Research Article

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Analytical method for the simultaneous estimation of Benzthiazide and Triamterene in pure form and its pharmaceutical dosage form

K.Vijaya, G.Sai kiran, N.Sriram

Holy Mary Institute of Science and Technology, (College of Pharmacy), Bogaram, Keesara, Hyderabad.

ABSTRACT

The primary objective of proposed work is developed new simple, sensitive, accurate and economical analytical method for the simultaneous estimation of Benzthiazide and Triamterene in pure form and its pharmaceutical dosage form. Also validate the proposed method in accordance with USP and ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the Benzthiazide and Triamterene in dosage form. In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Benzthiazide and Triamterene in bulk drug and pharmaceutical dosage forms. The analytical method was developed by studying different parameters. Maximum absorbance was found to be at 238 nm and the peak purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Develosil C18 (4.6mm×250mm) 5µm particle size Column because it was giving good peak. Ambient temperatures were found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase is acetonitrile and acetate buffer (pH-4.3) (35:65% v/v) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Run time was selected to be 6 min because analyze gave peak around 2.179, 3.610 ±0.02 min respectively and to reduce the total run time. The percent recovery was found to be 98.0-102% was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. The analytical method was found linearity over the range 10-30mg/ml of Benzthiazide and 30-90mg/ml of Triamterene of the target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

Keywords: Analytical method development, Benzthiazide and Triamterene

INTRODUCTION

Method development

Analytical method development is considered as a critical process in pharmaceuticals. Availability of the different types of columns, operating parameters, mobile phase composition, diluent and pH values make it critical to develop an analytical method. A good analytical method should be simple, used column, mobile phase and buffer should be common.

Author for Correspondence:
Vijaya
Holy Mary Institute of Science and Technology, (College of Pharmacy),
Bogaram, Keesara, Hyderabad.

Method validation

Validation is a fundamental piece of value affirmation; it includes the deliberate study of frameworks, offices and procedures went for figuring out if they perform their planned capacities sufficiently and reliably as determined [1, 2]. An accepted procedure is one which has been shown to give a high level of affirmation that uniform bunches will be created that meet the needed particulars and has in this manner been formally affirmed. Validation does not enhance forms but rather affirms that the procedures have been legitimately created what's more, are under control.

Methods and materials

In view of the need for a suitable RP-HPLC method for routine analysis of Benzthiazide and Triamterene in formulations, attempts were made to develop simple, precise and accurate analytical method for simultaneous estimation of Benzthiazide and Triamterene and extend it for their determination in formulation.

Validation is a necessary and important step in both framing and documenting the capabilities of the developed method. The utility of the developed method to determine the content of Benzthiazide and Triamterene in commercial formulation was also demonstrated. Validation of the method was done in accordance with USP and ICH guideline for the assay of active ingredient. The method was validated for parameters like system suitability, linearity, precision, accuracy, specificity, ruggedness and robustness, limit of detection and limit of quantification. This method provides means to quantify the component. This proposed method was suitable for the analysis of Pharmaceutical dosage forms.

EXPERIMENTAL METHODS

Hplc method development

Preparation of standard solution

Accurately weigh and transfer 10 mg of Benzthiazide and Triamterene working standard into a 10 ml of clean dry volumetric flasks add about 7 ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.2 ml of Benzthiazide and 0.6ml of Triamterene from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile phase optimization

Initially the mobile phase tried was Methanol: Water and ACN: Water with varying proportions. Finally, the mobile phase was optimized to Acetonitrile and Acetate buffer (pH-4.3) in proportion 35:65 v/v respectively.

VALIDATION

Preparation of mobile phase

Preparation of mobile phase

Accurately measured 350ml of Acetonitrile (35%) of and 650ml of Acetate buffer (65%) were mixed and degassed in a digital ultra sonicater for 20 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 Benzthiazide and Triamterene working standard into a 10 ml 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 out 0.2 ml of Benzthiazide and 0.6 ml of Triamterene from the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with Diluent.

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 Benzthiazide and Triamterene 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 out 0.2ml of Benzthiazide and 0.6ml of Triamterene from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Sample Solution

Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Benzthiazide and Triamterene 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. Filter the sample solution by using injection filter which contains 0.45µ pore size.

Further pipette out 0.2ml of Benzthiazide and 0.6ml of Triamterene Sample solution from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure

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

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

PRECISION

Repeatability

Preparation of benzthiazide and triamterene product solution for precision

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

Further pipette out 0.2 ml of Benzthiazide and 0.6ml of Triamterene from the above stock solutions into a 10 ml 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

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 Benzthiazide and Triamterene working standard into a 10 ml of clean dry volumetric flasks add about 7 ml of diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette out 0.1 ml of Benzthiazide and 0.3 ml of Triamterene from the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluent.

