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Stability Indicating RP-HPLC Method Development and Validation of Anti-Diuretic Drugs in a Combined Dosage Forms

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Abstract: ***Eprosartan is as Angiotensin Conversion enzyme inhibitor, Hydrochlorothiazide is a benzo-thiadiazine acts as diuretic. Very less amount of research was carried out in the combined dosage of these drugs an attempt has been developed using RP-HPLC for combined dosage form and the method is validated as per ICH guidelines. Chromatographic separation of Eprosartan and Hydrochlorothiazide was achieved on Shimadzu HPLC with Phenomenex C18 column of having 5 µ particle size, 50% methanol and 50% of O-phosphoric acid was used as mobile with 0.8ml/min flow rate constats were detected by using UV-visible detector at a 232 nm Hydrochlorothiazide and Eprosartan are 2.5 and 4.3 minutes respectively were detected from the retention time. Further stress conditions like Photostability, acid stability, alkaline and oxidation conditions were detected for identification of degradation. Optimized method was a good approach for obtaining reliable results and found to be suitable for the routine analysis of Hydrochlorothiazide and Eprosartan in combined dosage form.***

Keywords: Eprosartan, Hydrochlorothiazide, degradation studies, RPHPLC

1. Introduction

Qualitativeanalysisconcernswiththeidentificationofelements, functionalgroupsorcompounds in a sample, whereas the quantitative analysis helps in the determination of amount of a particular element, species or compound in the given sample[4], which enable us to maintain permissible limits of impurities in the sample. A very few reports were available in the literature and no validated method was reported earlier for the drugs Eprosartan and Hydrochlorothiazide in their combined dosage form. Made an attempt to develop a new stability indicating method for the simultaneous determination of hydrochlorothiazide and eprosartan in combined dosage form. Eprosartan (fig:1) is considered as Angiotensin Conversion enzyme inhibitor there by it acts as anti-hypertensive drug it acts by dilating the smooth muscle and controls the blood pressure, Hydro chlorothiazide(fig:2) is a benzo-thiadiazine derivative and is used as diuretic

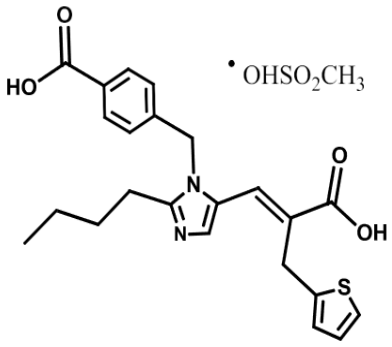

Figure 1: Structure of Eprosartan

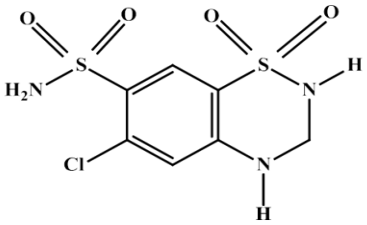

Figure 2: Structure of Hydrochlorthiazide

Both these drugs combination available in the tablet form with the trade name TEVETEN by Abbott Company at 12.5 mg Hydrochlorothiazide and 600mg of Eprosartan helps for reducing oedema and hypertension conditions at simultaneously. Brand name of hydrochlorothiazide and eprosartan combined dosage formulation and its composition which is available in the market.

2. Materials and Methods

All the chemicals and pure active pharmaceutical ingredients which are used in the study were of Analytical grade. Shimadzu VP Binary pump LC-10 10ADvp autosampler was used. PhenomenoxC18 column was used

Instrument and Chromatographic Condition:
The Chromatographic equipment consists of a Shimadzu Class VP binary pump LC- 10ATvp, SIL-10ADvp auto sampler, CTO-10Avp column temperature oven, SPD-10Avp UV-visible detector. All the constituents of the system were controlled by using SCL- 10Avp system controller. Data acquisition was done by using the software LC solutions.

Preparation of diluents solution: 50% of methanol and 50% of milli-Q water were mixed well to obtain 50:50 %

(v/v) of methanol and water, and is degassed by using sonication process.

Blank preparation: Diluents used as blank for the experiment.

Preparation of standard solutions: Stock solutions of Eprosartan and Hydrochlorothiazide (5 mg/mL) were prepared separately in a volumetric flask and labeled accordingly. The calibration curve containing 6 non-zero standards for each drug were prepared by using diluents solution in the concentration range of 5.01-50.16µg/m Land5.03 - 50.31 for Eprosartan and Hydrochlorothiazide respectively.

