Development and Validation of Spectrophotometric and Chromatographic Analytical methods of Triamterene and Hydrochlorothiazide in Dosage form

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ABSTRACT

The UV and RP-HPLC method describes developed simple, rapid, specific, selective, accurate and precise method for the simultaneous estimation of triamterene and hydrochlorothiazide in dosage form. Hydrochlorothiazide diuretic agents whereas triamterene RP-HPLC method for triamterene and hydrochlorothiazide were developed using mobile phase Phosphate Buffer: Acetonitrile (70:30), flow rate 1 ml/min, injection volume 20µL, temperature 25ºC detection wavelength 222nm. Linearity range was found to be 0.66-29.8µg/ml for triamterene and 0.60-18.2µg/ml for triamterene. LOD and LOQ values were found to be 0.127 and 0.390 µg/ml for triamterene and 0.144 and 0.434 µg/ml for hydrochlorothiazide respectively. The assay result found to be 99.75% and 99.97% for triamterene and hydrochlorothiazide respectively. The developed method was validated according to ICH guidelines.

Keywords: *Triamterene, Hydrochlorothiazide, HPLC, ICH guidelines.*

1. INTRODUCTION

Analytical method development and validation has the important role in the synthesis, development and manufacturing of the pharmaceutical products[1]. As a increasing in the globalisation there are newer diseases increases day by day and there where has a greater challenge to stop such diseases or to treat such diseases by using drugs or pharmaceutical products. The diseases are very complicated and complex in nature so as to treat such diseases the single drug is not effective therefore it requires combination of drugs. For the development of pharmaceutical products with combination of two or more drugs the analytical method validation has the greater role[2]. By validating such methods we can produce the new pharmaceutical products with combination. Triamterene is a potassium sparing diuretic drug and

hydrochlorothiazide is a thiazide containing diuretics[3]. Hydrochlorothiazide is a drug which is act by decreasing the water level of the kidney and by decreasing the blood volume, and by decreasing the cardiac output. Triamterene is a potassium sparing diuretic when used with hydrochlorothiazide it acts by sparing the potassium in the blood and avoid the loss of the potassium from the blood stream due to this the hypokalemia condition can be treated by using potassium sparing diuretics[4]. In the patients with blood pressure there is causes of the hypertension are increases the sodium level in the body due to the wrong diet or diet with high salty intake. In such conditions hydrochlorothiazide can not be used singly it is always used in combination with potassium sparing diuretics. In the market there are various combinations of thiazide and potassium sparing diuretics e.g. Torsemide and spironolactone but the combination of triamterene and hydrochlorothiazide is a newer combination. The combination of such drug is called as water pills. Such combinations are effective in the patients with hypertension, edema, hypokalemia, congestive heart failure, diabetes insipidus, renal failure, etc. Therefore the present study focused on combination of drug triamterene and Hydrochlorothiazide. Therefore the all over study gives the idea about development and validation of simple, accurate and precise method for the determination of triamterene and hydrochlorothiazide in combination[5].

2. MATERIALS AND METHODS

All the chemicals were procured from Cipla Healthcare LTD, Mumbai and Fischer Scientific Chemicals Pvt. Ltd Mumbai. Dyazide Capsules were used as formulation procured from GlaxoSmithKline (GSK) company.

1) Identification of Drug

a) ***Melting Point Determination:*** Melting point of drug is determined to identify the purity and quality. Melting point of drug is determined by open capillary tube method.

b) ***IR-Interpretation:*** The standard Triamterene and Hydrochlorothiazide powders IR spectra is obtained from IR instrument and interpretation of spectra is carried out to determine the number of functional group present in the drug with respect to their wave numbers.

2) Analysis of drug by using UV Spectrophotometric technique

a) ***Solubility Studies:*** Triamterene and hydrochlorothiazide were found soluble in the 0.1 N HCl and methanol. Therefore such solvents are used for solvent system.

b) ***Selection of Wavelength:*** For the validation of method by using UV Spectrophotometry of Triamterene and Hydrochlorothiazide the suitable wavelength is selected in between 250-400 nm for

both the samples. The absorbance is recorded at 271.5 nm and cannot be maximum because of overlapped spectrum.

c) ***Preparation of Stock Solution:*** The stock solutions of Triamterene and Hydrochlorothiazide were prepared by dissolving the required quantity of Triamterene and Hydrochlorothiazide in the methanol. The solution of Triamterene and Hydrochlorothiazide gives the concentration of 400 µg/ml. This solutions are working as a standards. The dilutions of Triamterene and Hydrochlorothiazide from this stock solutions is made by using 0.1 N HCl[6].

➢ **Validation of Methods As per ICH guidelines**

The above Prepared solution is validated by using the parameters of the ICH guidelines.

1. ***Linearity Study***
2. ***Accuracy & Precision***
3. ***Limit of Detection & Limit of Quantitation:*** The Limit of Detection (LOD) and Limit of Quantitation (LOQ) for the above method is calculated using following formula[7].

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

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

Where,

σ = Standard deviation

S = Slope of Calibration curve.

4. ***Robustness***
5. ***Ruggedness***

3) Method Validation by Using Reverse Phase High Performance Liquid Chromatography

1. ***Fixed Chromatographic Condition***

Table 1. Fixed Chromatographic Condition

Column	**Grace smart RP-18 (4.6X250), 5µm**
Wavelength	267 nm
Temperature	40 degree Centigrade
Injection	20µl
Flow Rate	1.0 ml/min
Mobile Phase	Phosphate Buffer : Acetonitrile (70:30)
Run Time	7 min

2. ***Preparation of Stock Solution and Calibration Curve***

Standard Stock solution of Triamterene and Hydrochlorothiazide was prepared by Weighing 37.4 mg of Triamterene and 25.8 mg of Hydrochlorothiazide in 200 ml Volumetric flask. In to this 150 ml of mobile phase of Phosphate buffer:methanol (70:30) is added and sonicated for 10 minutes. Allow this solution at room temperature and diluted up to mark with mobile phase. From this solution 20µl of each concentration was injected into the HPLC system under optimized chromatographic conditions and calibration curve was plotted by using the peak areas against to the concentration.

