Journal of Pharmacreations

PharmaCreations

ISSN: 2348-6295

Pharmacreations | Vol.7 | Issue 4 | Oct - Dec- 2020

Journal Home page: www.pharmacreations.com

Research article *Open Access*

Method Development And Validation For The Simultaneous Determination Of Benazepril And Hydrochlorothiazide In Bulk Form And Marketed Pharmaceutical Combined Dosage Form By Using Reverse Phase-Hplc

Shravan, G.Saikiran, R. Hemalatha

Department of Pharmaceutcial Analysis, Holy Mary College of Pharmacy, Bogaram, Hyderabad, India.

Corresponding author: Shravan

ABSTRACT

A novel, precise, accurate, rapid and cost effective isocratic reverse phase high performance liquid chromatographic (RP-HPLC) method was developed, optimized and validated for the estimation of Benazepril (BEN) and Hydrochlorothiazide (HYD) in bulk and pharmaceutical dosage forms. The drugs were estimated using Phenomenex Gemini C18 (4.6mm×150mm, 5µm) particle size column. A mobile phase composed of tri ethylamine buffer and methanol in proportion of 32:68 v/v, at a flow rate of 1.0 ml/min was used for the separation. Detection was carried out at 248nm. The linearity range obtained was 30-70µg/ml for Benazepril and 10-50µg/ml for Hydrochlorothiazide with retention times (Rt) of 3.297min and 5.405min for Benazepril and Hydrochlorothiazide respectively. The correlation coefficient values were found to be 0.999 & 0.999. Precession studies showed % RSD values less than 2 % for both the drugs in all the selected concentrations. The percentage recoveries of Benazepril (BEN) and Hydrochlorothiazide (HYD) were found to be 100.1873% for Benazepril and 100.748% for Hydrochlorothiazide respectively. The assay results of Benazepril (BEN) and Hydrochlorothiazide (HYD) were found to be 99.82%. The limit of detection (LOD) and limit of quantification (LOQ) were 2.6µg/ml and 7.8µg/ml for Benazepril and 3.4µg/ml 10.2µg/ml for Hydrochlorothiazide respectively. The proposed method was validated as per the International Conference on Harmonization (ICH) guidelines. The proposed validated method was successfully used for the quantitative analysis of commercially available dosage form.

Keywords; Benazepril and Hydrochlorothiazide, RP-HPLC, ICH Guidelines, Validation.

INTRODUCTION

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

Specificity/Selectivity

Specificity is ability to assess unequivocally the analyte in the presence of components that may be expected to be present. The terms selectivity and specificity are often used interchangeably. According to ICH the term specific generally refers to a method that produces a response for a single analyte only while the term selectivity refers to a method that provides responses for a number of chemical entities that may or may not be distinguished from each other. If the response is distinguished from all other responses, the method is said to be selective. Since there are very few methods that respond to only one analyte, the term selectivity is usually more appropriate.

Specificity is the ability of a method to discriminate between the analytes of interest and other components that

are present in the sample. Studies are designed to evaluate the degree of interference, if any which can be attributed to other analytes, impurities, degradation products, reagent "blanks" and excipients. This provides the analyst with a degree of certainty that the response observed is due to the single analyte of interest. The degree of specificity testing varies depending on the method type and the stage of validation. Specificity should be evaluated continually through the drug development process. Specificity is sometimes used interchangeably with the term "selectivity". The argument over which term is more correct is one of semantics. Although there is some dissention, the term "specificity" has been adopted by the regulatory guidance documents and should be used to prevent further confusion.

- Blank solution to show no interference with any HPLC system.
- Placebo to demonstrate the lack of interference from excipients.
- Drug substance to show that all significant related substances are resolved from the drug substance.
- Authentic samples of critical related substances to show that all known related substances are resolved from each other.

Accuracy

The Accuracy of analytical procedure expresses the closeness of agreement between the value that is accepted either as a conventional true value or as an accepted reference value and value found.

Accuracy may be inferred once precision, linearity and specificity have been established. Accuracy for the area percent method should be established from 50% of the ICH reporting limit to the nominal concentration of drug substance in the sample solution. For the high–low and external standard methods, determine accuracy from 50% of the ICH reporting level to 150% of the proposed shelf life specification of the related substances. In addition for the area percent and high–low methods, it is necessary to determine the accuracy of the related substances and the drug substance. For the external standard method only the accuracy of related substances is required. Since the response of the drug substance in the sample solution is not used in the external standard calculation it is not necessary to determine accuracy for the drug substance. Typically known amounts of related substances and the drug substance in placebo are spiked to prepare an accuracy sample of known concentration of related substance. According to the ICH accuracy should be determined using a minimum of nine determinations over a minimum of three concentration levels covering the range.

Precision

ICH defines the precision of an analytical procedure as the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions. ICH has defined precision to contain three components: repeatability, intermediate precision and reproducibility. Ruggedness as defined in USP XXII <1225>, 1990 incorporates the concepts described under the terms *"intermediate precision", "reproducibility"* and *"repeatability"* of this guide.

Linearity

Linearity of an analytical procedure as its ability (within a given range) to obtain test results that are directly proportional to the concentration (amount) of analyte in the sample.

Concentration range

The concentration range used for linearity should be large enough to encompass the desired range of the method. A minimum of five concentration ranges should be investigated and a plot of the detector response vs. the sample concentration should be generated. It is important that the concentration ranges selected for the linearity study are relatively equally spaced throughout the range of the method (e.g., 25%, 50%, 75%, 100%, 125% and 150%), and not clustered, as this will provide a skewed estimation of linearity.

Acceptance criteria

Solutions of known concentrations are used to determine the linearity. A plot of peak area versus concentration (in percent related substance) is used to demonstrate the linearity. Authentic samples of related substances with known purity are used to prepare these solutions. In most cases, for the linearity of a drug product, spiking the related substance authentic sample into excipients is not necessary, as the matrix effect should be investigated in method accuracy. Visual inspection is the most sensitive method for detecting nonlinearity. Therefore, the plot has to be linear by visual inspection. In addition, according to ICH guidelines, the following results should be reported: slope, correlation coefficient, y-intercept, and residual sum of squares. Under most circumstances, regression coefficient (r^2) is 0.999. Intercept and slope should be indicated.

Limit of detection

Limit of detection (LOD) is the lowest concentration of analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. With UV detectors, it is difficult to assure the detection precision of low level compounds due to potential gradual loss of sensitivity of detector lamps with age or noise level variation by detector manufacturer. At low levels, assurance is needed that the LOD and LOQ limits are achievable with the test method each time. With no reference standard for a given impurity or means to assure detectability, extraneous peak(s) could "disappear / appear." A crude method to evaluate the feasibility of the extraneous peak detection is to use the percentage claimed for LOD from the area counts of the analyte. Several approaches for determining the LOD are possible, depending on whether the procedure is a non-instrumental or instrumental.

- Based on visual evaluation
- Based on signal-to-noise

❖ Based on the standard deviation of the response and the slope

The LOD may be expressed as:

$$LOD = 3.3\ \sigma / S$$

Where,
σ = Standard deviation of Intercepts of calibration curves
S = Mean of slopes of the calibration curves
The slope S may be estimated from the calibration curve of the analyte.

Limit of quantification

Limit of quantitation (LOQ) is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. Several approaches for determining the LOQ are possible depending on whether the procedure is a non-instrumental or instrumental.

❖ Based on visual evaluation
❖ Based on signal-to-noise Approach
❖ Based on the standard deviation of the response and the slope

The LOQ may be expressed as

$$LOQ = 10\ \sigma / S$$

Where,
σ = Standard deviation of Intercepts of calibration curves
S = Mean of slopes of the calibration curves
The slope S may be estimated from the calibration curve of the analyte.

Robustness

The robustness of an analytical procedure is defined as a measure of its capacity to obtain comparable and acceptable results when perturbed by small but deliberate variations in specified experimental conditions. Robustness provides an indication of the test method's suitability and reliability during normal use. During a robustness study, conditions are intentionally varied to see if the method results are affected. The key word in the definition is deliberate. Example HPLC variations are illustrated for isocratic and gradient methods, respectively.

System Suitability

According to the USP, system suitability tests are an integral part of chromatographic methods. These tests are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. The purpose of the system suitability test is to ensure that the complete testing system is suitable for the intended application. Similar to the analytical method development, the system suitability test strategy should be revised as the analysts develop more experience with the assay. In general consistency of system performance, and chromatographic suitability. (Tailing factor, column efficiency and resolution of the critical pair, detector sensitivity) are the main components of system suitability.

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 2.25ml of the above Benazepril and 0.45ml of the 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.

Mobile Phase Optimization

Initially the mobile phase tried was Methanol: Water, Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: TEA buffer pH 4.8 in proportion 32:68 v/v respectively.

Optimization of Column

The method was performed with various columns like C18 column, X- bridge column, Xterra. Phenomenex Gemini C18 (4.6mm×150mm, 5.0 µm) particle size 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.
Column	:	Phenomenex Gemini C18 (4.6mm×150mm, 5.0 µm) particle size
Column temperature	:	38˚C
pH	:	4.8
Mobile phase	:	Methanol: TEA buffer pH 4.8 (32:68v/v)
Flow rate	:	1ml/min
Wavelength	:	248nm
Injection volume	:	20µl
Run time	:	7 min

METHOD VALIDATION

PREPARATION OF MOBILE PHASE

Preparation of mobile phase

Accurately measured 320ml (32%) of HPLC Methanol and 680ml of TEA buffer (68%) were mixed and degassed in a digital ultra sonicater for 15 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 Benazepril and Hydrochlorothiazide 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 0.5ml of the above Benazepril and 0.3ml of the Hydrochlorothiazide stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

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 Benazepril and Hydrochlorothiazide 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 0.5ml of the above Benazepril and 0.3ml of the Hydrochlorothiazide stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

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 Benazepril and Hydrochlorothiazide 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 0.5ml of the above Benazepril and 0.3ml of the Hydrochlorothiazide stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Precision

Repeatability

Preparation of Benazepril and Hydrochlorothiazide Product Solution for Precision

Accurately weigh and transfer 10 mg of Benazepril and Hydrochlorothiazide 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 0.5ml of the above Benazepril and 0.3ml of the Hydrochlorothiazide stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

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

DAY 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.

