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RESEARCH ARTICLE

Analytical Method Development and Validation of Hydrochlorothiazide and Eposartan in API and its Dosage form by RP-HPLC

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ABSTRACT

On the basis of experimental results, the proposed method is suitable for the quantitative determination of Hydrochlorothiazide and Eposartan in pharmaceutical dosage form. The method provides great sensitivity, adequate linearity and repeatability. The estimation of Hydrochlorothiazide and Eposartan was done by RP-HPLC. The Phosphate buffer was pH 4.6 and the mobile phase was optimized which consists of MEOH: Phosphate buffer mixed in the ratio of 70:30 % v/ v. A Symmetry C18 (4.6 x 150mm, 5µm, Make XTerra) column used as stationary phase. The detection was carried out using UV detector at 273 nm. The solutions were chromatographed at a constant flow rate of 1.0 ml/min. the linearity range of Hydrochlorothiazide and Eposartan were found to be from 25-125 µg/ml. Linear regression coefficient was not more than 0.999.The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 97-102% of Hydrochlorothiazide and Eposartan LOD and LOQ was found to be within limit. The proposed method is precise, simple and accurate to determine the amount of Hydrochlorothiazide and Eposartan in formulation. High percentage of recovery shows that the method is free from the interference of excipients used in the formulation. So the method can be useful in the routine quality control of these drugs.

Keywords: Symmetry C18, Hydrochlorothiazide and Eposartan RP-HPLC

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CONTENTS

1. Introduction

Hydrochlorothiazide is a diuretic medication often used to treat high blood pressure and swelling due to fluid buildup. Other uses include treating diabetes insipidus and renal tubular acidosis and to decrease the risk of kidney stones in those with a high calcium level in the urine

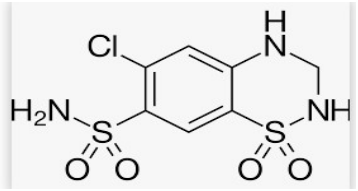

Figure 1: Hydrochlorothiazide

Eprosartan is an angiotensin II receptor antagonist used for the treatment of high blood pressure. It is marketed in the United States as Teveten by Abbvie, the spin-off of the pharmaceutical discovery division of Abbott Laboratories.

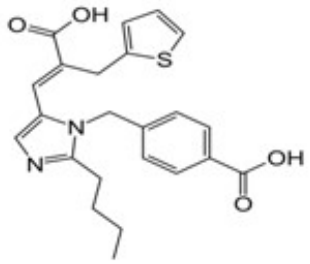

Figure 2: Eprosartan

2. Materials and Methods

Instrumentation

System Alliance Waters 2690 separation module, Pump Analytical HPLC isocratic pump, Detector Photo diode array detector, Software Empower 2 software, Column Agilent (250×4.6mm, 5µ) C-18 RP-column, Sonicator Analytical Technologies Limited- Ultrasonic cleaner. U.V double beam spectrophotometer LABINDIA, UV 3000^{+}pH meter, Weighing machine

Chemicals:

Hydrochlorothiazide and Eposartan, KH_2PO_4, Water and Methanol for HPLC, Acetonitrile for HPLC, Ortho phosphoric Acid.

Optimized chromatogram is obtained by following conditions

Column : Symmetry C18 (4.6 x 150mm, 5µm, Make: XTerra) or equivalent

Buffer pH : 4.6

Mobile phase: 70% Meoh: 30% phosphate buffer ph-4.6

Flow rate : 1 ml per min

Wavelength : 273 nm

Temperature : ambient.

Run time : 7min.

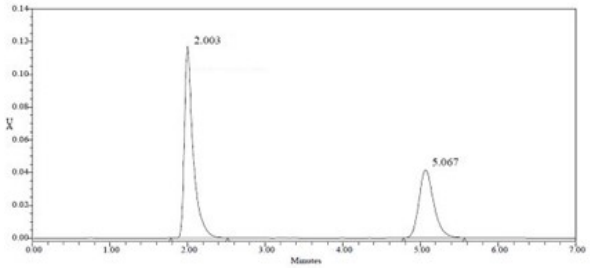

Figure 3: Chromatogram for Hydrochlorothiazide and Eprosartan

From the above chromatogram it was observed that the Hydrochlorothiazide and Eprosartan peaks are well separated

Preparation of Sample Solution: (Tablet)

Accurately 10 tablets are weighed and crushed in mortar and pestle and weight equivalent to 10 mg of Eposartan and Hydrochlorothiazide (marketed formulation) sample into a 10mL clean dry volumetric flask and about 7mL of Diluents is added and sonicated to dissolve it completely and made volume upto the mark with the same solvent. (Stock solution) Further 3 ml of above stock solution was pipetted into a10ml volumetric flask and diluted upto the mark with diluant.