3. *Selection of Analytical Wavelength*

For Reverse Phase HPLC method the analytical wavelength is selected from UV Spectrophotometer showing the maximum absorbance at 267 nm

4. Optimization of Reverse Phase HPLC method

The mobile phase such as Phosphate buffer: Acetonitrile with pH 3.5. Various mobile phases are prepared with different compositions such as 80:20, 90:10, 60:40, 70:30, etc but from that concentrations best result is obtained at the ratio of 70:30[8].

Table 2. Optimization of Mobile Phase for Triamterene & Hydrochlorothiazide

Sr. no	Mobile Phase	(A:B)	Detection Wavelength	RT	Observation	Inference
1.	Phosphate buffer: Acetonitrile	(80:20)	267	2.5 (TRMT) 3.5 (HCTZ)	Peak resolves but assymetric	Rejected
2.	Phosphate buffer: Acetronitrile	(90:10)	267	2.2 (TRMT) 3.2(HCTZ)	Peaks not resolve properly	Rejected
3.	Phosphate buffer: Acetronitrile	(60:40)	267	2.7 (TRMT) 3.8 (HCTZ)	Peaks not resolve properly	Rejected
4.	Phosphate buffer: Acetonitrile	(70:30)	267	3.2 (TRMT) 4.6 (HCTZ)	Good peak resolution	Accepted

Table 3. System Suitability Parameters

Sr. No.	Parameters	Triamterene	Hydrochlorothiazide
1.	Retention Time	4.0	5.7
2.	Theoretical Plates	16227	8008
3.	Asymmetry	1.20	1.21
4.	Limit of Detection (LOD) (µg/ml)	0.127	0.144
5.	Limit of Quantitation (LOQ)(µg/ml)	0.390	0.434
6.	Tailing Factor	1.26	1.15

Validation of Analytical method as per ICH guidelines:

1. **Linearity Study:** The standard calibration curve for Triamterene and Hydrochlorothiazide was plotted separately As peak area Vs Concentration.
2. **Precision Study:** The data was obtained within the limits.
3. **Accuracy Study (Recovery Study):** The method was carried out by using drug sample of known amount of 80, 100, and 120% of Triamterene and Hydrochlorothiazide. Three determinations of such concentration were performed and % drug recovery was calculated[9].
4. **Robustness:** The robustness study on the sample was performing by the changing in the operational conditions such as changing in the flow rate or changing in the wavelength. The data will be obtained on the different flow rate e.g. 0.8 ml, 1.2 ml and different wavelengths are used such as 265 nm and 269 nm[10].
5. **Specificity & Repeatability:** The Specificity and Repeatability is nothing but the validation parameter in which the repeated validations of the samples are carried out for checking[11-12].

3. RESULT & DISCUSSIONS

1) Identification of Drugs

a) Melting point determination

Table 4. Melting point of pure triamterene and hydrochlorothiazide

Sr. No.	Name of drugs	Observed Melting Ranges
1	Triamterene	316^0C
2	Hydrochlorothiazide	274^0C

b) ***FTIR-Spectra Interpretation:*** The FTIR spectra of pure Triamterene and Hydrochlorothiazide is interpreted with respect to observed wave numbers. The FTIR spectrum of Triamterene and Hydrochlorothiazide will be present in figure.

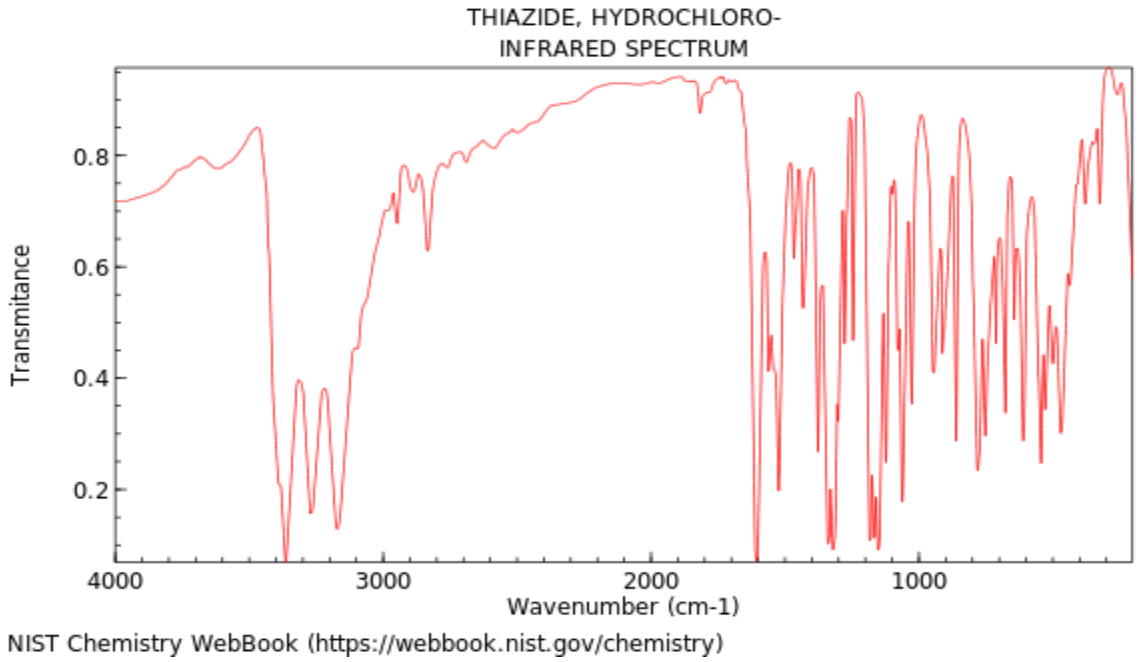

Figure 1. FTIR Spectra of Triamterene

Table 5. Interpretation of IR Spectra of Triamterene

Sr. no.	Observed Wavenumbers	Standard Wavenumbers	Name of functional group present
1	3444	3372	OH Streching
2	2962	2934	CH Streching
3	1741	1730	C=O Streching
4	1539	1526	OC-NH Streching
5	1386	1380	CH plane bending