DAY 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 Benazepril and Hydrochlorothiazide 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 0.25ml of the above Benazepril and 0.15ml of the Hydrochlorothiazide stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 100% Standard stock 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 Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.5ml of the above Benazepril and 0.3ml of the Hydrochlorothiazide stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 150% Standard stock 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 Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.75ml of the above Benazepril and 0.45ml of the Hydrochlorothiazide stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

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 Benazepril and Hydrochlorothiazide 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 Benazepril and Hydrochlorothiazide 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 0.5ml of the above Benazepril and 0.3ml of the Hydrochlorothiazide stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Effect of Variation of flow conditions

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 20µl 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: TEA buffer pH 4.8 was taken in the ratio and 27:73, 37:63 instead of 32:68, remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION

Trails

Trail 1

Column (4.6mm×250mm) 5µm particle size	:ZorbaxC18
Column temperature	: Ambient
Wavelength	: 248nm
Mobile phase ratio (40:60 v/v)	: Methanol: Acetonitrile
Flow rate	: 1ml/min
Injection volume	: 20µl
Run time	:10minutes

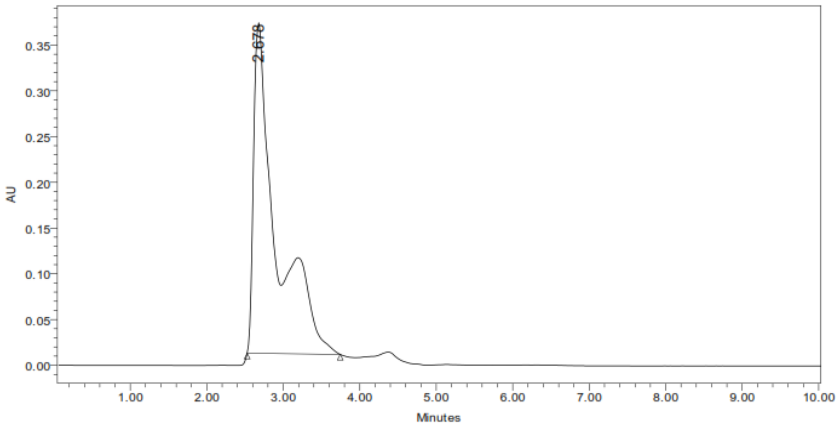


Fig.1 Chromatogram for Trail 1

Observation

In this trial it shows less plate count and improper separation of two peaks in the chromatogram. So, it's required more trials to obtain good peaks.

Trail 2

Column	: Develosil ODS C18 (4.6mm×250mm) 5µm
Column temperature	: 37˚C
Wavelength	: 248nm
Mobile phase ratio	: Acetonitrile: Phosphate Buffer (30:70 v/v)
Flow rate	: 0.8ml/min
Injection volume	: 10µl
Run time	: 8minutes

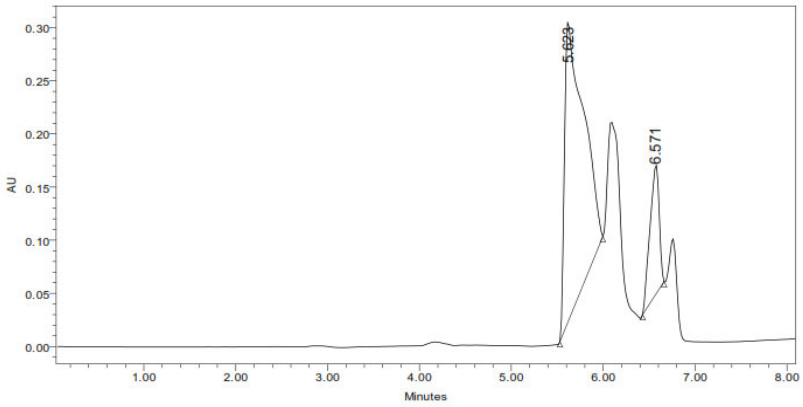


Fig . 2 Chromatogram for Trail 2

Observation

In this trail it shows improper separation of two peaks, shows less plate count and improper baseline in the chromatogram. It's required more trails to get optimized peaks.

Trail 3

Column	: Symmetry ODS C18 (4.6×250mm) 5µm
Column temperature	: 35˚C
Wavelength	: 248nm
Mobile phase ratio	: Methanol: Phosphate buffer (40:60 v/v)
Flow rate	: 1ml/min
Injection volume	: 20µl
Run time	: 8.50minutes

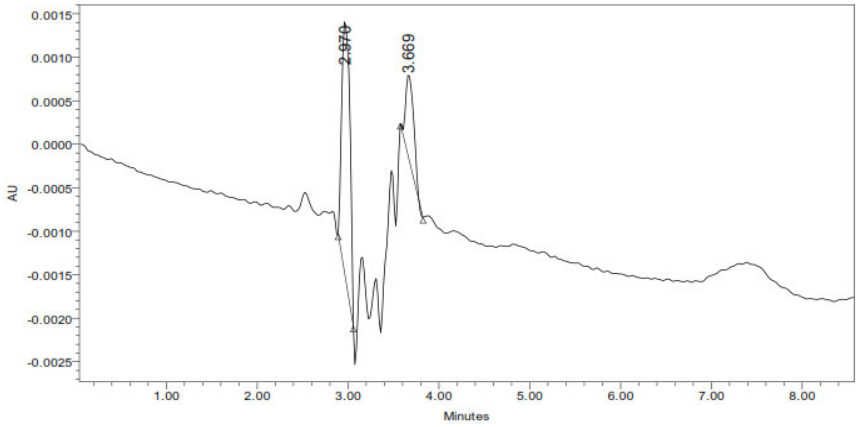


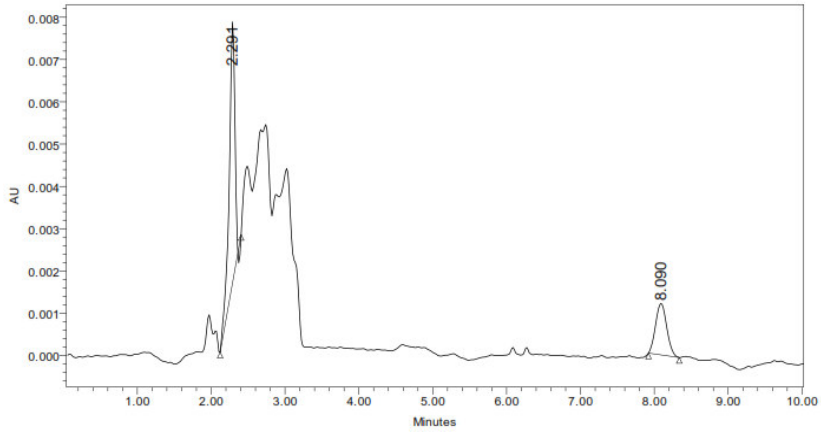
Fig . 3 Chromatogram for Trail 3

Observation

From the above chromatogram it was observed that it shows less plate count and more tailing, improper separation of two sample peaks in the chromatogram. More trails required to get optimized chromatogram.

Trail 4

Column	: Phenomenex Gemini C18 (4.6mm×150mm, 5.0 µm) particle size
Column temperature	: 40˚C
Wavelength	: 248nm
Mobile phase ratio	: Methanol: TEA buffer pH 3.6 (20:80 v/v)
Flow rate	: 1ml/min
Injection volume	: 20µl
Run time	: 10minutes



Observation

From the above chromatogram it was observed that it shows less plate count and more tailing, improper separation of two sample peaks in the chromatogram. More trails required to get optimized chromatogram.

Trail 5 (Optimized Chromatogram)

Column	: Phenomenex Gemini C18 (4.6mm×150mm, 5.0 µm) particle size
Column temperature	: 38˚C
Wavelength	: 248nm
Mobile phase ratio	: Methanol: TEA buffer pH 4.8 (32:68v/v)
Flow rate	: 1ml/min
Injection volume	: 20µl
Run time	: 7minutes

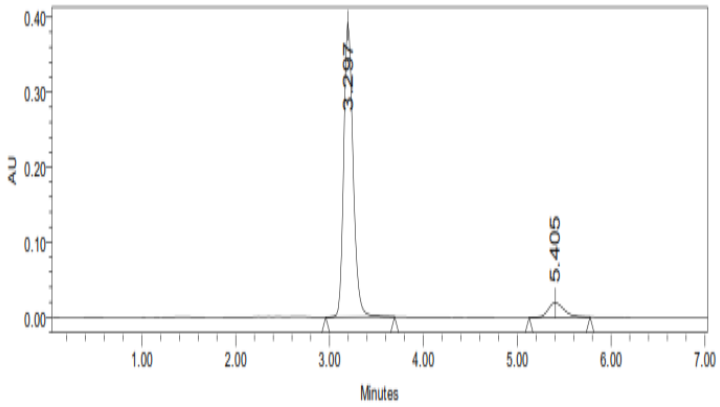


Fig . 5 Optimized Chromatogram (Standard)

Observation

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)

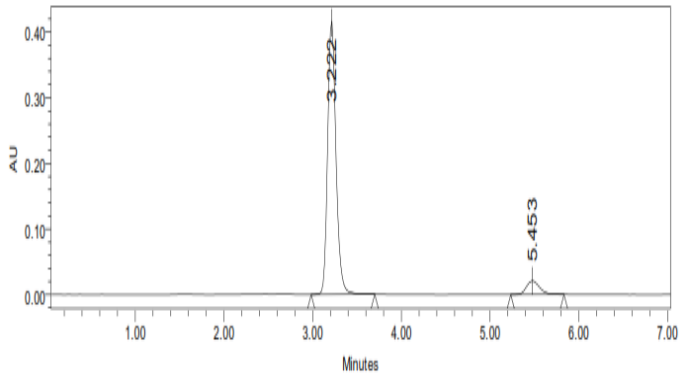


Fig . 6 Optimized Chromatogram (Sample)

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.