For preparation of 100% Standard stock solution

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

Further pipette out 0.2 ml of Benzthiazide and 0.6ml of Triamterene from the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 150% Standard stock solution

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

Further pipette out 0.3ml of Benzthiazide and 0.9 ml of Triamterene from the above 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 Benzthiazide and Triamterene 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 Benzthiazide and Triamterene working standard into a 10 ml 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 out 0.2 ml of Benzthiazide and 0.6 ml of Triamterene from the above stock solutions into a 10 ml 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 1 ml/min, remaining conditions are same. 20 µl 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. Acetonitrile: Acetate buffer was taken in the ratio 40:60 and 30:70 instead of 35:65 remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION

Column : Hypersil C18 (4.6mm×250mm) 5µm Particle size
Column temperature: 28ºc
Wavelength : 238nm
Mobile phase ratio: Acetonitrile: Water (70:30) V/V
Flow rate : 0.8ml/min
Injection volume : 20µl
Run time : 10minutes

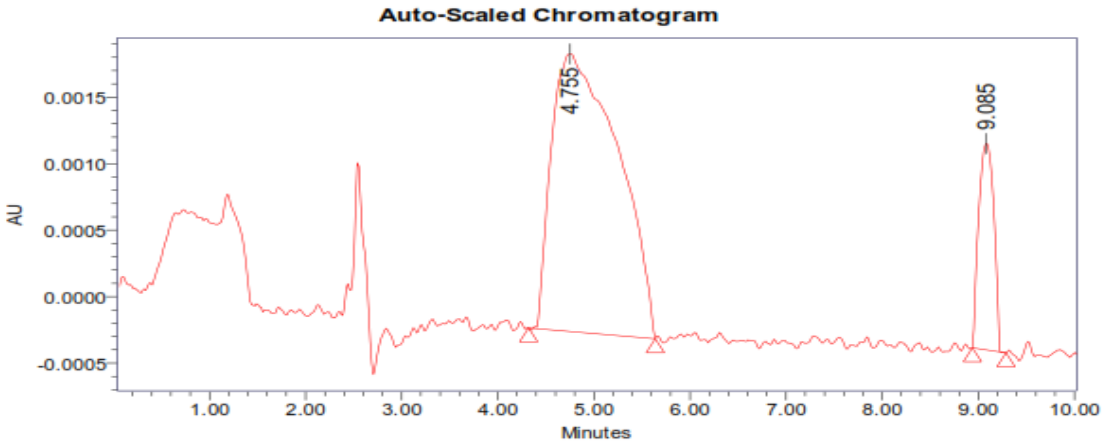


Figure: Chromatogram for trail 1

Table: Peak Results for trail 1

S.No	Peak Name	R_t	Area	Height	USP Tailing	USP Plate count
1	Benzthiazide	4.755	63156	10236	1.7	710
2	Triamterene	9.085	1167502	46055	2.6	415