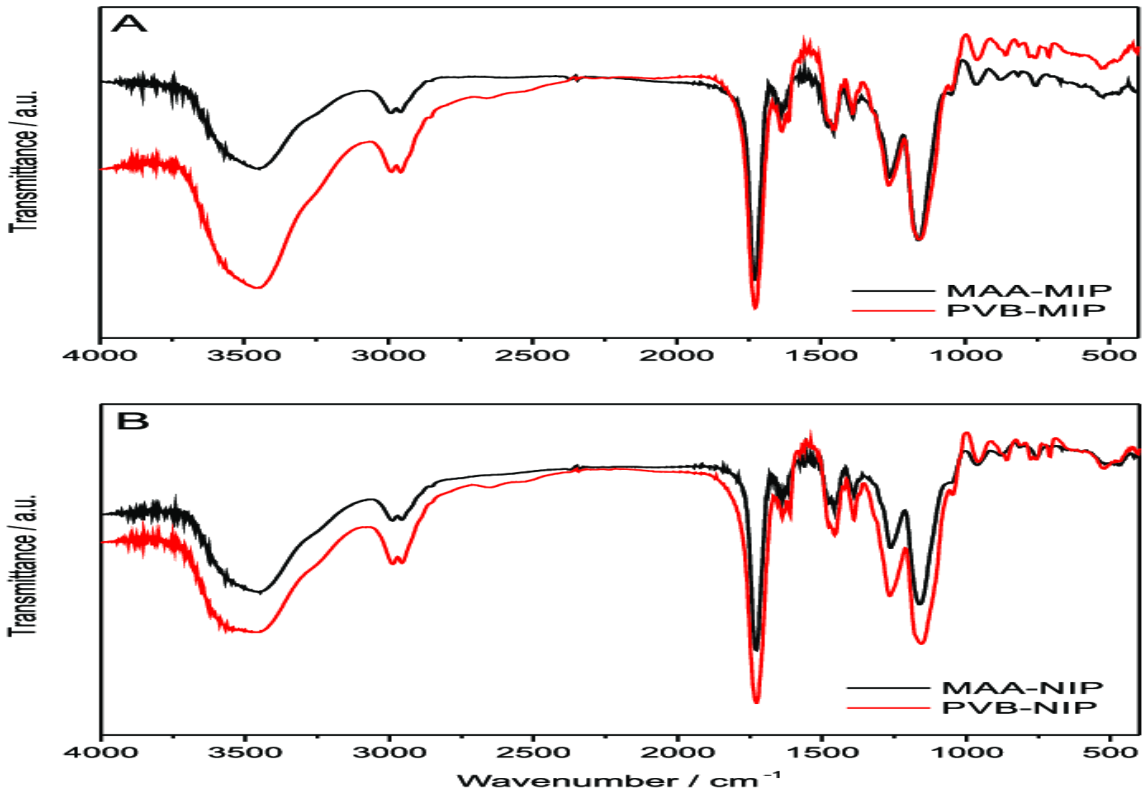

Figure 2. FTIR Spectra of Hydrochlorothiazide

Table 6. Interpretation of IR Spectra of Hydrochlorothiazide

Sr. no.	Observed Wavenumbers	Standard Wavenumbers	Name of functional group present
1	3353	3500	Phenol Alcohols
2	2929	2500	Carboxylic Acid
3	1702	1750	Ketones
4	1639	1470	Aldehydes
5	1454	1370	Alkanes

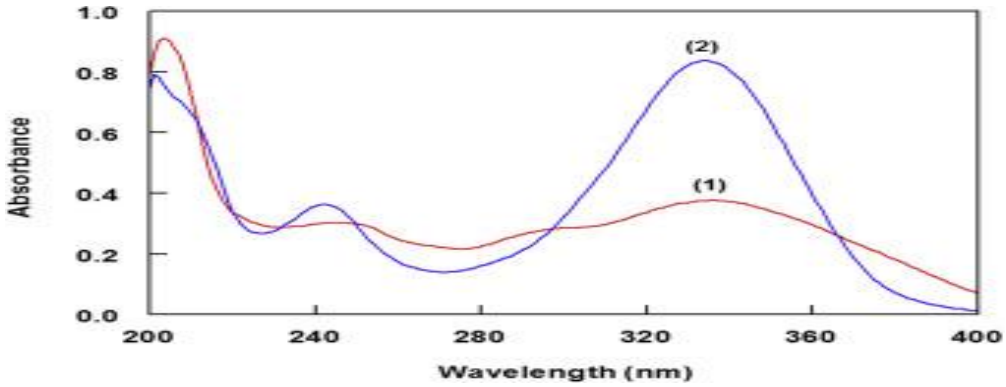

Figure 3. Isobestic points of the Triamterene and Hydrochlorothiazide (271nm)

1.1 UV SPECTROPHOTOMETRY

a) ***Linearity:*** The concentrationLinearity is found in between 0.66-29.8µg/ml at 280 nm for Hydrochlorothiazide and concentration linearity is found in between 0.60-18.2µg/ml at 379 nm for Triamterene. Both the solutions of drug Triamterene and Hydrochlorothiazide shows the linearity.