Preparation of Mobile Phase: 50 parts of methanol is mixed with 50 parts of 0.1% orthophosphoric acid to obtain 50:50% (v/v) of Methanol and 0.1% orthophosphoric acid.

Assay process: 20 tablets were taken, weighed and made into a fine powder of uniform size by using mortar and pestle. From this the average weight of the tablet was calculated. Now from the accurately weighed portion 600 mg of Eprosartan and 12.5 mg of Hydrochlorothiazide were taken and transferred to 100 mL a volumetric flask which contains 20 mL of methanol. For complete solubility of the drugs, the contents of the flasks were sonicated for about 20 min and made up the mark with mobile phase. Then the mixture is filtered by passing through 0.45 µ membrane filter. From this solution, 2 mL aliquot was taken into a separate 10 mL volumetric flask and made up to the mark with mobile phase and mixed well. The above solution (20 µL) was then injected eight times into the column. The mean peak areas of the drugs were calculated and the drug content in the formulation was calculated by the regression equation of the method. The % assay results are shown in the Table 8.

Method development:
In order to achieve satisfactory separation-resolution of Hydrochlorothiazide and eprosartan in various mobile phases with varying buffer ratios and organic phase utilizing phenomenex 250 X 4.6 mm id 5 µm, several studies were carried out. A mixture of buffer (20m M potassium dihydrogen orthophosphate, pH 4.75) and methanol in the proportion 50:50 v/v orthophosporic acid was discovered to be the optimal mobile phase. The resolution of HCTZ and EPRO produced by this mobile phase during isocratic elution was excellent and extremely satisfactory. A pH adjustment of 0.3 in the mobile phase did not significantly alter the duration of each analyte's retention. At a flow rate of 0.8 ml min-1, the analytical column's HCTZ and EPRO retention times were assessed. The injection had a 20µl volume. For HCTZ and EPRO, the standard and sample retention times were adequate and had high resolution.

Method validation: The International Conference on Harmonization (ICH) criteria [17–19] were used to validate the method. Specificity, linearity, and other characteristics for method validation were examined. Accuracy, precision, detection and quantification limits, and robustness**.**

Linearity determination using calibration curve: Calibration curve was drawn by measuring the concentration range of 5.01- 50.15 µg/ml obtained data was shown in fig 4 and 5 for hydrochlorothiazide and eprosartan respectively

Precision determination: Inter day precision and intraday precision was determined by results were given in Table:2 and Table:3

Limit of Detection and Limit of Quantification (LOD and LOQ) : It is detected by using linearity curve method by using slope and intercept of the calibration curve. Obtained results were given in Table 4 and Table: 5

Ruggedness: The prepared MQC mix was injected and performed by different analysts

Robustness: Robustness was detected by checking the varying the mobile phase.

Stability: By leaving the sample on the bench top for at least 12 hours at room temperature, the stability of stock dilution is demonstrated. Then, a reference sample is created using a fresh sample with a similar concentration. After 12 hours, both samples are examined using the established HPLC method.

System suitability: Six replicate injections of the tested working standard solutions were used to conduct a system suitability investigation. For the obtained peak areas, the% RSD was determined. The system suitability data in Table 6(% RSD 2) confirms the instrument's reproducible performance.

Stress degradation studies: The stress degradation studies were performed by treating samples under acid, light (UV), oxidation, acid and alkaline conditions. The stress studies involving acid, light (UV) and oxidation revealed that Hydrochlorothiazide and Eprosartan were not completely degraded. In alkaline conditions (0.1N NaOH), the drugs were unstable and the degradation peak was eluted earlier followed with a drastic peak distortion and increased tailing. Except in alkaline conditions, the drugs were indicating stability for all stress conditions within 95 - 105% and specificity of the analytical method to differentiate the degradation peaks the results were given in Table:7