Observation

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

Validation

System suitability

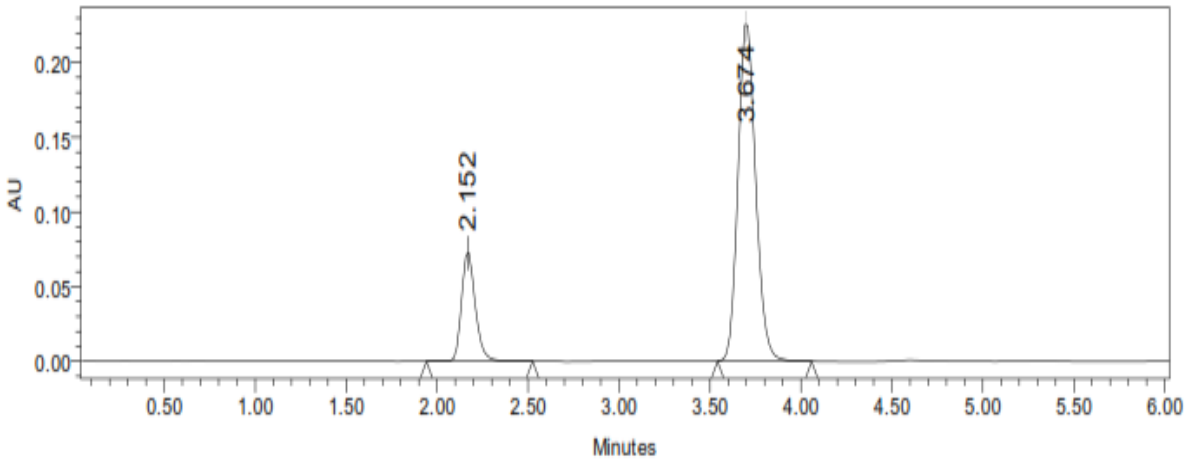


Fig-: Chromatogram showing injection -1

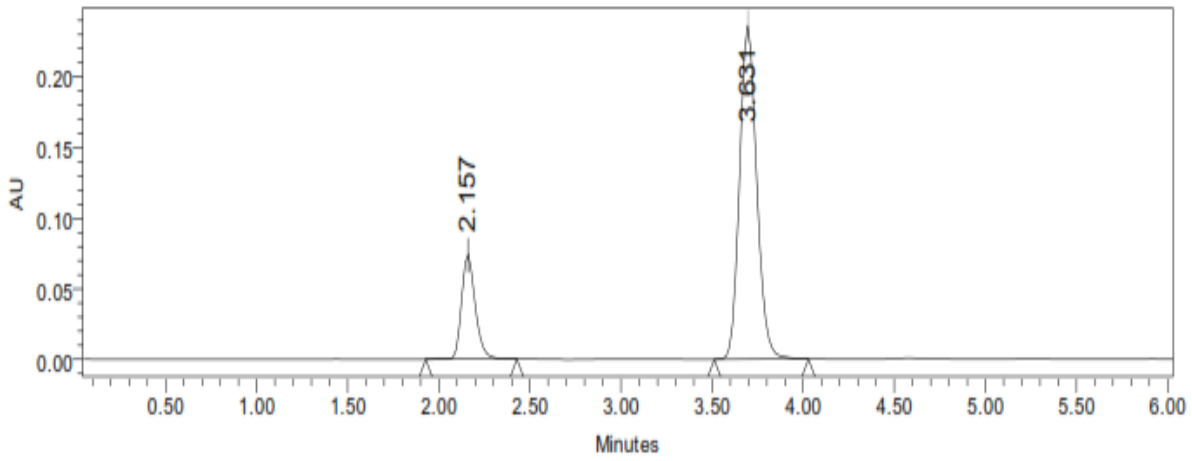


Table-: Results of system suitability for Benzthiazide

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Benzthiazide	2.152	513652	78542	4698	1.2
2	Benzthiazide	2.157	513524	78654	4785	1.2
3	Benzthiazide	2.141	513425	78541	4682	1.2
4	Benzthiazide	2.133	513647	78454	4854	1.2
5	Benzthiazide	2.166	514824	78655	4872	1.2
Mean			513814.4			
Std. Dev.			572.2004			
% RSD			0.111363			

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: Results of system suitability for Triamterene

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	Resolution
1	Triamterene	3.674	1635285	265421	7985	1.1	10.1
2	Triamterene	3.631	1635241	265484	7898	1.1	10.1
3	Triamterene	3.625	1652547	253498	7954	1.1	10.1
4	Triamterene	3.692	1658458	265241	7965	1.1	10.1
5	Triamterene	3.629	1652894	265348	7985	1.1	10.1
Mean			1646885				
Std. Dev.			10865.58				
% RSD			0.659766				

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 quantitated Benzthiazide and Triamterene in drug product.

Assay (standard)

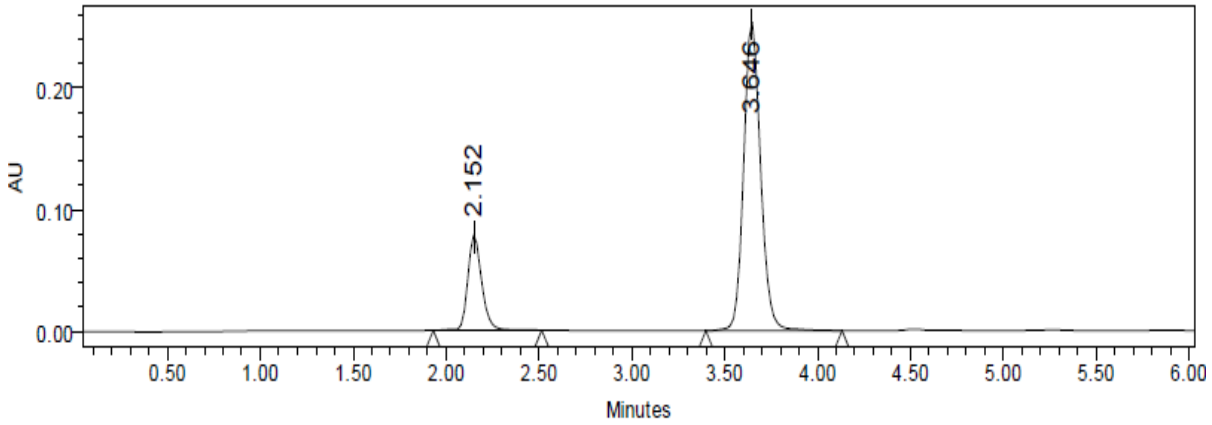
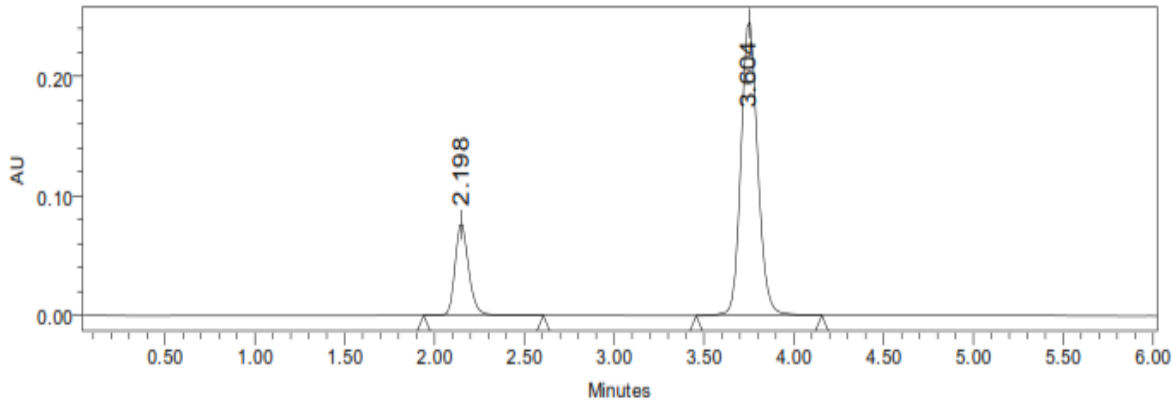


Fig-: Chromatogram showing assay of standard injection -1



Assay (sample)