Table 7. Statistical Data for graph calibration

Method	Analyte	Wavelength selected (nm)	Linearity range µg/ml	Correlation coefficient (R2)	LOD µg/ml	LOQ µg/ml
DS	HCTZ	1D 280 nm	0.66-29.8	0.9994	0.144	0.434
DS	TRMT	1D 379 nm	0.60-18.2	0.9989	0.127	0.390

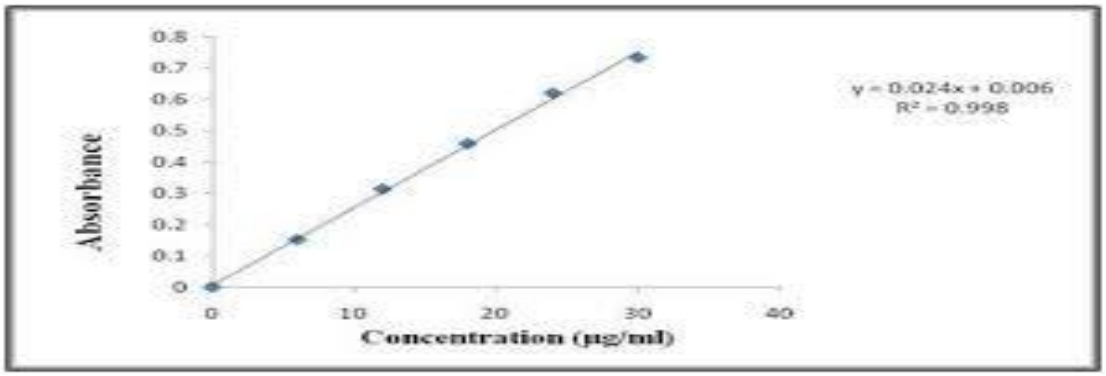

Figure 4. Data of Calibration Curve of Triamterene at 379nm

Conc (µg/ml)	Absorbance
05	0.107
10	0.184
12	0.267
15	0.359
20	0.441

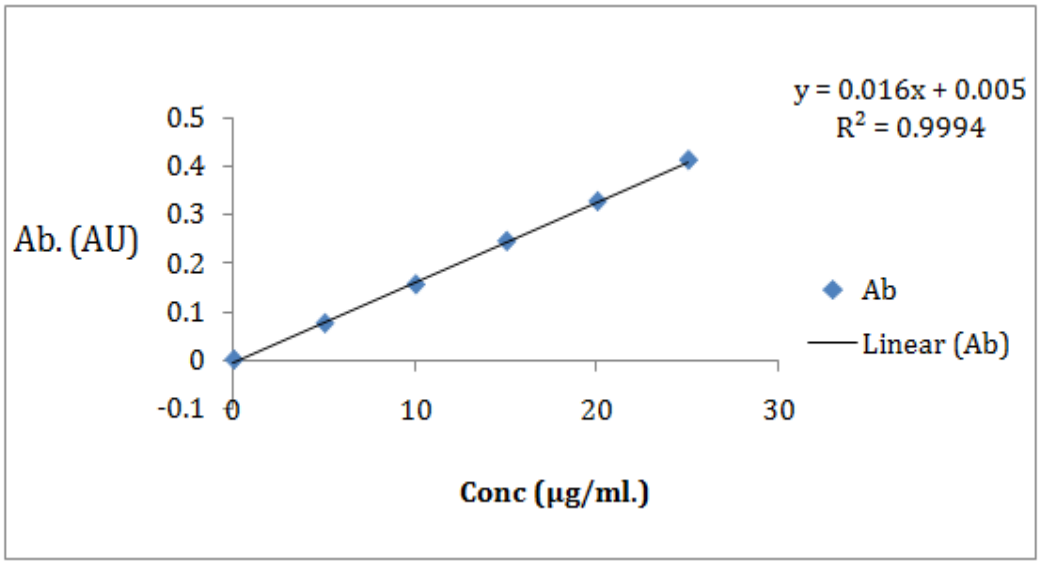

Figure 5. Data of Calibration Curve for Hydrochlorothiazide at 379nm

Conc (µg/ml)	Absorbance
05	0.143
10	0.264
15	0.391
20	0.529
25	0.554

b) ***Accuracy & Precision:*** The Accuracy and precision was performing by carried out five determinations on three different concentrations for the solutions of Triamterene and Hydrochlorothiazide. The Precision RSD% and Accuracy% will be shown in table

Table 8. Validation data of Accuracy & Precision

Method	Sample	Theoretical concentration µg/ml	Observed concentration µg/ml	SD µg/ml	Precision RSD%	Accuracy %
DS	HCTZ	12.70	12.79	0.0723	0.559	100.60
		19.10	19.00	0.0884	0.458	99.38
		28.70	28.86	0.0639	0.215	100.11
DS	TRMT	0.70	0.69	0.0154	2.066	98.65
		6.30	6.28	0.0444	0.702	99.59
		19.10	19.11	0.1844	0.955	100.00

c) ***Limit of Detection (LOD) & Limit of Quantitation (LOQ):*** Limit of Detection (LOD) And Limit of Quantitation (LOQ) for Triamterene and Hydrochlorothiazide was found to be as shown in table below.

Table 9. Validation Data of LOD & LOQ

Sample	LOD (µg/ml)	LOQ (µg/ml)
Triamterene (TRMT)	0.144	0.434
Hydrochlorothiazide (HCTZ)	0.127	0.390

d) ***Ruggedness Study:*** Ruggedness study on the analytical sample was performing by carried out the analysis of the solution of sample of Triamterene and Hydrochlorothiazide by two different analysts and comparing the data. The data for the Triamterene and Hydrochlorothiazide will be present in Table

Table 10. Validation Data for Ruggedness Study

Sample		**Dyazide (GSK Labs)**
Label Claim		25mg (HCTZ), 37.5(TRM)
		20 µg/ml
Analyst-I	**Amount Found**	19.12±0.0315
	% Recovery (Mean)	99.93(TRM) 99.32(HCTZ)
	%RSD	0.02(TRM) 0.05(HCTZ)
Analyst-II	**Amount Found**	19.17±0.010
	% Recovery	99.98(TRM) 99.33(HCTZ)
	%RSD	0.61(TRM) 0.58(HCTZ)

e) ***Robustness Study:*** The Robustness study was carried out by changing the wavelength and by changing the mobile phase compositions. The effect of changing wavelength and changing the effect of changing composition of mobile phase was showing in the table

