothiazide peaks are well separated and they show proper retention time, resolution, peak tail and plate count. So, it's optimized trial.

**Optimized Chromatogram (Sample)**

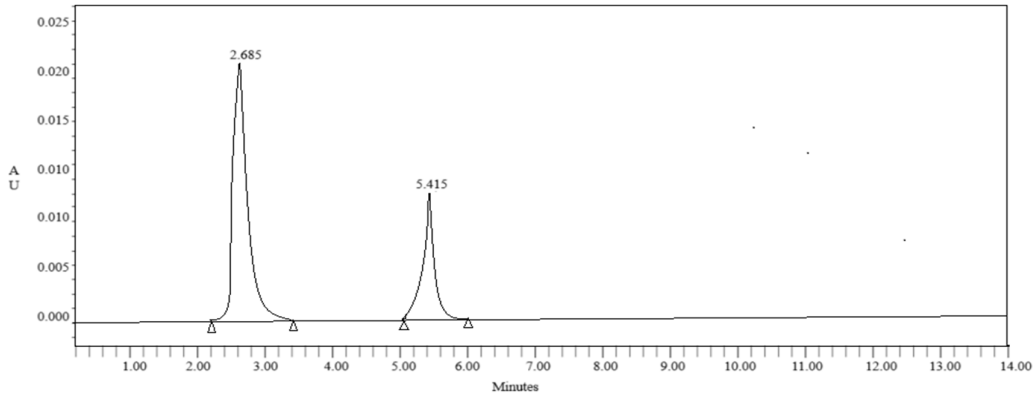



**Fig 2: Optimized Chromatogram (Sample)**

**Table 2: Optimized Chromatogram (Sample)**

| S.No | Name | RT | Area | Height | USP Tailing | USP Plate Count | USP Resolution |
|---|---|---|---|---|---|---|---|
| 1 | Benazepril | 3.213 | 2381649 | 391846 | 1.2 | 9472 | |
| 2 | Hydrochlorothiazide | 5.478 | 191057 | 8104 | 1.1 | 8936 | 7.5 |

**Acceptance criteria:**

- Resolution between two drugs must be not less than 2
- 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**