METHOD VALIDATION

Blank

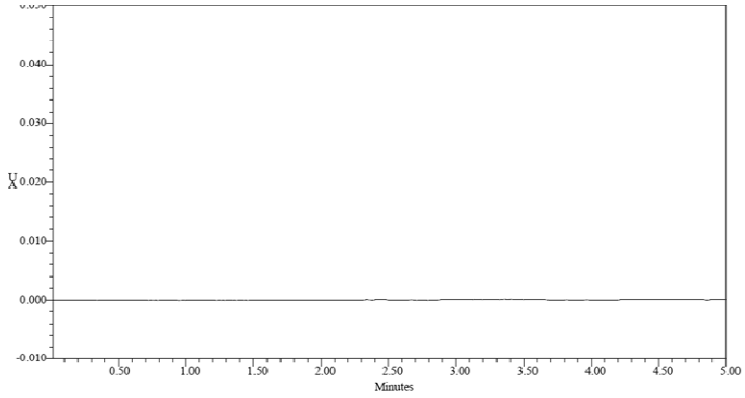


Fig . 7 Chromatogram showing blank (mobile phase preparation)

System Suitability

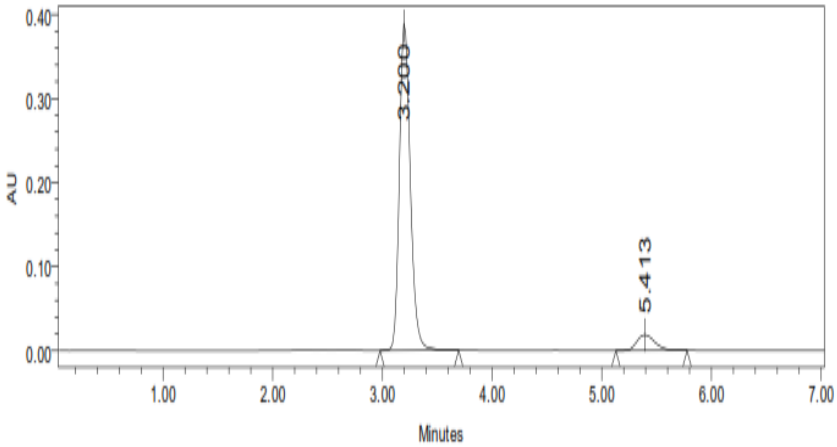


Fig. 8 Chromatogram showing injection -1

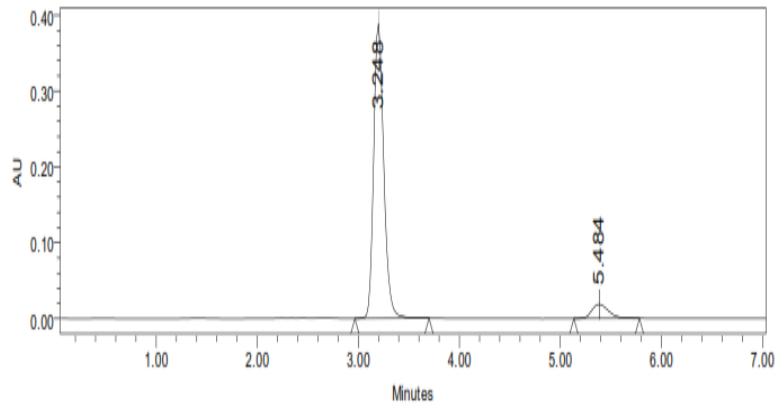


Fig . 9 Chromatogram showing injection -2

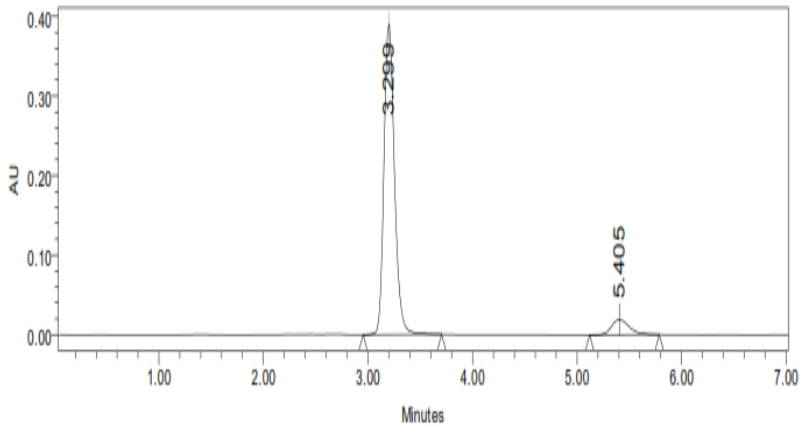


Fig . 10 Chromatogram showing injection -3

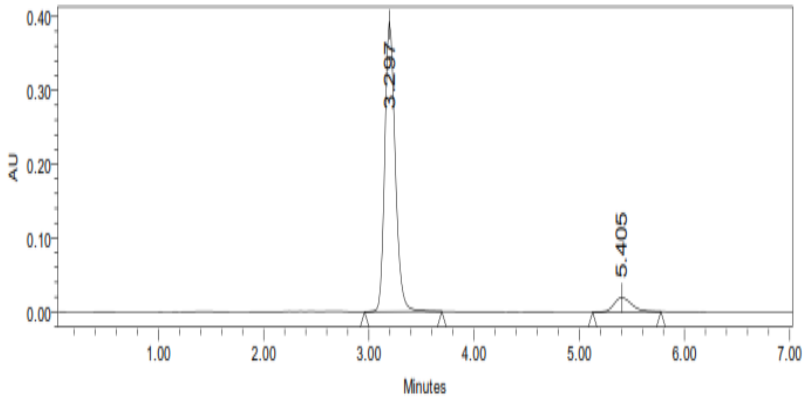


Fig . 11 Chromatogram showing injection -4

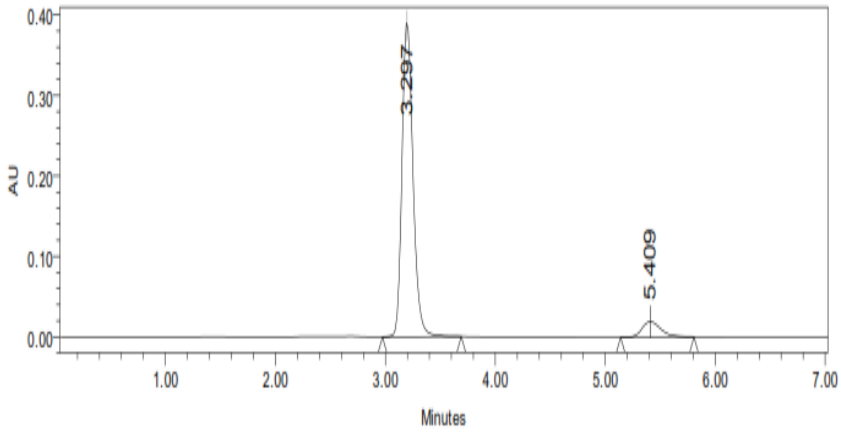


Fig . 12 Chromatogram showing injection -5

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.

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)

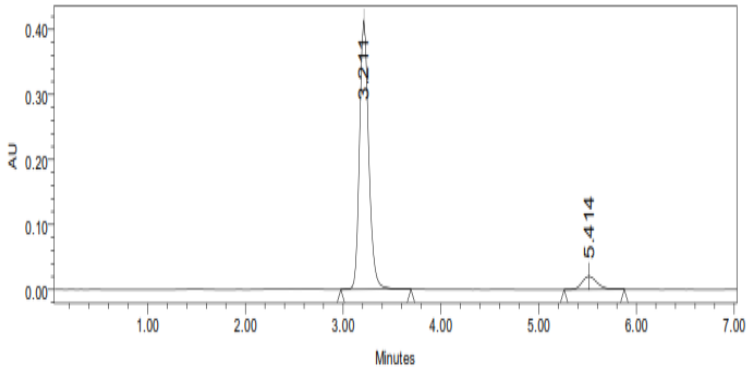


Fig . 13 Chromatogram showing assay of standard injection -1

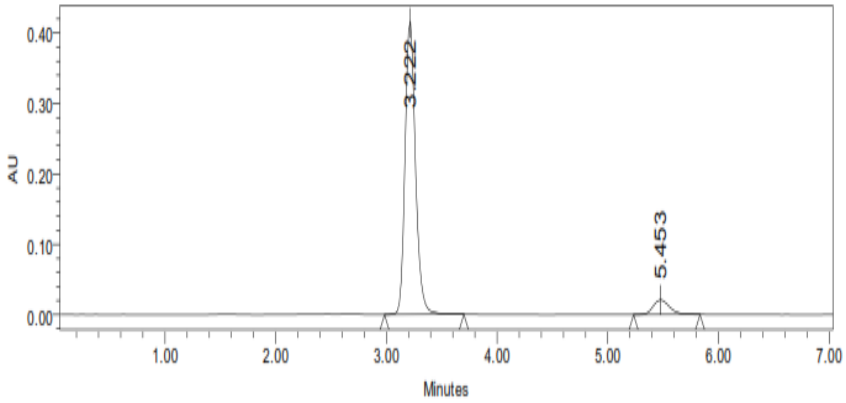


Fig . 14 Chromatogram showing assay of standard injection -2

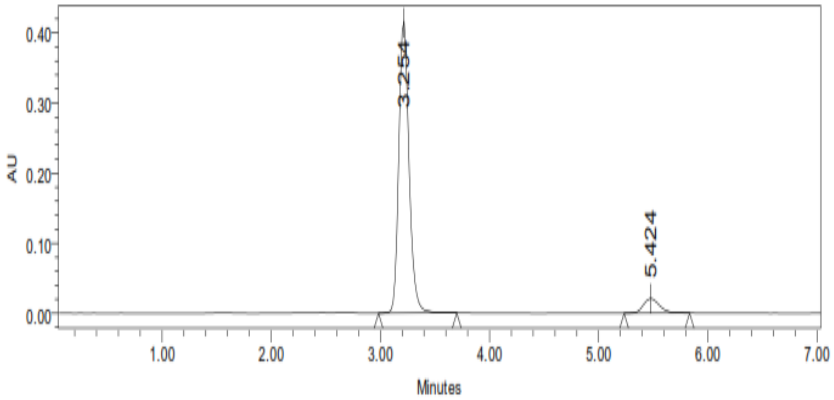


Fig . 15 Chromatogram showing assay of standard injection -3

Assay (Sample)