Table 11. Validation Data of Robustness study

Change in wavelength	**265**	**269**	**267**
Concentration	10	10	10
Amount Found±SD	9.99(TRM ±0.0444 9.93(HCT)±0.0884	9.87(TRM)±0.0154 9.94(HCT)±0.0884	9.95(TRM)±0.0444 9.96(HCT)±0.0884
% Amount Recovered	99.9 (TRM) 99.3(HCT)	98.7(TRM) 99.4 (HCT)	99.5 (TRM) 99.6 (HCT)
%RSD	0.61 (TRM) 0.58 (HCT)	0.61 (TRM) 0.58 (HCT)	0.05 (TRM) 0.58 (HCT)

f) ***Analysis of Marketed Formulations*****:** It is important to determine the marketed formulation of combination of Triamterene and Hydrochlorothiazide. The data for the analysis of marketed formulation of triamterene and hydrochlorothiazide will be shown in table no.

Table 12. Data for analysis of marketed Formulation

Formulation	Label CLAIM mg/cap	Amount Found mg/cap	Percentage Label Claim	Average %±SD
Dyazide (gsk)	HCTZ(25mg) TRMT(37.5mg)	24.4(HCTZ) 37.5(TRMT)	97.6 100	97.6±0.0884 100±0.1844

1.2 Reverse Phase HPLC: Number of theoretical plates was found to be more than 2000. Other parameters was found to be within the acceptance limit. Therefore proposed RP-HPLC method shows system suitability for estimation of Triamterene and Hydrochlorothiazide. In the RP-HPLC method the peak for Triamterene and Hydrochlorothiazide was clearly separated from other excipients of the formulations within retention time range of 4.0min for Triamterene and 5.7 min for Hydrochlorothiazide.

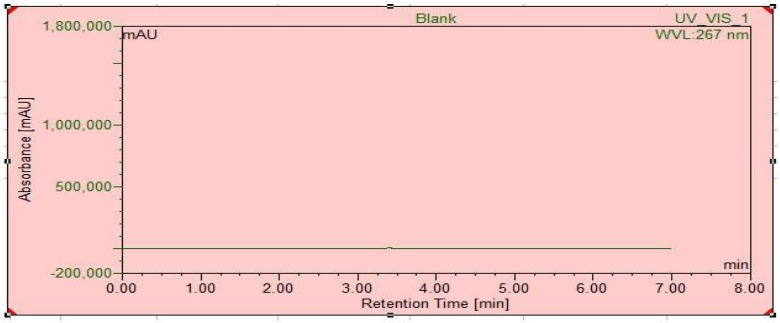

Figure 6. Chromatogram of the blank sample

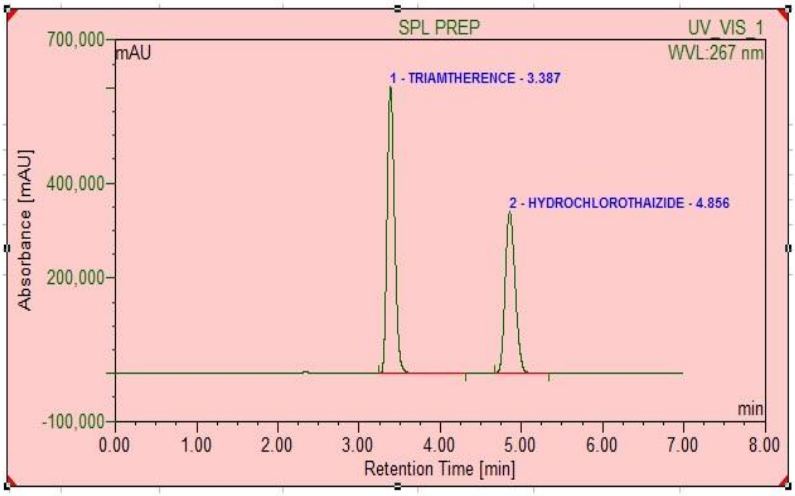

Figure 7. Chromatogram of the Triamterene and Hydrochlorothiazide

a) ***Linearity:*** The linearity is carried out by using the nine different determinations of the sample of Triamterene and Hydrochlorothiazide in the solution form. From the data given in the table was found to be that the both solutions of Triamterene and Hydrochlorothiazide was showing linearity.