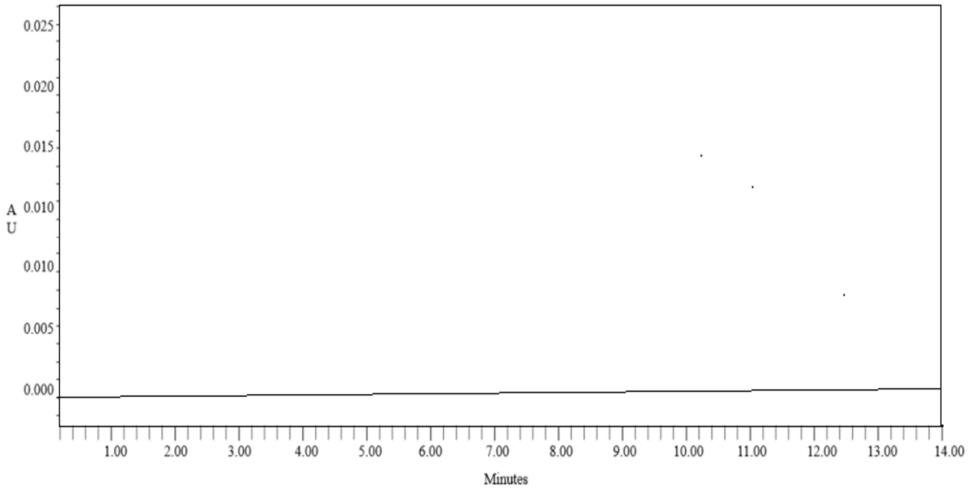



**Fig 3: Chromatogram showing blank (mobile phase preparation)**

**System suitability**

**Table 3: Results of system suitability for Benazepril**

| S. No | Peak Name | RT | Area (µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Benazepril | 2.698 | 2391746 | 394171 | 8952 | 1.2 |
| 2 | Benazepril | 2.696 | 2391647 | 381946 | 9561 | 1.2 |
| 3 | Benazepril | 2.690 | 2381647 | 391746 | 6572 | 1.2 |
| 4 | Benazepril | 2.688 | 2385631 | 386562 | 6452 | 1.2 |
| 5 | Benazepril | 2.694 | 2385635 | 389164 | 7452 | 1.2 |
| **Mean** | | | 2387261 | | | |
| **Std. Dev.** | | | 4363.771 | | | |
| **% RSD** | | | 0.182794 | | | |

**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.

**Table 4: Results of system suitability for Hydrochlorothiazide**

| S. No | Peak Name | RT | Area (µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Hydrochlorothiazide | 5.425 | 198362 | 7917 | 5272 | 1.1 |
| 2 | Hydrochlorothiazide | 5.423 | 197486 | 7486 | 6291 | 1.1 |
| 3 | Hydrochlorothiazide | 5.420 | 198354 | 7859 | 6184 | 1.1 |
| 4 | Hydrochlorothiazide | 5.419 | 197352 | 7926 | 7145 | 1.1 |
| 5 | Hydrochlorothiazide | 5.422 | 198453 | 7946 | 6946 | 1.1 |
| **Mean** | | | 198001.4 | | | |
| **Std. Dev.** | | | 535.1774 | | | |
| **% RSD** | | | 0.27029 | | | |

**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 Benazepril and Hydrochlorothiazide in drug product.

**Assay (Standard)**

**Table 5: Peak results for assay standard**

**Benazepril**

| S. No | Name | RT | Area | Height | USP Tailing | USP Plate Count |
|---|---|---|---|---|---|---|
| 1 | Benazepril | 2.698 | 2397162 | 397161 | 1.2 | 9472 |
| 2 | Benazepril | 2.696 | 2394721 | 389173 | 1.2 | 9745 |
| 3 | Benazepril | 2.690 | 2389461 | 391723 | 1.2 | 8917 |

**Hydrochlorothiazide**

| S.No | Name | RT | Area | Height | USP Tailing | USP Plate Count | Resolution |
|---|---|---|---|---|---|---|---|
| 1 | Hydrochlorothiazide | 5.425 | 198462 | 7811 | 1.1 | 8492 | 7.49 |
| 2 | Hydrochlorothiazide | 5.423 | 198472 | 8193 | 1.1 | 8916 | 7.52 |
| 3 | Hydrochlorothiazide | 5.420 | 198735 | 7972 | 1.1 | 9372 | 7.44 |

**Assay (Sample)**

**Table 6: Peak results for Assay sample-**

**Benazepril**

| S. No | Name | RT | Area | Height | USP Tailing | USP Plate Count |
|---|---|---|---|---|---|---|
| 1 | Benazepril | 5.420 | 2391741 | 381612 | 1.2 | 9472 |
| 2 | Benazepril | 5.419 | 2389166 | 391746 | 1.2 | 8927 |
| 3 | Benazepril | 5.422 | 2361731 | 381634 | 1.2 | 9017 |

**Hydrochlorothiazide**

| S. No | Name | RT | Area | Height | USP Tailing | USP Plate Count | Resolution |
|---|---|---|---|---|---|---|---|
| 1 | Hydrochlorothiazide | 5.420 | 198641 | 8174 | 1.1 | 9284 | 7.18 |
| 2 | Hydrochlorothiazide | 5.419 | 196547 | 8942 | 1.1 | 8974 | 7.44 |
| 3 | Hydrochlorothiazide | 5.422 | 194027 | 7294 | 1.1 | 9017 | 7.38 |