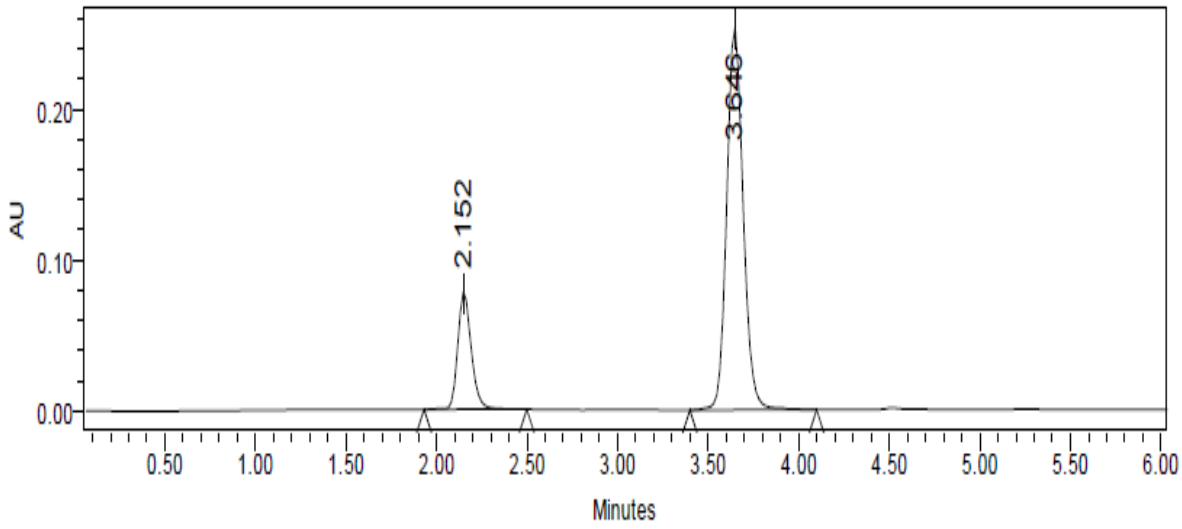


Fig-: Chromatogram showing assay of sample injection-1

Table-: Peak results for assay sample of Benzthiazide

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Benzthiazide	3.651	513265	78548	1.2	4582	1
2	Benzthiazide	2.150	513254	78547	1.2	4658	2
3	Benzthiazide	2.187	513873	78498	1.2	4597	3

Table-: Peak results for Assay sample of Triamterene

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Triamterene	3.646	162528	478569	1.1	7985	1
2	Triamterene	3.651	162461	378547	1.1	7898	2
3	Triamterene	3.601	162587	478462	1.1	7854	3

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

The % purity of Benzthiazide and Triamterene in pharmaceutical dosage form was found to be 99.57%

Linearity

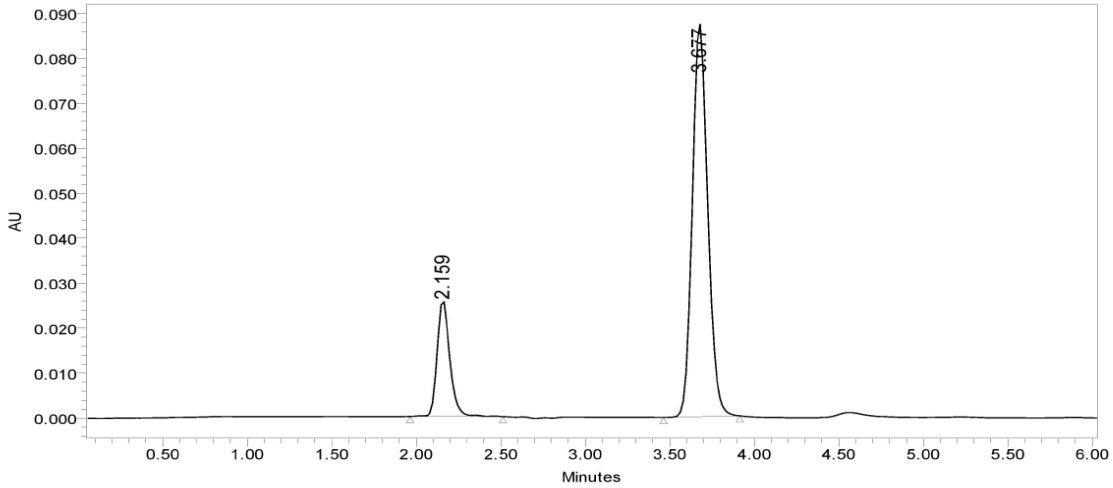


Fig-: Chromatogram showing linearity level-1

Chromatographic data for linearity study of benzthiazide

Concentration µg/ml	Average Peak Area
10	245899
15	365687
20	481526
25	589854
30	705882