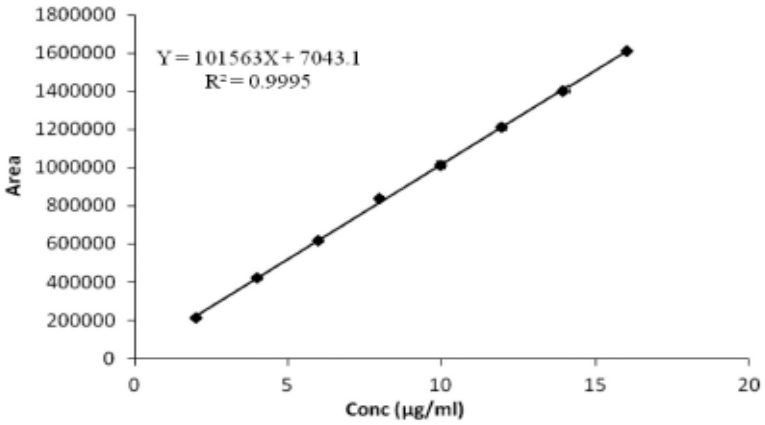

Figure 8. Calibration Curve of Triamterene by RP-HPLC Method

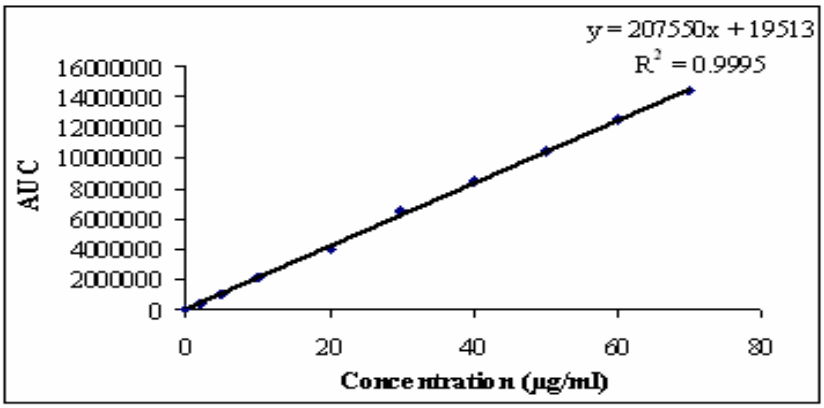

Figure 9. Calibration Curve of Hydrochlorothiazide by RP-HPLC Method

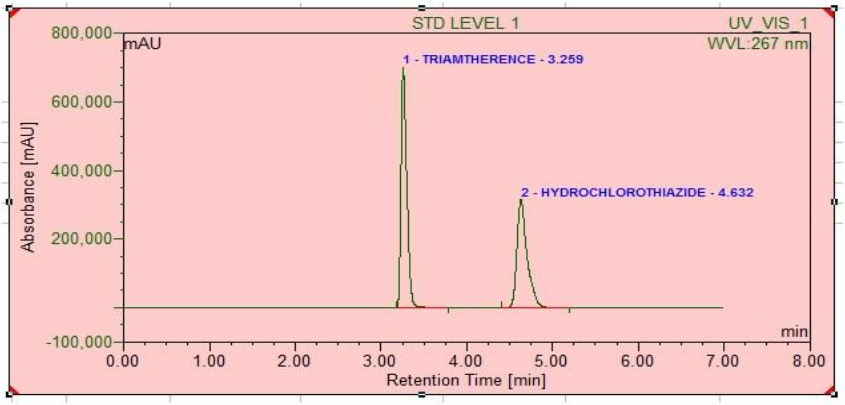

Figure 10. Chromatogram showing Linearity of Sample of Level 1

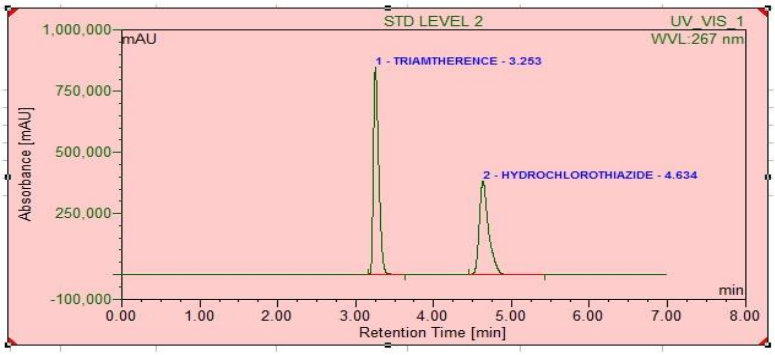

Figure 11. Chromatogram Showing Linearity of Sample of Level 2

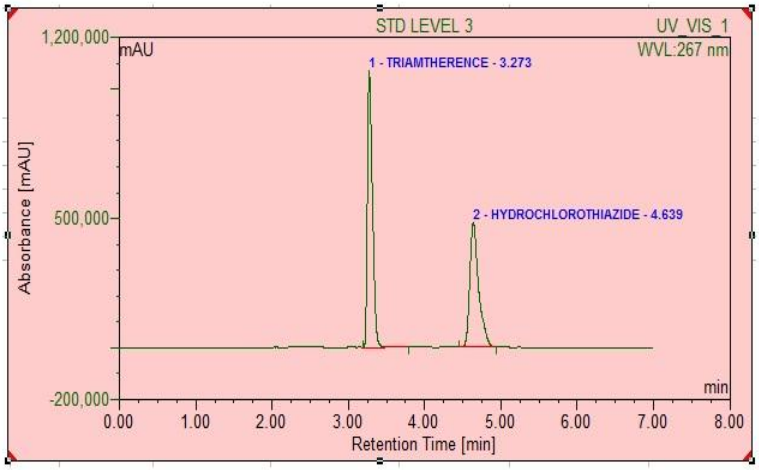

Figure. 12. Chromatogram showing Linearity of Sample of Level 3

Table 13. Data of Calibration curve for Triamterene (TRMT)

Sr. no.	Conc. ppm	Area	Average
1	60.4	54440.540	54477.16333
2	60.4	54483.731	
3	60.4	54507.219	
4	75.40	66470.915	66496.481
5	75.40	66484.960	
6	75.40	66533.568	
7	90.60	82339.271	80324.22933
8	90.60	82389.508	
9	90.60	82243.909	

Table 14. Data of Calibration Curve for Hydrochlorothiazide (HCTZ)

Sr. No.	Conc. ppm	Area	Average
1	40.60	41852.178	41870.86467
2	40.60	41902.516	
3	40.60	41857.9	
4	50.80	52555.045	52550.67133
5	50.80	52476.073	
6	50.80	52620.896	
7	61.20	65188.934	65241.46833
8	61.20	65382.593	
9	61.20	65152.878	

b) ***Precision:*** The precision study will be carried out by analyzing the solutions by intraday or interday. The six different determinations of the samples are taking in the one day and repeated it for second day.