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

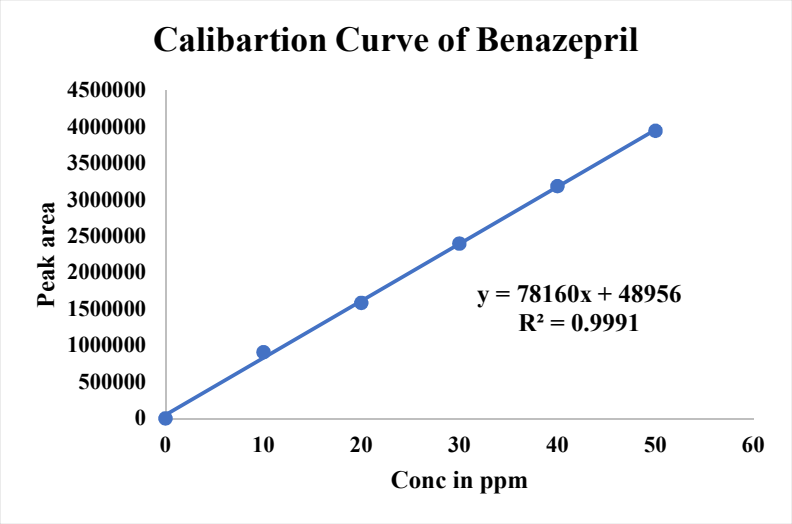
The % purity of Benazepril and Hydrochlorothiazide in pharmaceutical dosage form was found to be 99.2%.

**LINEARITY**

**CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:**

**Benazepril**

| Concentration µg/ml | Average Peak Area |
|---|---|
| 10 | 909889 |
| 20 | 1583641 |
| 30 | 2395378 |
| 40 | 3185089 |
| 50 | 3943725 |

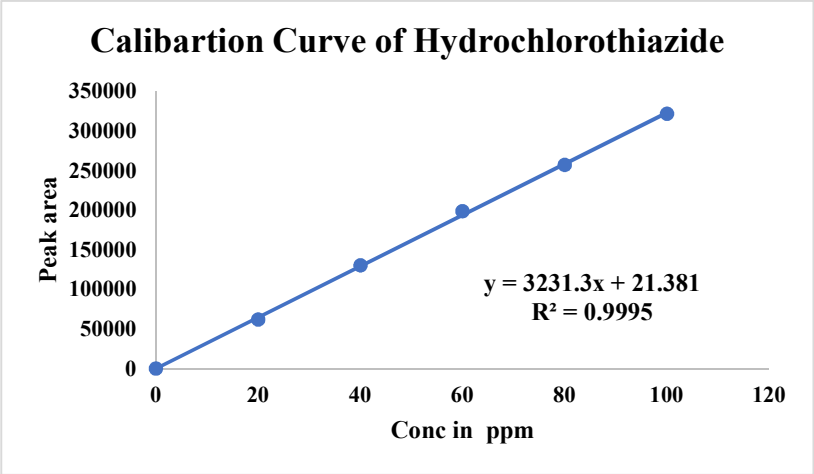



**LINEARITY PLOT**

Correlation Coefficient (r) is 0.99, and the intercept is 48956. These values meet the validation criteria.

**Hydrochlorothiazide**

| Concentration µg/ml | Average Peak Area |
|---|---|
| 20 | 61953 |
| 40 | 130213 |
| 60 | 198697 |
| 80 | 257002 |
| 100 | 321658 |




**LINEARITY PLOT**

Correlation Coefficient (r) is 0.99, and the intercept is 21.381. 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.

**Table 7: Results of repeatability for Benazepril**

| S. No | Peak name | Retention time | Area(µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Benazepril | 2.688 | 2397164 | 381741 | 8155 | 1.2 |
| 2 | Benazepril | 2.694 | 2391741 | 371742 | 9174 | 1.2 |
| 3 | Benazepril | 2.698 | 2371846 | 391746 | 7154 | 1.2 |
| 4 | Benazepril | 2.696 | 2361748 | 391847 | 9917 | 1.2 |
| 5 | Benazepril | 2.690 | 2371649 | 384622 | 9247 | 1.2 |
| **Mean** | | | 2378830 | | | |
| **Std.dev** | | | 14958 | | | |
| **%RSD** | | | 0.628797 | | | |