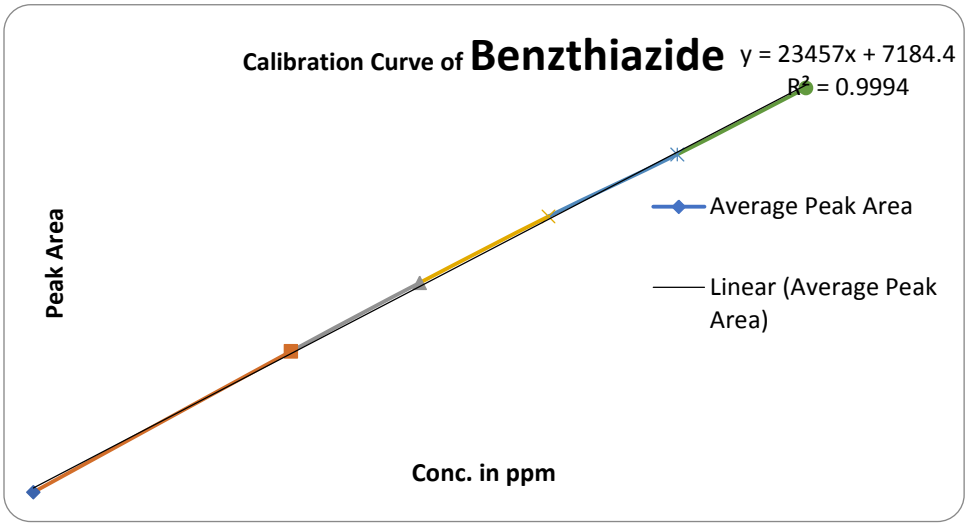


Fig-: Calibration Graph of Benzthiazide

Linearity plot

The plot of concentration (x) versus the average peak area (y) data of Benzthiazide is a straight line.

- Y = mx + c
- Slope (m) = 23457
- Intercept (c) = 7184
- Correlation Coefficient (r) = 0.999

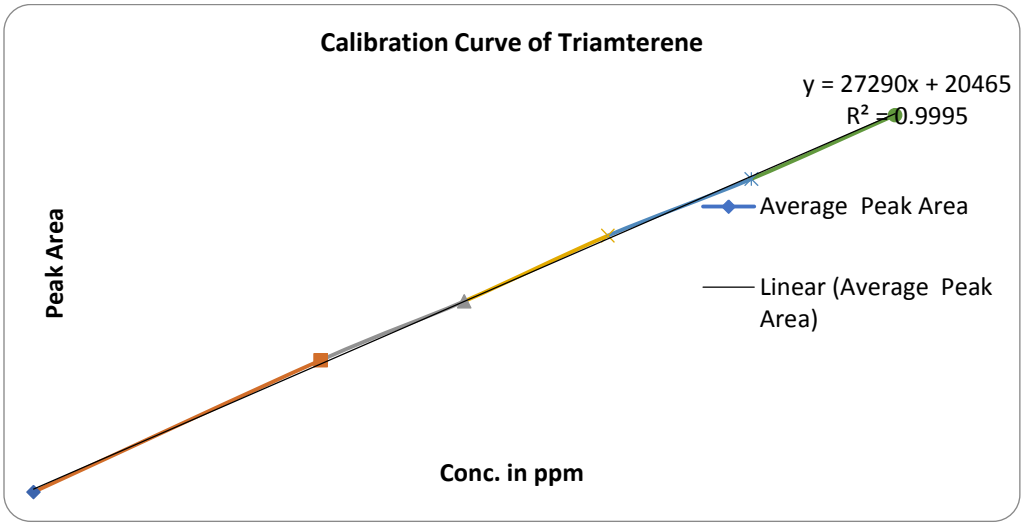


Fig-: Calibration Curve of Triamterene

Linearity plot

The plot of concentration (x) versus the average peak area (y) data of Triamterene is a straight line.

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 replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