Table 15. Data showing the precision study

Sample	Area TRMT	Triamterene		Area HCTZ	Hydrochlorothiazide	
Sample-1	64181.152	37.81mg/cap	100.83%	48098.394	24.66mg/cap	98.65%
Sample-2	64125.49	37.38mg/cap	99.68%	48190.869	24.45mg/cap	97.80%
Sample-3	64174.029	37.36mg/cap	99.62%	48172.294	24.41mg/cap	97.63%
Sample-4	64202.762	37.64mg/cap	100.38%	48128.789	24.56mg/cap	98.25%
Sample-5	64294.335	37.27mg/cap	99.38%	48187.326	24.31mg/cap	97.25%
Sample-6	64201.913	37.41mg/cap	99.77%	48222.933	24.46mg/cap	97.84%

c) ***Accuracy and Recovery study:*** The accuracy and recovery study was performing by analyzing the sample solutions of known amount of 80%, 100%, 120% the resulting solution was injected into he chromatogram and from that the % recovery will be the calculated.

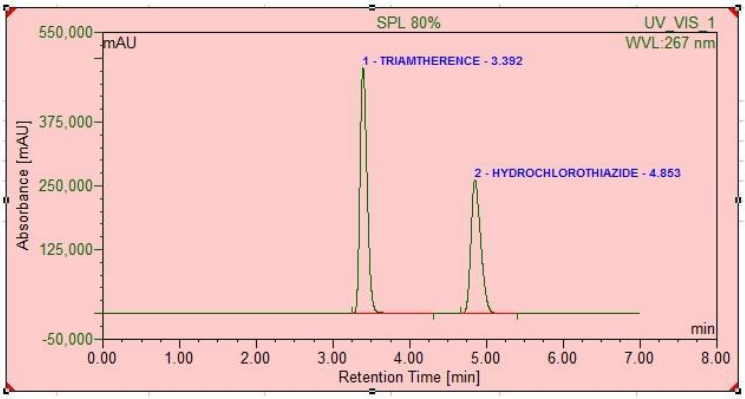

Figure 13. Chromatogram Showing Accuracy for Sample 80%

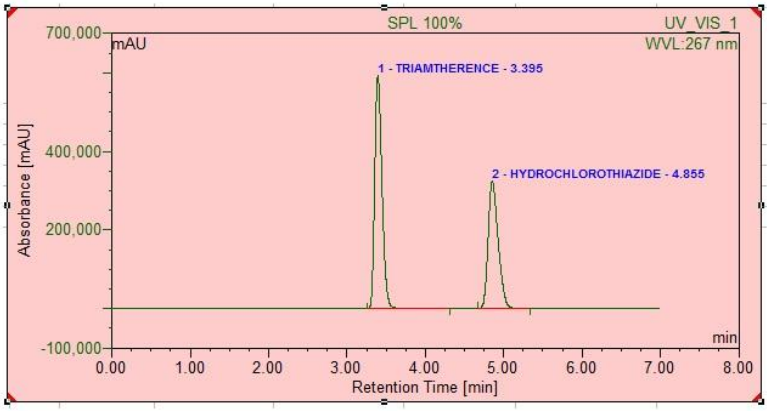

Figure 14. Chromatogram Showing Accuracy for Sample 100%

Table 16. Data Showing the Accuracy (Recovery Study) of Triamterene

Sample	% Assay Threotical	% Assay Actual	% Recovery	%RSD
Wt of sample-1 80% (gm)	98.94	100.17	101.24	0.34
Wt of sample-2 80% (gm)	98.49	100.25	101.78	
Wt of sample-3 80% (gm)	98.90	100.02	101.13	
Wt of sample-1 100% (gm)	99.29	99.27	99.98	0.05
Wt of sample-2 100% (gm)	99.31	99.25	99.93	
Wt of sample-3 100% (gm)	99.41	99.45	100.04	
Wt of sample-1 120% (gm)	99.57	98.35	98.78	0.61
Wt of sample-2 120% (gm)	99.47	98.45	98.97	
Wt of sample-3 120% (gm)	98.97	98.89	99.91	

Table 17. Data showing Accuracy of Hydrochlorothiazide

Sample	% Assay Threotical	% Assay Actual	% Recovery	%RSD
Wt of sample-1 80% (gm)	96.95	97.55	100.62	0.41
Wt of sample-2 80% (gm)	96.51	97.62	101.15	
Wt of sample-3 80% (gm)	96.91	97.23	101.33	
Wt of sample-1 100% (gm)	97.29	96.66	99.33	0.02
Wt of sample-2 100% (gm)	97.31	96.64	99.32	
Wt of sample-3 100% (gm)	97.40	96.71	99.29	
Wt of sample-1 120% (gm)	97.56	96.91	99.33	0.58
Wt of sample-2 120% (gm)	97.47	96.88	99.40	
Wt of sample-3 120% (gm)	96.98	97.32	100.36	

d) ***Robustness:*** The robustness study was performed by changing in the flow rate and by changing in the wavelength and analyzing the samples. The different flow rate such as 0.8, 1.2 and different wavelengths such as 265 nm and 269 nm will be used and analyzing the samples.