**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.

**Table 8: Results of repeatability for Hydrochlorothiazide**

| S. No | Peak name | Retention time | Area(µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Hydrochlorothiazide | 5.419 | 198464 | 7291 | 6274 | 1.1 |
| 2 | Hydrochlorothiazide | 5.422 | 193643 | 7219 | 6592 | 1.1 |
| 3 | Hydrochlorothiazide | 5.425 | 196462 | 7194 | 6028 | 1.1 |
| 4 | Hydrochlorothiazide | 5.423 | 194644 | 8174 | 6927 | 1.1 |
| 5 | Hydrochlorothiazide | 5.420 | 198464 | 8653 | 5920 | 1.1 |
| **Mean** | | | 196335.4 | | | |
| **Std.dev** | | | 2190.191 | | | |
| **%RSD** | | | 1.115536 | | | |

**Intermediate precision:**

**Day 1:**

**Table 9: Results of Intermediate precision for Benazepril**

| S.No | Peak Name | RT | Area (µV*sec) | Height (µV) | USP Plate count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Benazepril | 2.689 | 2389572 | 395275 | 9375 | 1.2 |
| 2 | Benazepril | 2.698 | 2391847 | 392175 | 9275 | 1.2 |
| 3 | Benazepril | 2.696 | 2319472 | 312947 | 8265 | 1.2 |
| 4 | Benazepril | 2.690 | 2306842 | 310585 | 6254 | 1.2 |
| 5 | Benazepril | 2.688 | 2375972 | 310694 | 9028 | 1.2 |
| 6 | Benazepril | 2.694 | 2396746 | 358373 | 8928 | 1.2 |
| **Mean** | | | 2363409 | | | |
| **Std. Dev.** | | | 39730.83 | | | |
| **% RSD** | | | 1.681082 | | | |

**Acceptance criteria:**

- %RSD of six different sample solutions should not more than 2

**Table 10: Results of Intermediate precision for Hydrochlorothiazide**

| S.No | Peak Name | RT | Area (µV*sec) | Height (µV) | USP Plate | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Hydrochlorothiazide | 5.418 | 197284 | 7194 | 8264 | 1.2 |
| 2 | Hydrochlorothiazide | 5.425 | 197849 | 7294 | 9174 | 1.2 |
| 3 | Hydrochlorothiazide | 5.423 | 196572 | 7147 | 9164 | 1.2 |
| 4 | Hydrochlorothiazide | 5.420 | 195028 | 7927 | 9733 | 1.2 |
| 5 | Hydrochlorothiazide | 5.419 | 199474 | 8238 | 9194 | 1.2 |
| 6 | Hydrochlorothiazide | 5.422 | 197482 | 7638 | 8973 | 1.2 |
| **Mean** | | | 197281.5 | | | |
| **Std. Dev.** | | | 1466.354 | | | |
| **% RSD** | | | 0.74328 | | | |

**Acceptance criteria:**

- %RSD of six different sample solutions should not more than 2

**Day 2:**

**Table 11: Results of Accuracy for concentration-50%**

**Benazepril**

| S.No | Name | RT | Area | Height | USP Tailing | USP Plate Count | Injection |
|---|---|---|---|---|---|---|---|
| 1 | Benazepril | 2.688 | 1214719 | 156568 | 1.1 | 6353 | 1 |
| 2 | Benazepril | 2.694 | 1218462 | 164774 | 1.2 | 8632 | 2 |
| 3 | Benazepril | 2.698 | 1218472 | 159664 | 1.1 | 7554 | 3 |

**Hydrochlorothiazide**

| S. No | Name | RT | Area | Height | USP Tailing | USP Plate Count | Injection |
|---|---|---|---|---|---|---|---|
| 1 | Hydrochlorothiazide | 5.419 | 98627 | 7462 | 1.1 | 7833 | 1 |
| 2 | Hydrochlorothiazide | 5.422 | 98634 | 7642 | 1.1 | 8264 | 2 |
| 3 | Hydrochlorothiazide | 5.425 | 98535 | 7814 | 1.1 | 7642 | 3 |