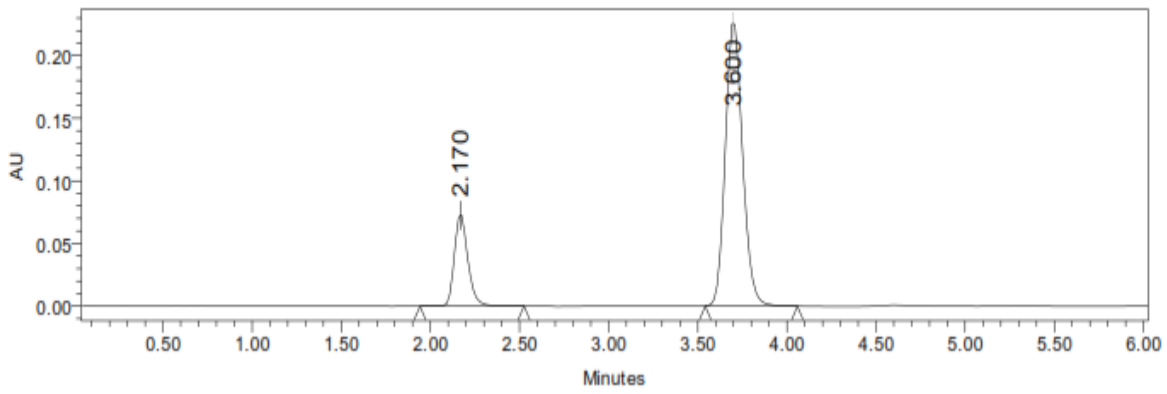


Fig-: Chromatogram showing precision injection -1

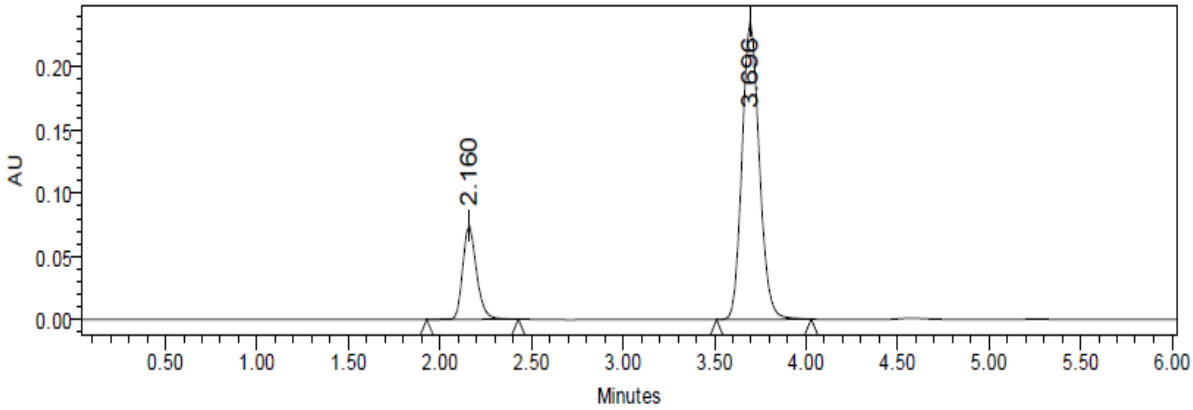


Table-: Results of repeatability for Benzthiazide:

S. No	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Benzthiazide	2.157	513568	78546	1.2	4528
2	Benzthiazide	2.159	513685	78541	1.2	4572
3	Benzthiazide	2.186	513659	79852	1.2	4598
4	Benzthiazide	2.160	513254	78498	1.3	4529
5	Benzthiazide	2.170	513647	77898	1.2	4572
Mean			513562.6			
Std.dev			177.9475			
%RSD			0.03465			

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: Results of repeatability for Triamterene:

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Triamterene	3.603	1635625	265325	1.1	7985
2	Triamterene	3.608	1658744	264588	1.1	7859
3	Triamterene	3.600	1652985	265985	1.2	7845
4	Triamterene	3.696	1645898	264898	1.1	7969

5	Triamterene	3.629	1652364	268489	1.1	7846
Mean			1649123			
Std.dev			8811.631			
%RSD			0.534322			

Intermediate precision

Day 1

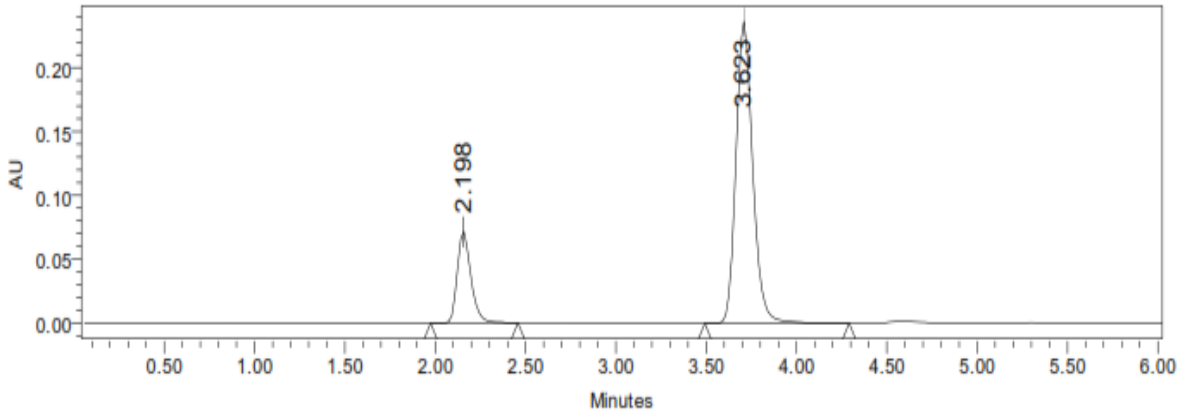


Fig-: Chromatogram showing Day1 injection -1

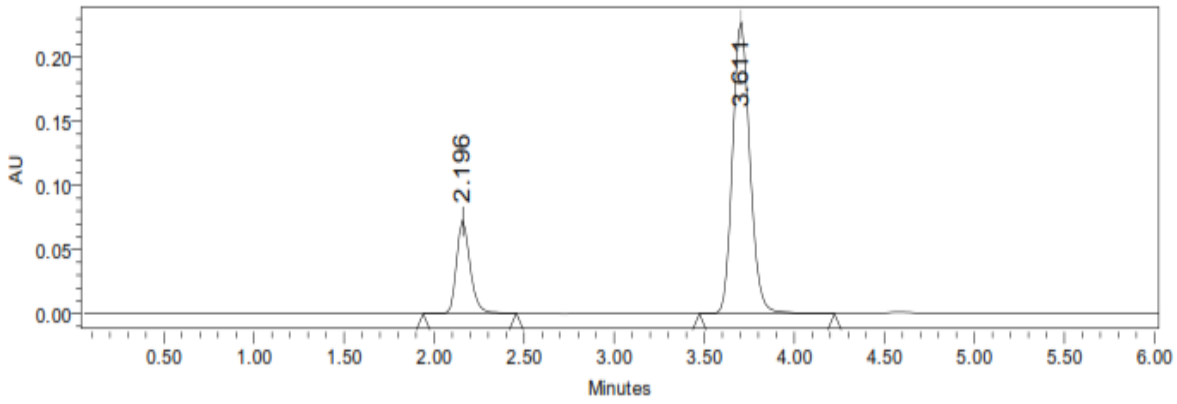


Table-: Results of Intermediate precision for Benzthiazide

S.No.	Peak Name	RT	Area(µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Benzthiazide	2.198	514658	78698	4658	1.2
2	Benzthiazide	2.196	514354	78599	4598	1.2
3	Benzthiazide	2.160	513985	79854	4652	1.2
4	Benzthiazide	2.160	514875	79879	4561	1.2
5	Benzthiazide	2.160	514658	79865	4659	1.2
6	Benzthiazide	2.186	516452	79854	4589	1.2
Mean			514830.3			
Std. Dev.			852.3705			
% RSD			0.165563			