Method Validation

- System Suitability
- Linearity
- Specificity
- Precision
- Intermediate Precision
- Accuracy
- Limit of Detection and Limit of Quantification
- Robustness

3. Results and Discussion

Table 1 Results of system suitability parameters for Hydrochlorothiazide and Eprosartan

S. No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Hydrochlorothiazide	2.003	920101	116666	12.0	1.6	2711.8
2	Eprosartan	5.067	552058	41531	11.0	1.3	3428.2

Table 2 Results of method precision for Hydrochlorothiazide

S. No	Sample area	Standard area	Percentage purity
1	983375	971536	101.04
2	985049	973007	101.03
3	982956	975717	100.54
4	985219	978909	100.44
5	994145	981422	101.09

Average			**100.84**
%RSD			**0.304**

Table 3 Results of method precision for Eprosartan

S. No	Sample area	Standard area	Percentage purity
1	592403	577531	101.36
2	592352	580381	101.85
3	592357	577723	102.32
4	592323	582190	101.44
5	596525	583378	101.09
Average			**101.24**
%RSD			**0.46**

Table 4 Results of Intermediate precision for Hydrochlorothiazide

S. No	Sample area	Standard area	Percentage purity
1	979556	984395	99.30
2	982467	984039	99.64
3	979717	983976	99.36
4	978909	984278	99.28
5	981432	973915	100.57
Average			**99.63**
%RSD			**0.54**

Table 5 Results of Intermediate precision for Eprosartan

S. No	Sample area	Standard area	Percentage purity
1	583416	593403	99.12
2	583657	594352	99.01
3	584731	593357	99.52
4	583594	592673	99.61
5	597649	593671	99.12
Average			**99.27**
%RSD			**0.27**

Table 6 Results of Accuracy

Sample concentration	Sample set no	Sample area		Assay		% Recovery	
		Hydrochlorothiazide	Eprosartan	Hydrochlorothiazide	Eprosartan	Hydrochlorothiazide	Eprosartan
50%	1	460064	276931	24.9	25.0	99.8	100
	2	460124	276694	24.6	24.9	99.6	99.6
	3	460216	276891	24.8	24.9	99.8	99.6
	Average Recovery					99.7%	99.7%
100%	1	923429	554156	49.9	50.0	99.8	100
	2	923654	554897	49.8	49.9	99.6	99.8
	3	923742	556371	49.8	49.9	99.6	99.8
	Average recovery					99.6%	99.8%
150%	1	1387901	828113	74.8	75.0	99.8	100
	2	1385360	828794	74.9	74.9	99.8	99.8
	3	1386984	828349	74.6	74.8	99.6	99.8
	Average recovery					99.7%	99.8%

Table 7 Area of different concentration of Hydrochlorothiazide and Eprosartan

Concentration (µg/ml)	Peak area of Hydrochlorothiazide	Peak area of Eprosartan
25	296800	179891
50	653819	387781
75	983775	599708
100	1342535	799619
125	1694286	1019614

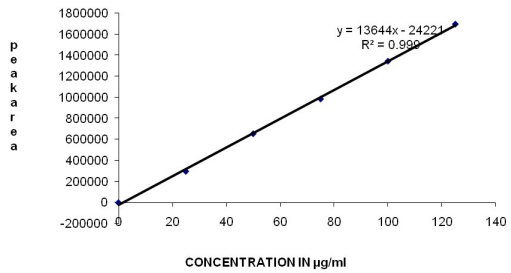

Figure 4 Calibration graph for Hydrochlorothiazide

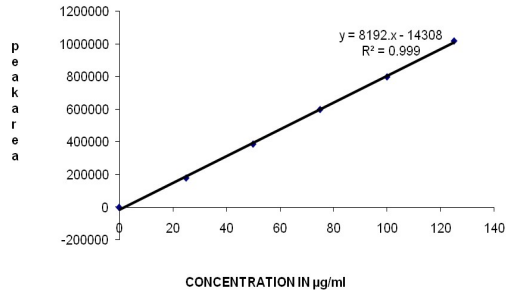

Figure 5 Calibration graph for Eprosartan

Table 8 Analytical performance parameters of Hydrochlorothiazide and Eprosartan

Parameters	Hydrochlorothiazide	Eprosartan
Slope (m)	13644	8192
Intercept (c)	24221	14308
Correlation coefficient (R^2)	0.999	0.999

Table 9: Results of Limit of Detection

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Hydrochlorothiazide	56	176	3.14
Eprosartan	56	154	2.75

Table 10: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Hydrochlorothiazide	56	563	10.05
Eprosartan	56	558	9.96

Table 11: Results for effect of variation in flow

S. No	peak area for Less flow (0.7 ml/min)		peak area for More flow (0.9 ml/min)	
	Hydrochlorothiazide	Eprosartan	Hydrochlorothiazide	Eprosartan
1	983465	575351	971563	592641
2	985134	580381	973021	592352
3	983467	587724	975674	595471
4	985217	583190	978974	594416
5	994245	584468	984542	583453
Mean	**986306**	**582223**	**976755**	**591667**
%RSD	**0.45**	**0.80**	**0.53**	**0.80**

Table 12: Results for effect of variation in mobile phase composition

S. No	peak area for Less organic(70%)		Peak area for More organic (90%)	
	Hydrochlorothiazide	Eprosartan	Hydrochlorothiazide	Eprosartan
1	984565	574371	981565	593761
2	986134	585481	983527	592462
3	984268	587627	985489	594491
4	986216	585362	987954	596316
5	995247	585448	994672	587353
Mean	**987286**	**583658**	**986641**	**592877**
%RSD	**0.45**	**0.90**	**0.51**	**0.57**

4. Conclusion

The validation study shows that the developed method is accurate, rapid, precise, reproducible and inexpensive with acceptable correlation co-efficient, RSD (%) and standard deviations which make it versatile and valuable for simultaneous determination of Hydrochlorothiazide and Eprosartan in pharmaceutical dosage forms.

5. References

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