**The accuracy results for Benazepril**

| %Concentration (at specification Level) | Area | Amount Added (ppm) | Amount Found (ppm) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 1217218 | 112.5 | 112.4 | 99.6 | 99.3 |
| 100% | 2397141 | 225 | 225 | 100 | |
| 150% | 3514547 | 337.5 | 332.5 | 98.5 | |

**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.

**The accuracy results for Hydrochlorothiazide**

| %Concentration (at specification Level) | Area | Amount Added (ppm) | Amount Found (ppm) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 98598.67 | 22.5 | 22.4 | 99.9 | 99.6 |
| 100% | 198359.7 | 45 | 45 | 100 | |
| 150% | 291512.3 | 67.5 | 66.8 | 99 | |

**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**

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

**Benazepril:**

**Result:**

=12.5µg/ml

**Hydrochlorothiazide:**

**Result:**

=3.7µg/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

**Benazepril:**

**Result:**

=38.1µg/ml

**Hydrochlorothiazide:**

**Result:**

=11.4µg/ml

**ROBUSTNESS**

**Benazepril**

| Parameter used for sample analysis | Peak Area | Retention Time | Theoretical plates | Tailing factor |
|---|---|---|---|---|
| Actual Flow rate of 1.0mL/min | 2391746 | 3.202 | 9028 | 1.2 |
| Less Flow rate of 0.9mL/min | 2371831 | 3.639 | 7381 | 1.2 |
| More Flow rate of 1.1mL/min | 2218319 | 2.859 | 9311 | 1.1 |
| Less organic phase (about 5 % decrease in organic phase) | 2294821 | 3.460 | 7462 | 1.2 |
| More organic phase (about 5 % Increase in organic phase) | 2394811 | 3.022 | 6817 | 1.1 |

**Acceptance criteria:**

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

**Table 12: Results for Robustness**

**Hydrochlorothiazide**

| Parameter used for sample analysis | Peak Area | Retention Time | Theoretical plates | Tailing factor |
|---|---|---|---|---|
| Actual Flow rate of 1.1mL/min | 194627 | 5.463 | 7398 | 1.1 |
| Less Flow rate of 0.9mL/min | 183738 | 6.250 | 6883 | 1.1 |
| More Flow rate of 0.8mL/min | 198373 | 4.863 | 9917 | 1.2 |
| Less organic phase (about 5 % decrease in organic phase) | 178471 | 6.196 | 8372 | 1.1 |
| More organic phase (about 5 % Increase in organic phase) | 189462 | 5.010 | 7716 | 1.2 |

**Acceptance criteria:**

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

## SUMMARY AND CONCLUSION

**Summary**

A reverse-phase high-performance liquid chromatography (RP-HPLC) method was successfully developed and validated for the simultaneous estimation of Benazepril and Hydrochlorothiazide HCl in pure form and pharmaceutical dosage formulations. The method was optimized for precise, accurate, and reproducible results.

The separation was achieved using a Waters HPLC system equipped with an autosampler and PDA detector (996 model). Chromatographic separation was carried out on an Altima C18 column (4.6×150 mm, 5 µm) maintained at 35°C. The optimized mobile phase composition of Water: Methanol: Acetonitrile (25:65:10 v/v/v) delivered at a flow rate of 1 mL/min provided good resolution and peak symmetry for both analytes. Detection was performed at a wavelength of 265 nm, with an injection volume of 10 µL and a total run time of 14 minutes.

The developed RP-HPLC method is simple, rapid, and reliable for the simultaneous determination of Benazepril and Hydrochlorothiazide HCl in bulk and pharmaceutical dosage forms. The method demonstrated excellent system suitability, specificity, and reproducibility, making it suitable for routine quality control analysis of these compounds in both research and pharmaceutical industries.

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