Acceptance criteria

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

Table-: Results of intermediate precision for Triamterene

S.No	Peak Name	Rt	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution
1	Triamterene	3.623	1645875	266589	7985	1.1	10.1
2	Triamterene	3.611	1658554	265898	8001	1.1	10.1
3	Triamterene	3.696	1649854	265415	7985	1.1	10.1
4	Triamterene	3.696	1659842	265154	7956	1.1	10.1
5	Triamterene	3.696	1645985	266598	7985	1.1	10.1
6	Triamterene	3.642	1659852	265341	8002	1.1	10.1
Mean			1653327				
Std. Dev.			6838.733				
% RSD			0.413635				

Acceptance criteria

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

Accuracy

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

Accuracy 50%

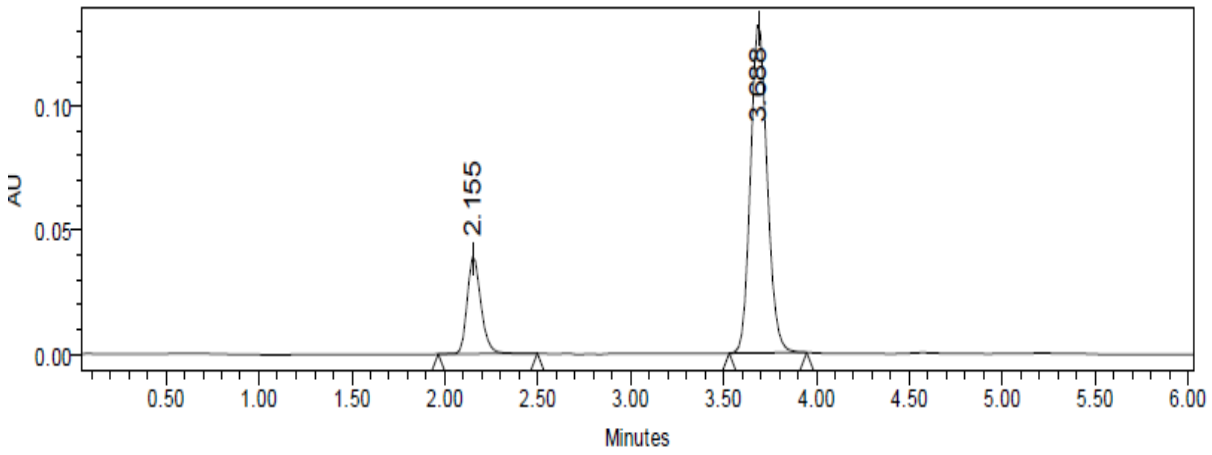


Fig-: Chromatogram showing accuracy-50% injection-1

Accuracy 100%

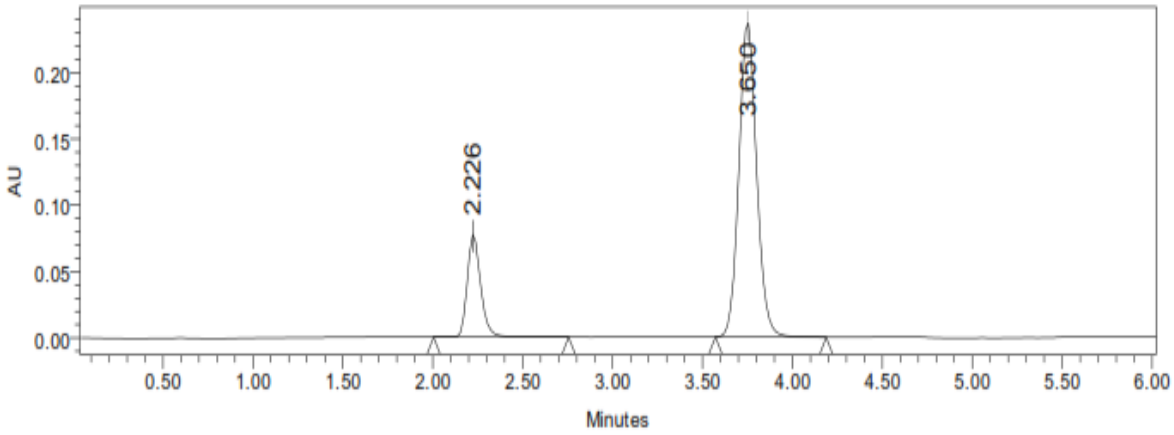
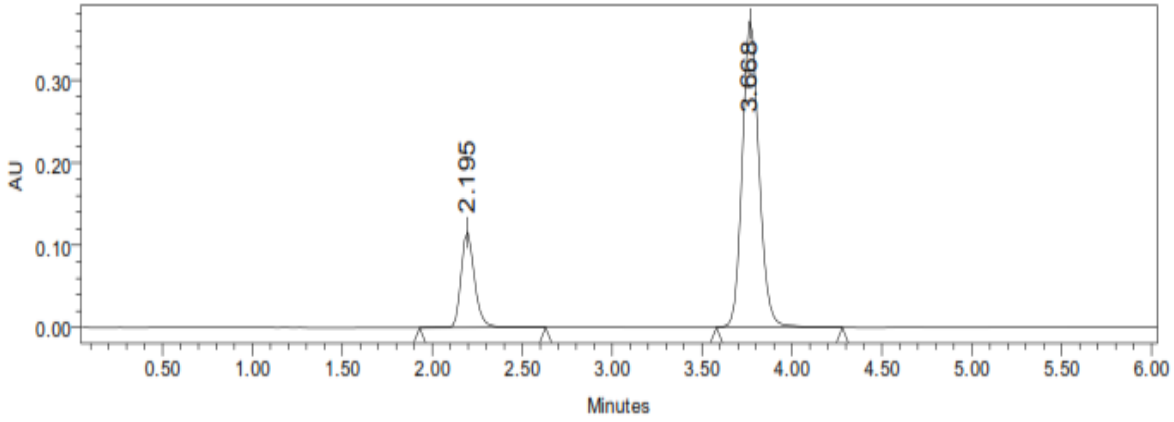