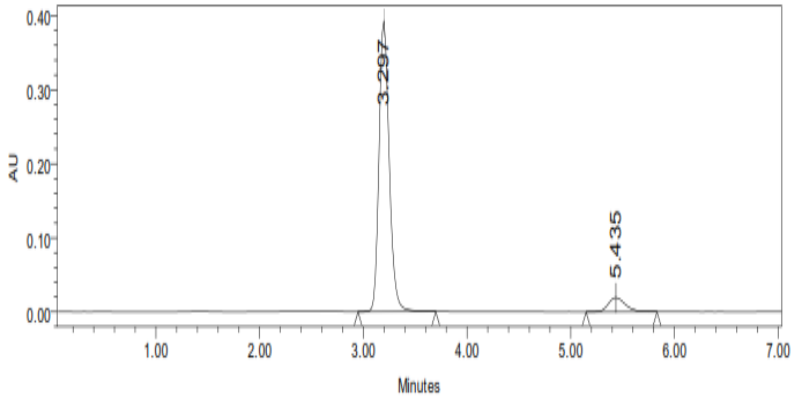


Fig . 17 Chromatogram showing assay of sample injection-1

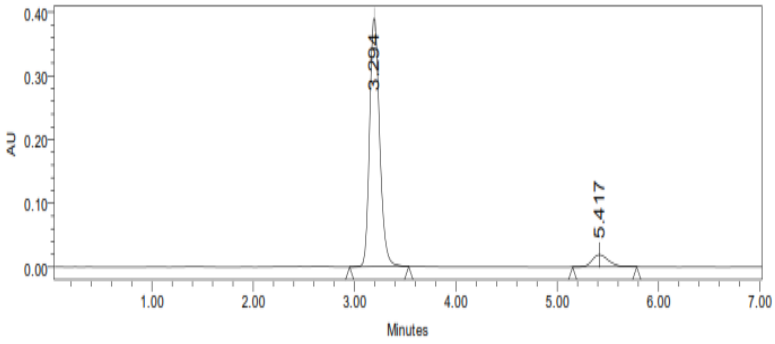


Fig. 18 Chromatogram showing assay of sample injection-2

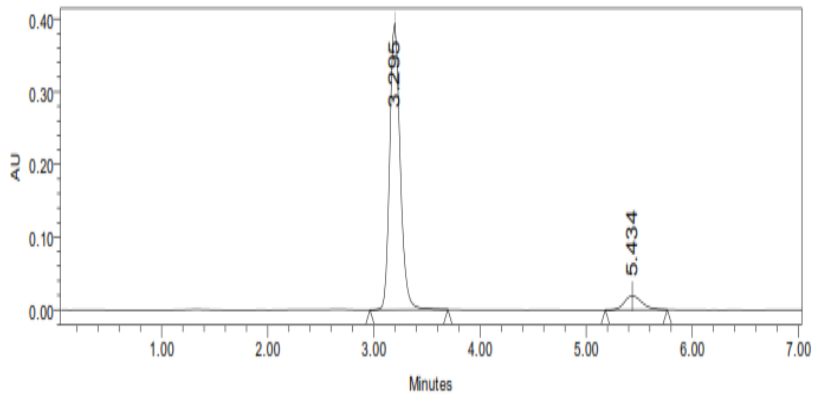


Fig . 19 Chromatogram showing assay of sample injection-3

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

The % purity of Benazepril and Hydrochlorothiazide in pharmaceutical dosage form was found to be 99.82%.

Linearity

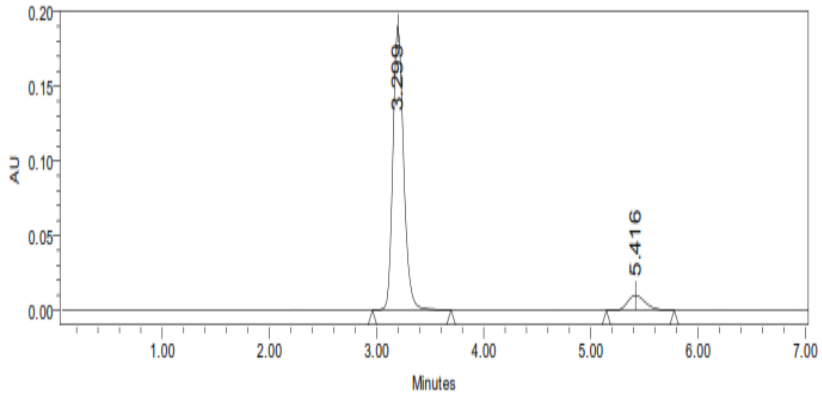


Fig. 20 Chromatogram showing linearity level-1

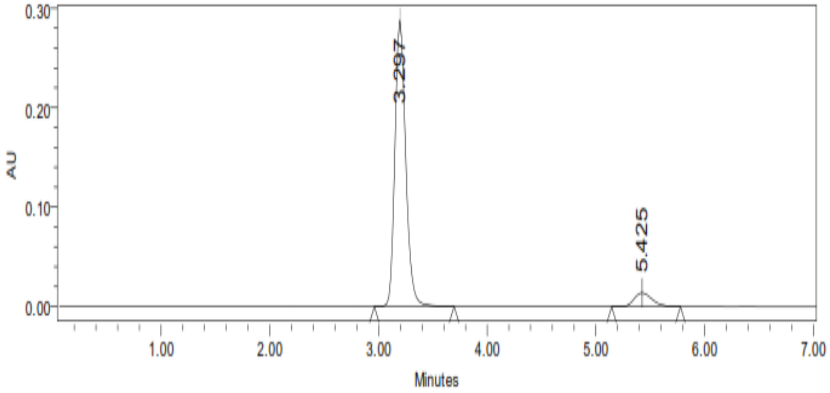


Fig . 21 Chromatogram showing linearity level-2

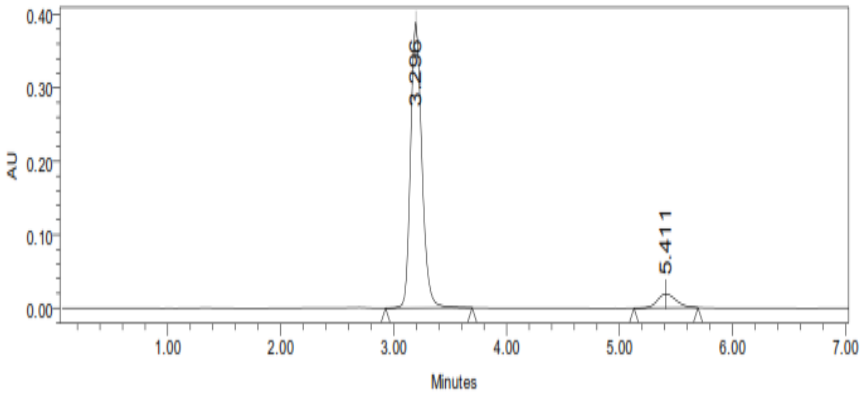


Fig . 22 Chromatogram showing linearity level-3

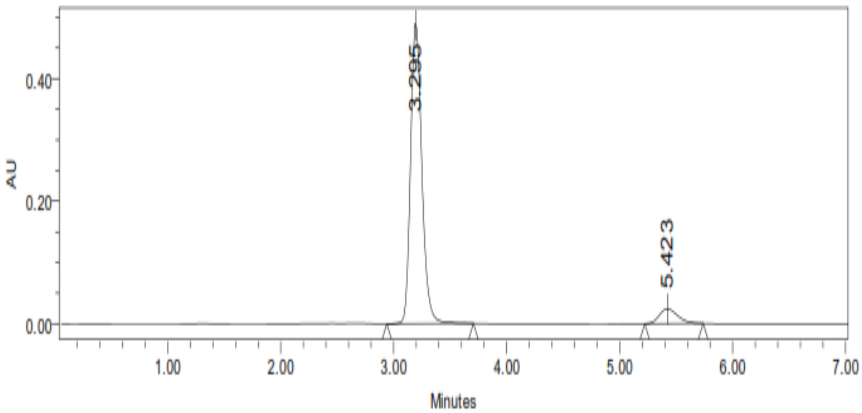


Fig .23 Chromatogram showing linearity level-4

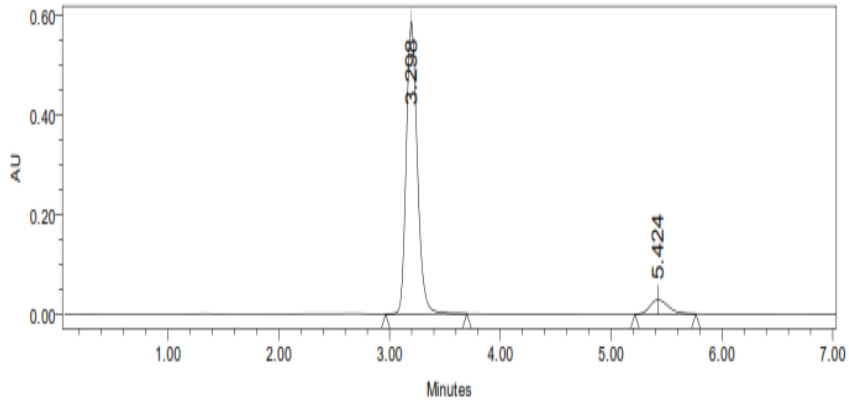


Fig . 24 Chromatogram showing linearity level-5

Chromatographic data for linearity study

Benazepril

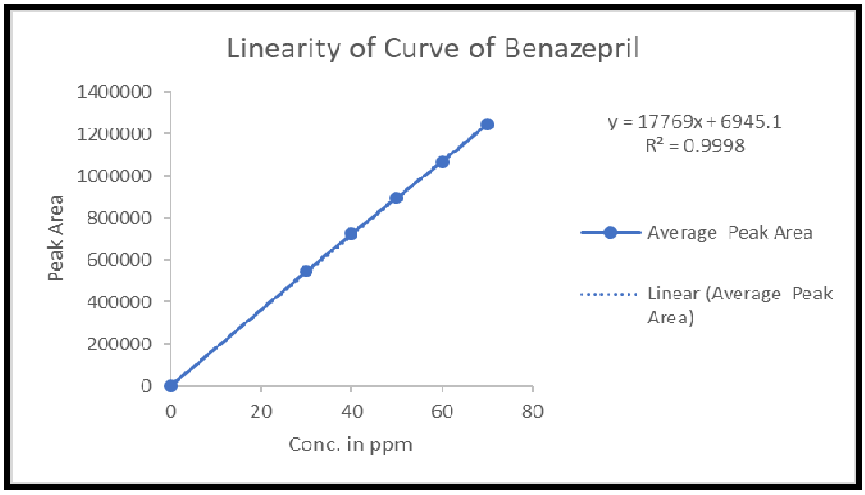


Fig .25 Calibration Curve of Benazepril

LINEARITY PLOT

The plot of Concentration (x) versus the Average Peak Area (y) data of Benazepril is a straight line.
Y = mx + c
Slope (m) =17769
Intercept (c) = 6945
Correlation Coefficient (r) = 0.999

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 6945. These values meet the validation criteria.