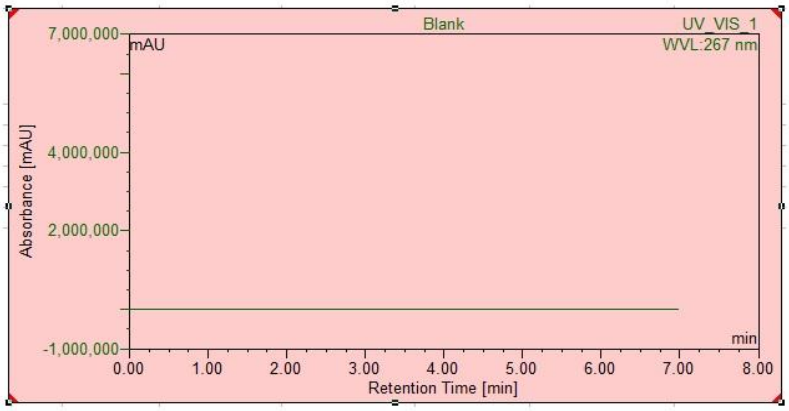

Figure 15. Chromatogram Showing the Robustness for Blank Sample

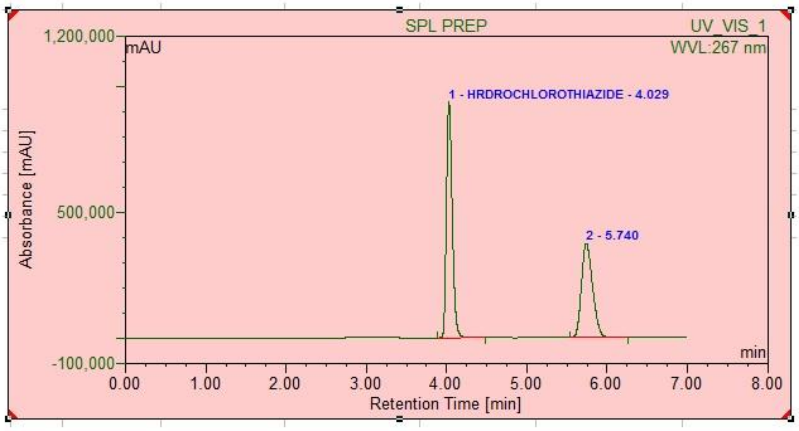

Figure 16. Chromatogram Showing Robustness for Sample

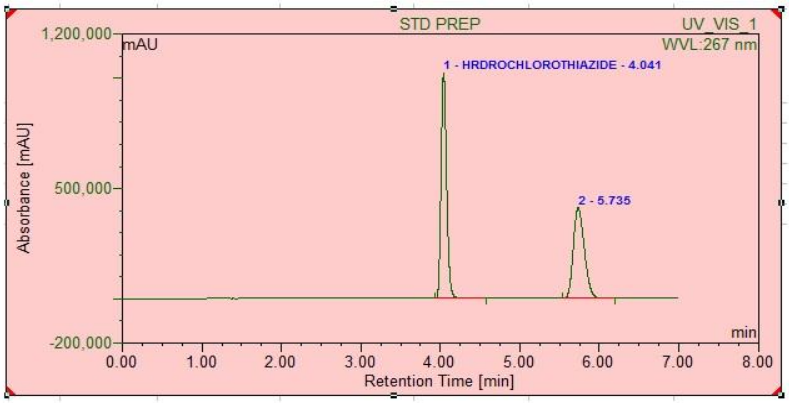

Figure 17. Chromatogram Showing the Robustness for Standard

Table 18. Table Showing Robustness study

Parameter	% Assay (TRMT)	% Assay (HCTZ)	%RSD (TRMT)	%RSD (HCTZ)
Flow Rate 0.8ml	100.43	97.84	0.35	0.47
Flow Rate 1.2ml	100.50	97.57	0.36	0.54
Wavelength 265 nm	100.26	97.38	0.35	0.60
Wavelength 269nm	100.80	97.58	0.38	0.54

e) Specificity & Repeatability: The Specificity was performed by weighing 37.4 mg of triamterene and 25.8 mg of Hydrochlorothiazide and added to the volumetric flask of 200 ml containing mobile phase. Then this solution is sonicated for 10 min for degas of solution. Allow the solution for room temperature and diluted up to mark with mobile phase .then pipette out 10 ml of solution to 25 ml volumetric flask containing mobile phase and from this solutions six different samples are prepared and used for analysis.

Table 18. Table of showing data for specificity

Sample No.	Sample wt	%Assay (TRMT)	% RSD (TRMT)	%Assay(HCTZ)	%RSD(HCTZ)
1	0.16524	100.67	0.35	98.90	0.40
2	0.16616	100.28		98.17	
3	0.16517	100.70		98.73	
4	0.16397	100.80		98.61	
5	0.16458	100.32		98.26	
6	0.16525	99.88		97.84	

f) Analysis Of Marketed formulations: The marketed formulation of Triamterene and Hydrochlorothiazide is analyzing by using RP-HPLC method.

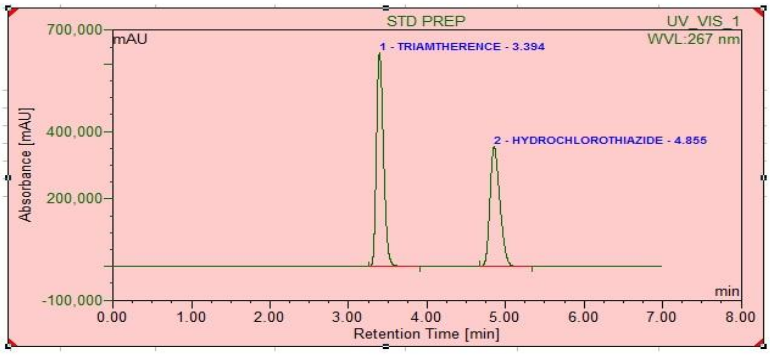

Figure 18. Chromatogram for Marketed formulation of Triamterene & Hydrochlorothiazide

Table 20. Table of Showing data for analysis of Marketed formulations

Formulation	Label CLAIM mg/cap	Amount Found mg/cap	Percentage Label Claim	Average %±SD
Dyazide (gsk)	HCTZ(25mg) TRMT(37.5mg)	24 (HCTZ) 37.5(TRMT)	96 100	97.6±0.0884 100±0.1844

4. CONCLUSION

The main target of this research was development and validation of RP-HPLC method for the combination of drug triamterene and hydrochlorothiazide in bulk drug and pharmaceutical formulation. This method gives a good resolution peak for both triamterene and hydrochlorothiazide with retention time of 4 min for triamterene and 5.7 min for hydrochlorothiazide. The developed method was validated by using parameteres of ICH guidelines such as Precision, Accuracy, Linearity, Specificity. All validation Parameters were found to be a good and within the acceptance criteria. From this it is concluded that the method was accurate, reproducible, repeatable, linear, precise and selective and proving the reliability of the method as per ICH guidelines. The results of this method shows that the method has a practical application as a quality control tool for the analysis of drug in to its dosage form in the quality control laboratories.

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