Fig-: Chromatogram showing accuracy-100% injection-1

Accuracy 150%



Acceptance criteria

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

Table: The accuracy results for Triamterene

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	842287	30	30.114	100.38%	100.26%
100%	1659744	60	60.068	100.113%	
150%	2483885	90	90.268	100.297%	

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

Benzthiazide

Result

= 1.0µg/ml

Triamterene

Result

= 11.0µg/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

Benzthiazide

Result

=3.1µg/ml

Triamterene

Result

=35.2µg/ml

Robustness

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

Variation in flow

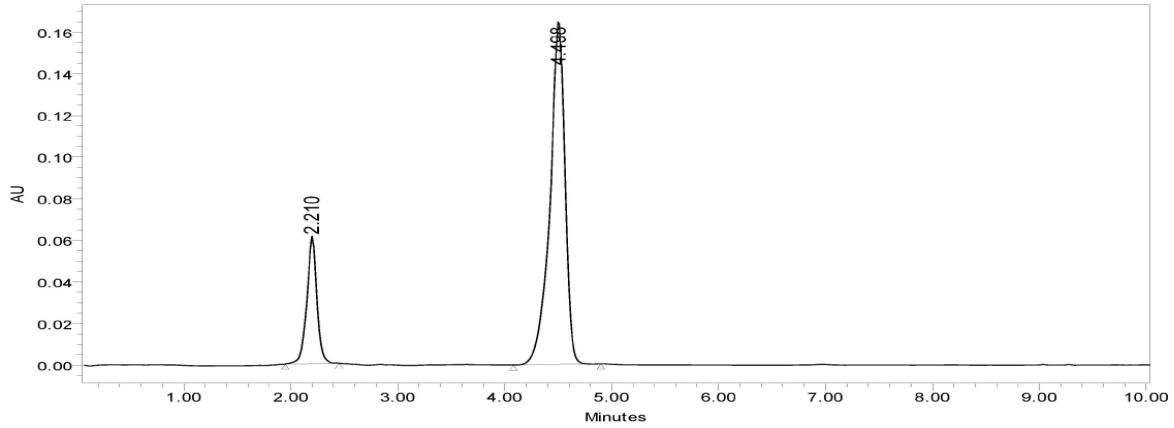


Fig-: Chromatogram showing less flow of 0.9ml/min

Benzthiazide

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	513567	2.179	4536	1.2
Less Flow rate of 0.9 mL/min	523652	2.210	4462.3	0.9
More Flow rate of 1.1 mL/min	502146	2.184	4325.1	1.0
Less organic phase	521574	2.200	4632.4	0.9
More Organic phase	502416	2.172	4190.8	0.8

Acceptance criteria

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

Triamterene

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1625892	3.610	4536	1.1
Less Flow rate of 0.9 mL/min	1758455	4.498	4426.4	0.9
More Flow rate of 1.1 mL/min	1742514	3.505	4421.5	0.8
Less organic phase	1726451	4.504	4355.1	0.9
More organic phase	1725466	3.512	4426.6	0.9

Acceptance criteria

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

SUMMARY

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 238 nm and the peak purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. Ambient temperatures were found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time.

Mobile phase is Acetonitrile and Acetate buffer (pH-4.3) (35:65% v/v) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Run time was selected to be 6 min because analyze gave peak around 2.179, 3.610 ±0.02min respectively and also to reduce the total run time.

The percent recovery was found to be 98.0-102% was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. The analytical method was found linearity over the range 10-30mg/ml of Benzthiazide and 30-90mg/ml of Triamterene of the target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

CONCLUSION

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

Benzthiazide is soluble in water, alcohol, chloroform or ether, and in alkaline solutions and soluble in dimethyl formamide, dimethyl sulfoxide, slightly soluble in methanol, ethanol and Triamterene is very slightly soluble in water, ethanol, and chloroform. It is practically insoluble in ether and soluble in formic acid. Very slightly soluble in water and in ethanol (96%). Soluble in DMSO, it is insoluble in water.

Acetonitrile and Acetate buffer (pH-4.3) (35:65% v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise.

The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Benzthiazide and Triamterene in bulk drug and in pharmaceutical dosage forms.

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