Hydrochlorothiazide

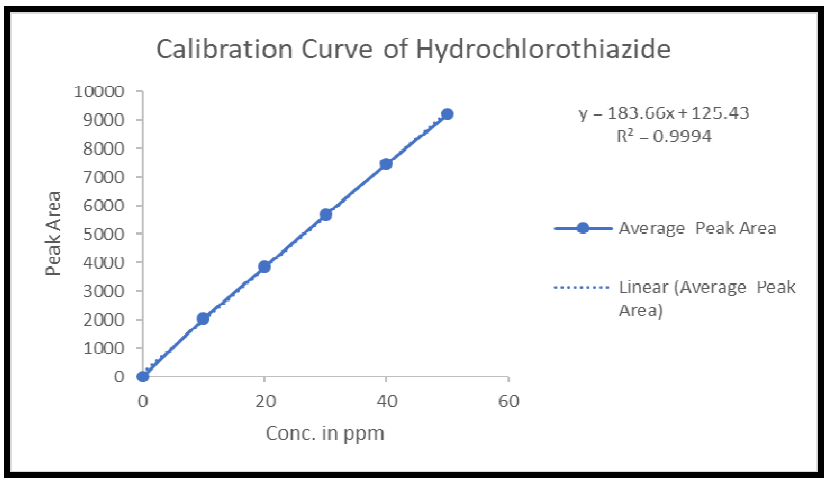


Fig . 26 Calibration Curve of Hydrochlorothiazide

Linearity plot

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

$Y = mx + c$

Slope (m) = 183.6

Intercept (c) = 125.4

Correlation Coefficient (r) = 0.999

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 125.4. 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.

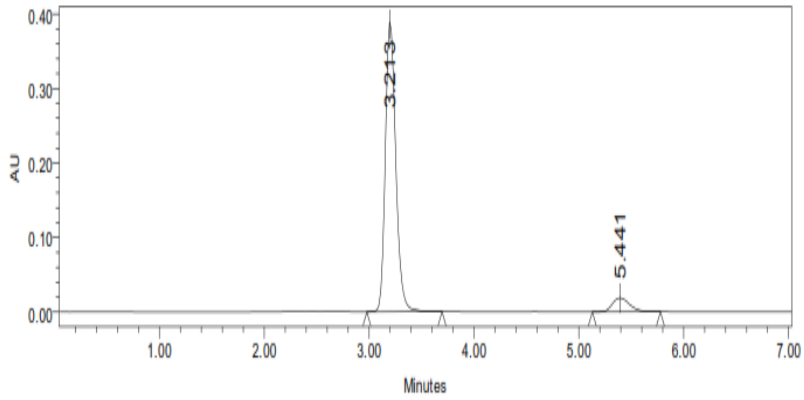


Fig . 27 Chromatogram showing precision injection -1

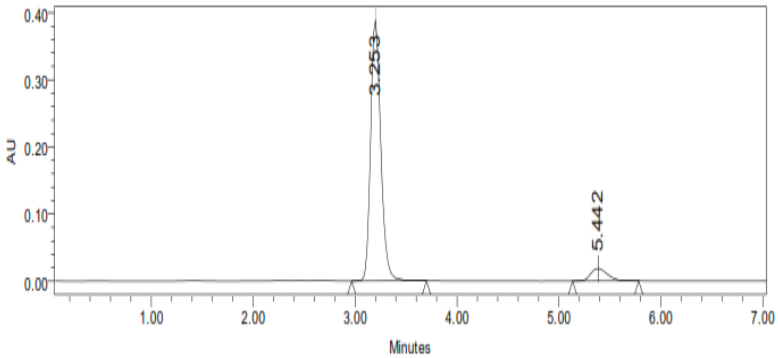


Fig . 28 Chromatogram showing precision injection -2

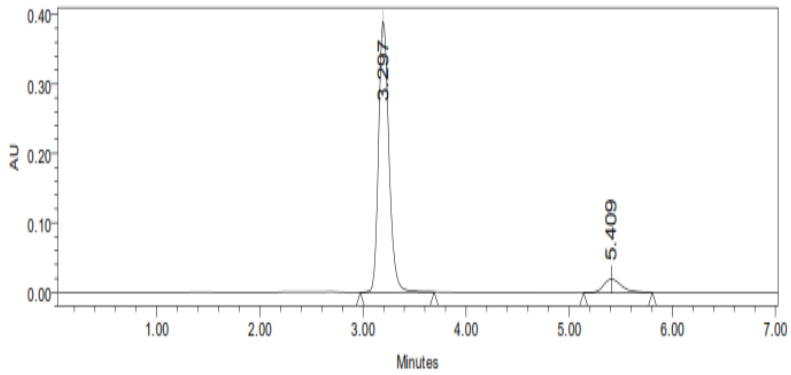


Fig . 29 Chromatogram showing precision injection -3

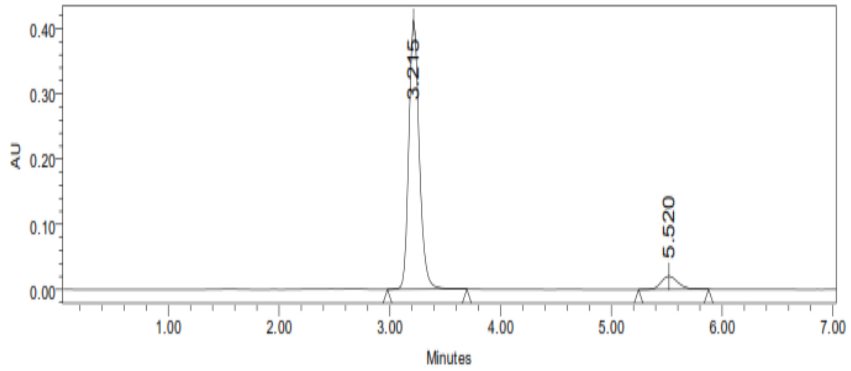


Fig .30 Chromatogram showing precision injection -4

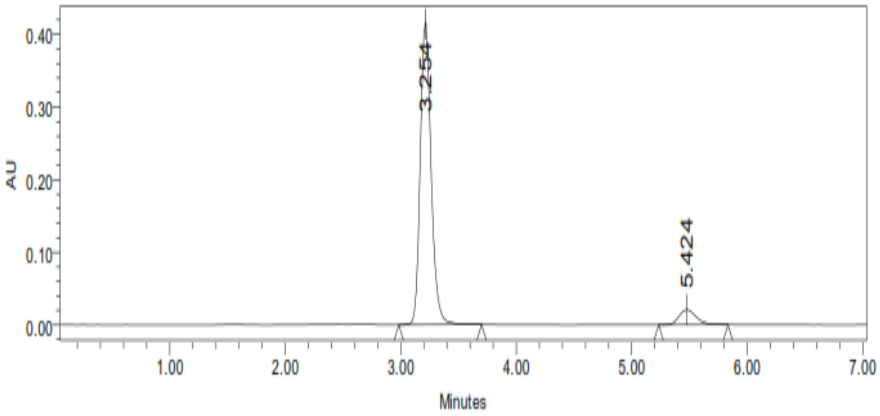


Fig . 31 Chromatogram showing precision injection -5

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

Day 1

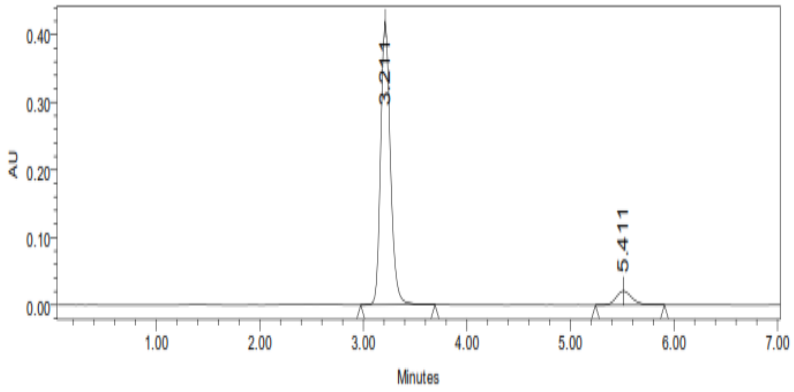


Fig . 32 Chromatogram showing Day1 injection -1

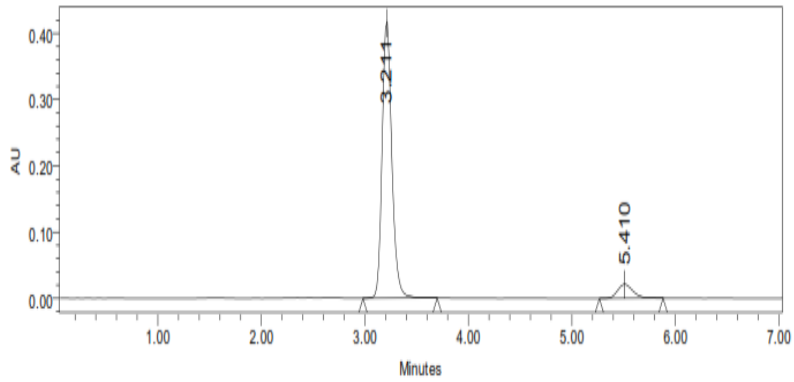


Fig . 33 Chromatogram showing Day1 injection -2

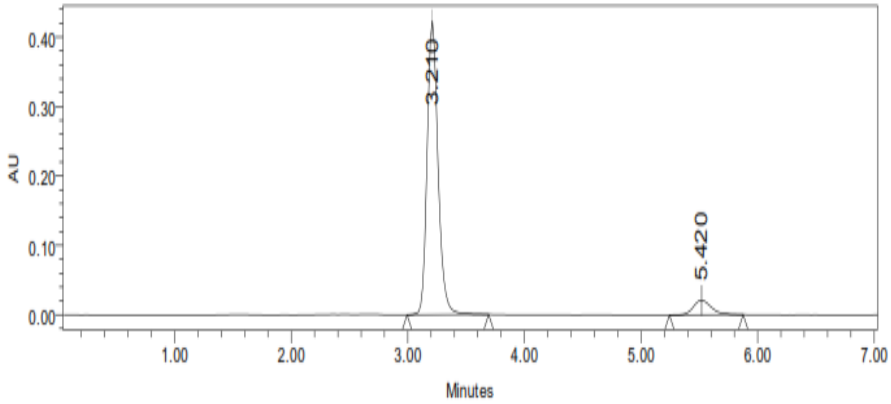


Fig 34 Chromatogram showing Day1 injection -3

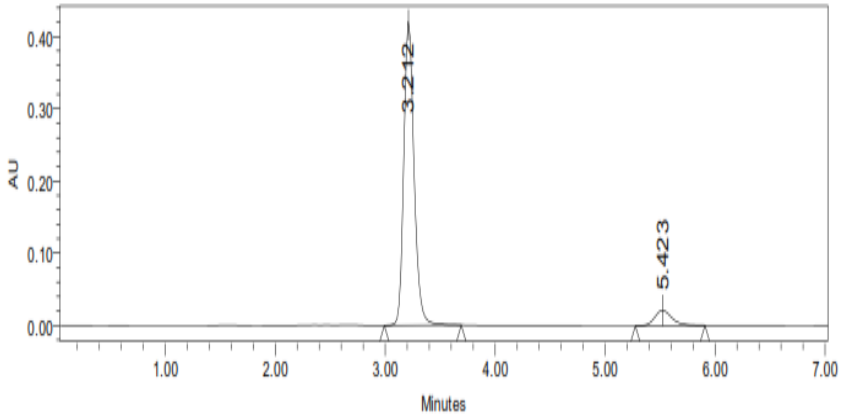


Fig .35 Chromatogram showing Day1 injection -4

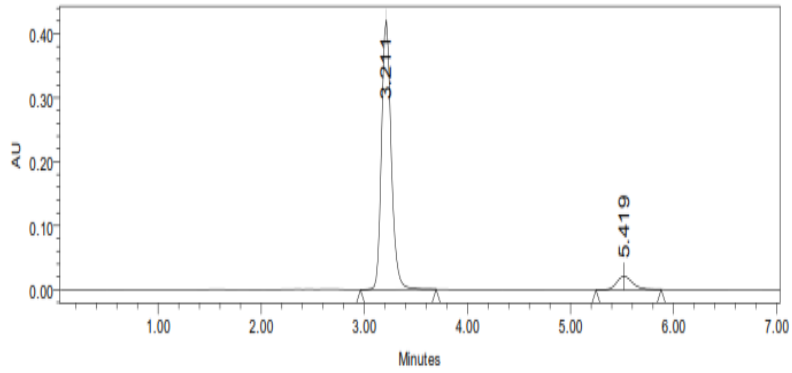


Fig . 36 Chromatogram showing Day1 injection -5

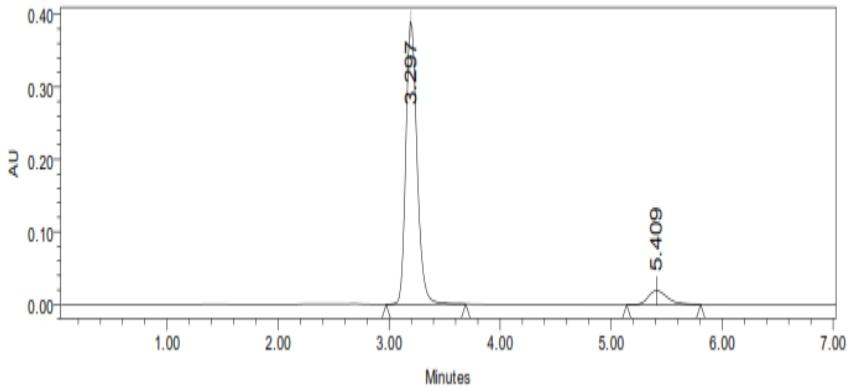


Fig . 37 Chromatogram showing Day1 injection -6

Acceptance criteria

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

Acceptance Criteria

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

Day 2

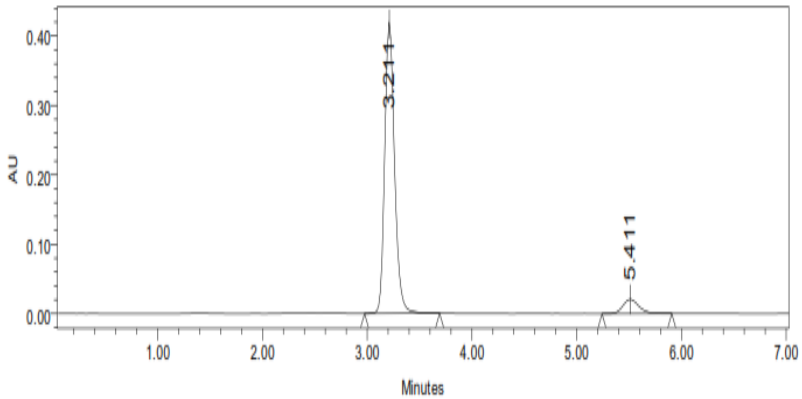


Fig .38 Chromatogram showing Day 2 injection -1

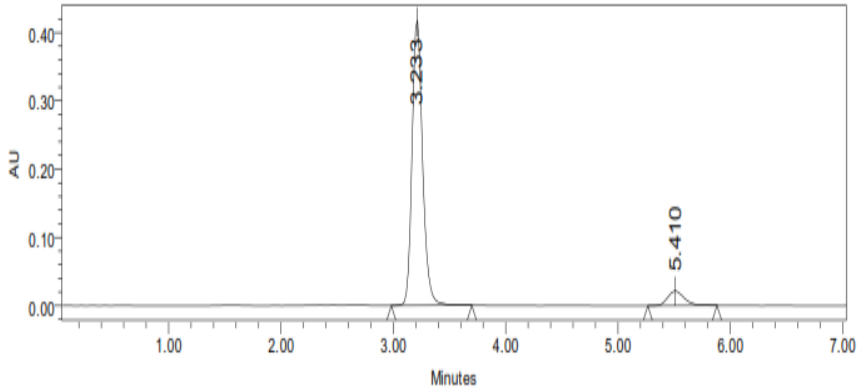


Fig . 39 Chromatogram showing Day 2 injection -2

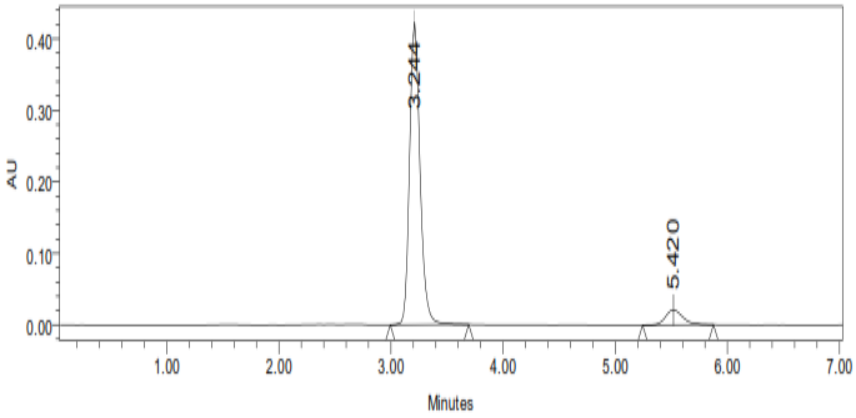


Fig . 40 Chromatogram showing Day 2 injection -3

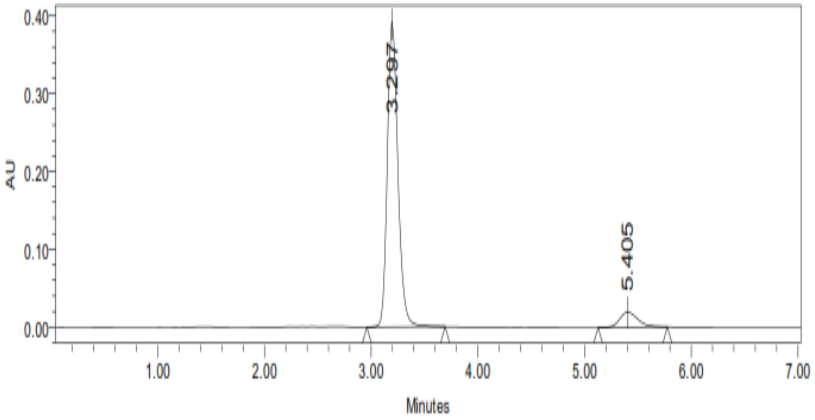


Fig .41 Chromatogram showing Day 2 injection -4

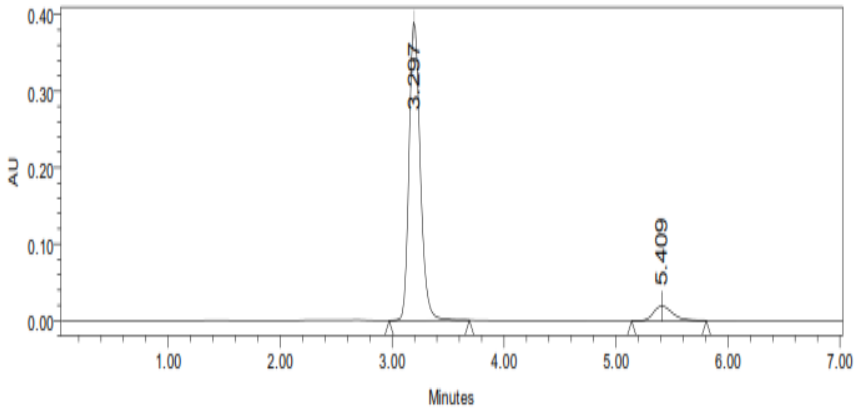


Fig . 42 Chromatogram showing Day 2 injection -5

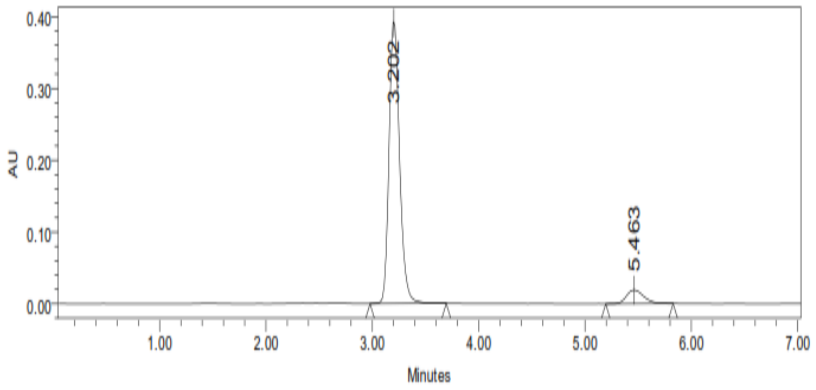


Fig . 43 Chromatogram showing Day 2 injection -6

Acceptance Criteria

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

6.3.4: ACCURACY

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

Accuracy 50%

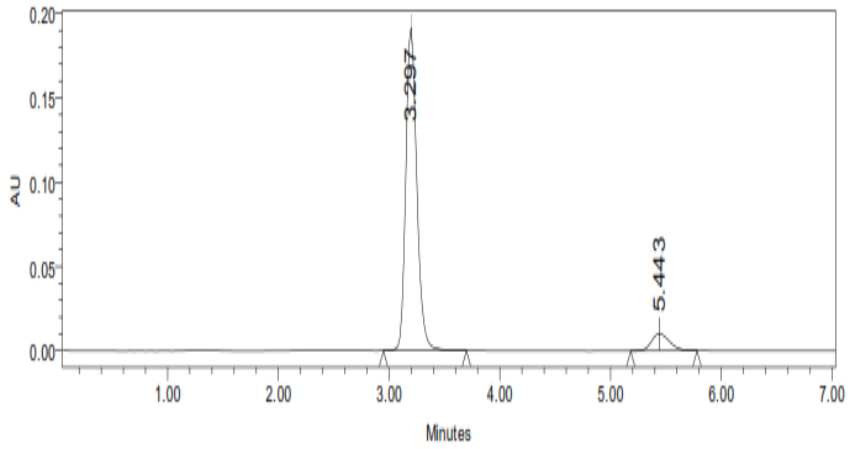


Fig . 44 Chromatogram showing accuracy-50% injection-1

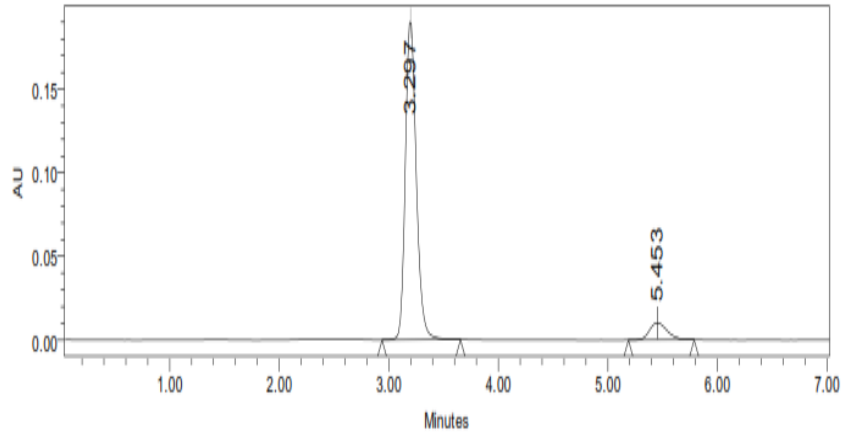


Fig . 45 Chromatogram showing accuracy-50% injection-2

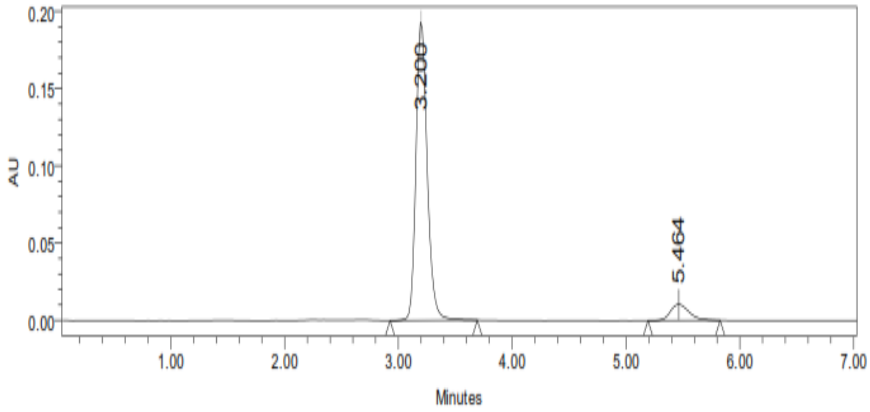


Fig . 46 Chromatogram showing accuracy-50% injection-3

Accuracy 100%

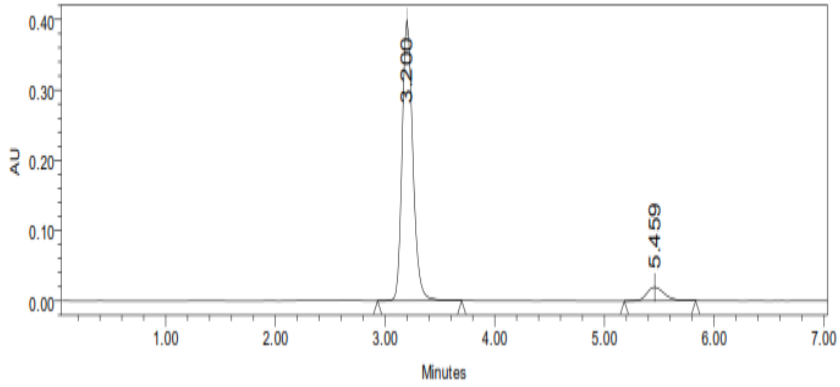


Fig . 47 Chromatogram showing accuracy-100% injection-1

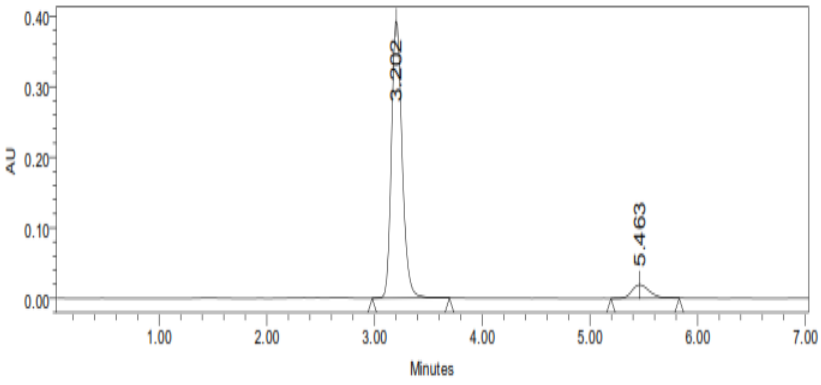


Fig .48 Chromatogram showing accuracy-100% injection-2

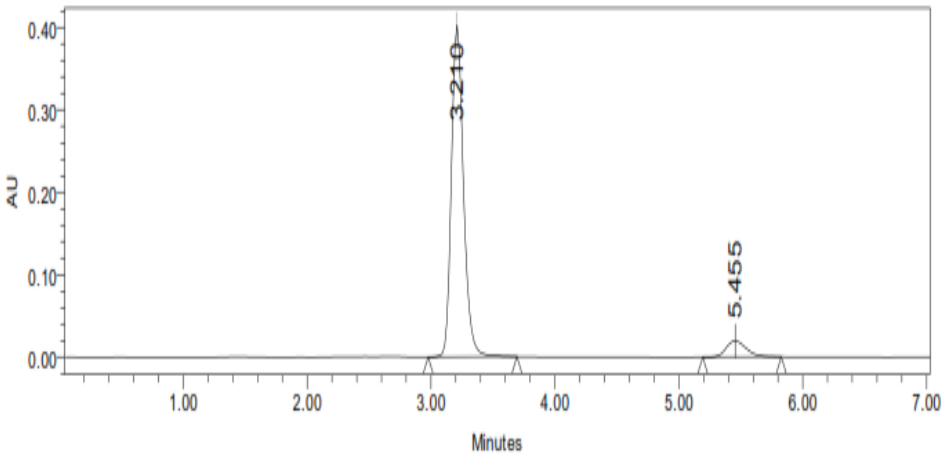


Fig . 49 Chromatogram showing accuracy-100% injection-3

Accuracy 150%

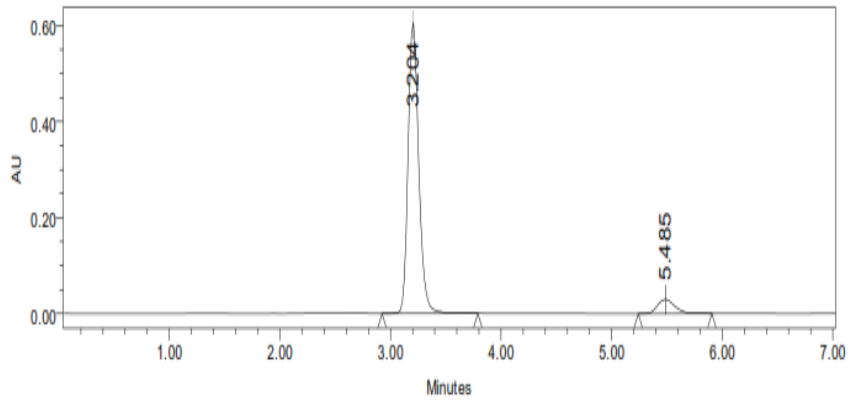


Fig . 50 Chromatogram showing accuracy-150% injection-1

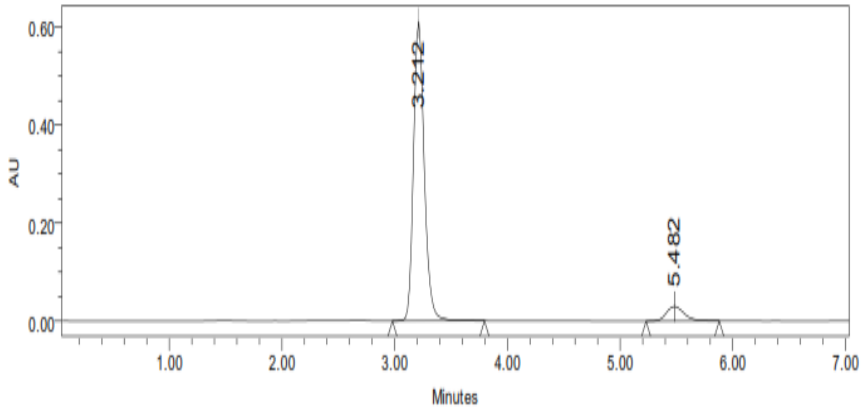


Fig . 51 Chromatogram showing accuracy-150% injection-2

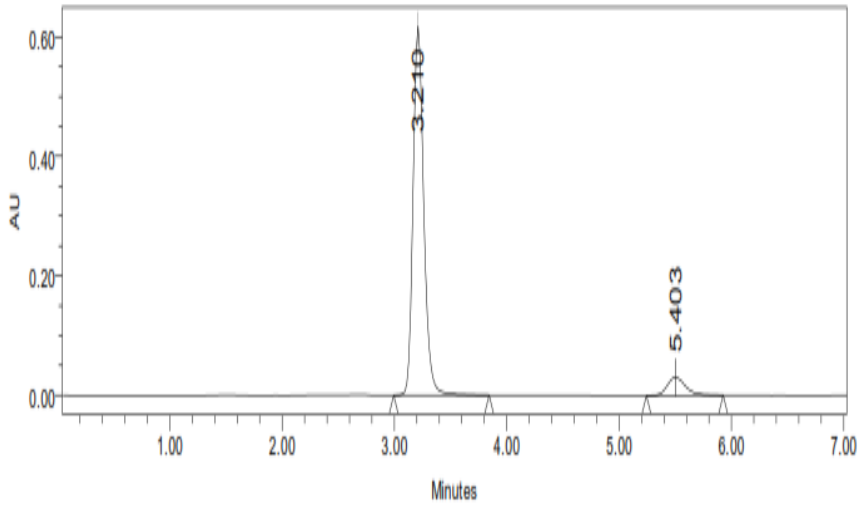


Fig . 52 Chromatogram showing accuracy-150% injection-3

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.

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.

$$LOD = 3.3 \times \sigma / s$$

Where
σ = Standard deviation of the response
S = Slope of the calibration curve

Benazepril:
Result: =2.6µg/ml
Hydrochlorothiazide:
Result: =3.4µg/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: =7.8µg/ml
Hydrochlorothiazide:
Result: =10.2µg/ml

Variation in flow

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 Benazepril and Hydrochlorothiazide. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard sample of Benazepril and Hydrochlorothiazide were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor and plate count.

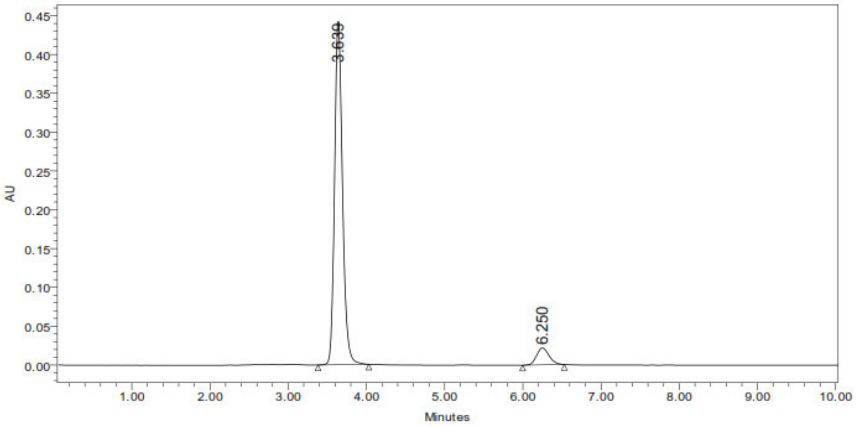


Fig . 53 Chromatogram showing less flow of 0.9ml/min

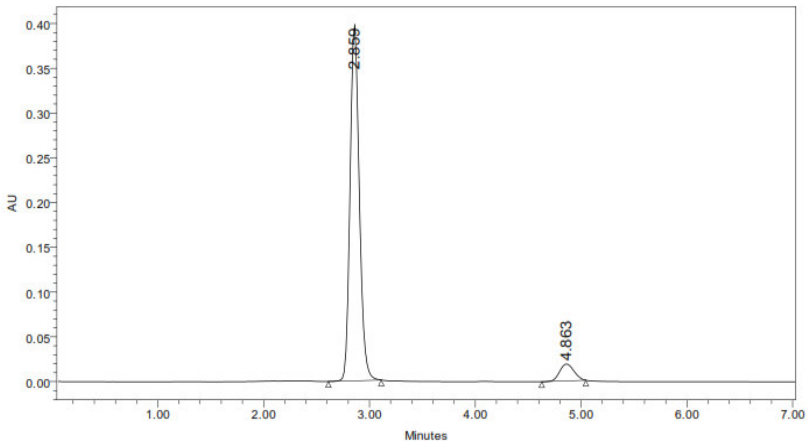


Fig . 54 Chromatogram showing more flow of 1.1 ml/min

Variation of mobile phase organic composition

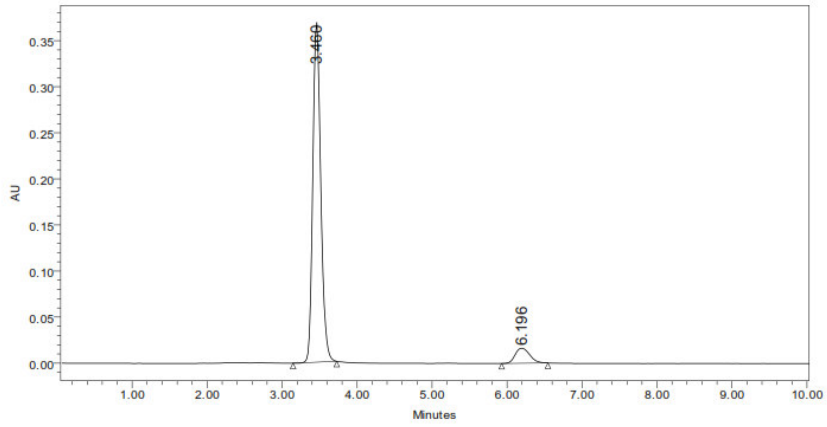


Fig . 55 Chromatogram showing less organic composition

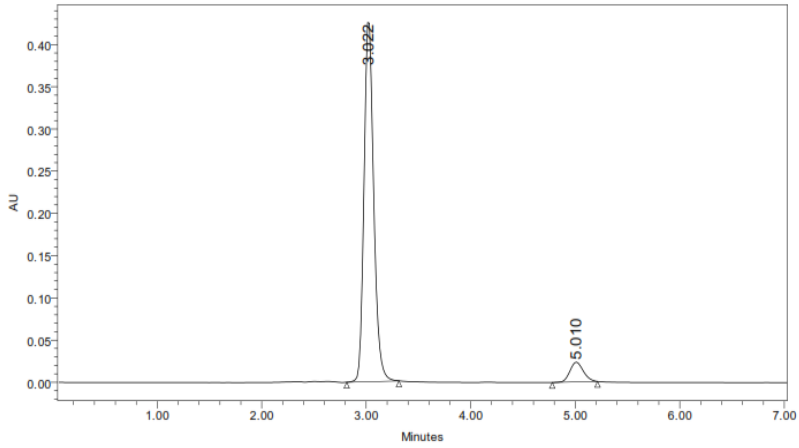


Fig . 55 Chromatogram showing more organic composition

Acceptance Criteria

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

Hydrochlorothiazide

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

High performance liquid chromatography is at present one of the most sophisticated tools of the analysis. The estimation of Benazepril and Hydrochlorothiazide was done by RP-HPLC. The TEA buffer was p^H 4.8 and the mobile phase was optimized with consists of Methanol: TEA buffer mixed in the ratio of 32:68 % v/v. A Phenomenex Gemini C18 (4.6mm×150mm, 5.0 µm) particle size or equivalent chemically bonded to porous silica particles was used as stationary phase. The solutions were chromatographed at a constant flow rate of 1.0 ml/min. The linearity range of Benazepril and Hydrochlorothiazide were found to be from 30-70µg/ml, 10-50µg/ml respectively. Linear regression coefficient was not more than 0.999, 0.999.

The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Benazepril and Hydrochlorothiazide. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

REFERENCES

1. Meyer V.R. Practical High-Performance Liquid Chromatography, 4th Ed. England, John Wiley & Sons Ltd, (2004), PP 7-8.
2. Sahajwalla CG a new drug development, vol 141, Marcel Dekker Inc., New York, (2004), PP 421–426.
3. Introduction to Column. (Online),URL:http://amitpatel745.topcities.com/index_files/study/column care.pdf
4. Detectors used in HPLC (online) URL:http://wiki.answers.com/Q/What_detectors_are_used_in_HPLC
5. Detectors (online) ,URL:http://hplc.chem.shu.edu/NEW/HPLC_Book/Detectors/det_uvda.html

6. Dr. Kealey and P.J. Haines, Analytical Chemistry, 1st edition, Bios Publisher, (2002), PP:1-7.
7. A. BraithWait and F.J. Smith, Chromatographic Methods, 5th edition, Kluwer Academic Publisher, (1996), PP 1-2.
8. Andrea Weston and Phyllis. Brown, HPLC Principle and Practice, 1st edition, Academic press, (1997), PP 24-37.
9. Yuri Kazakevich and Rosario Lobrutto, HPLC for Pharmaceutical Scientists, 1st edition, Wiley Interscience A JohnWiley & Sons, Inc., Publication, (2007), PP 15-23.
10. Chromatography, (online). URL:http://en.wikipedia.org/wiki/Chromatography.