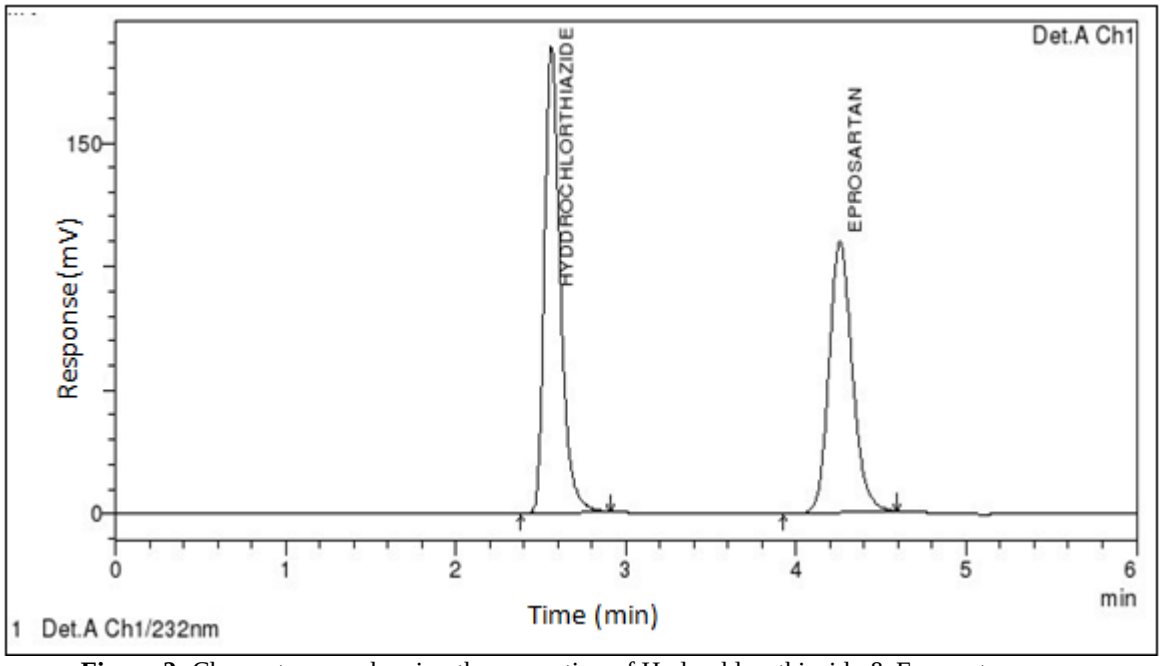

Figure 2: Chromatogram showing the separation of Hydrochlorothiazide & Eprosartan

Table 1: Optimized Chromatographic Conditions for Hydrochlorothiazide &Eprosartan

S. No	Parameter	Description
1	Column	phenomenex 250 X 4.6 mm id 5 µm
2	Mobile Phase	50:50 % (v/v) Methanol : 0.1% Orthophosphoric acid
3	Flow rate	0.8 mL / min
4	Run time	6 minutes
5	Column Temperature	25 ± 1 ^{o}C
6	Injection Volume	20 µL
7	Detection wavelength	232 nm
8	Retention times • Eprosartan • Hydrochlorothiazide	 4.3 minutes 2.5 Minutes

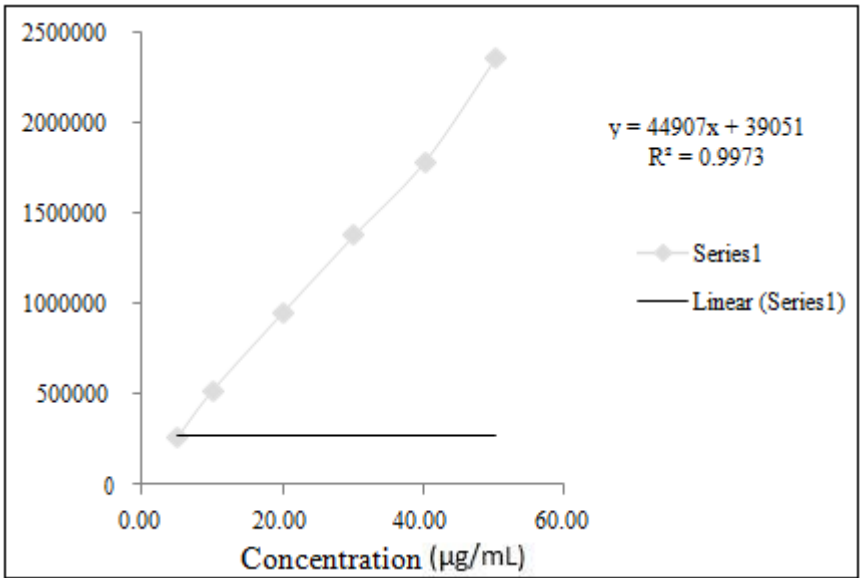

Figure –3. Linearity curve of Hydrochlorothiazide

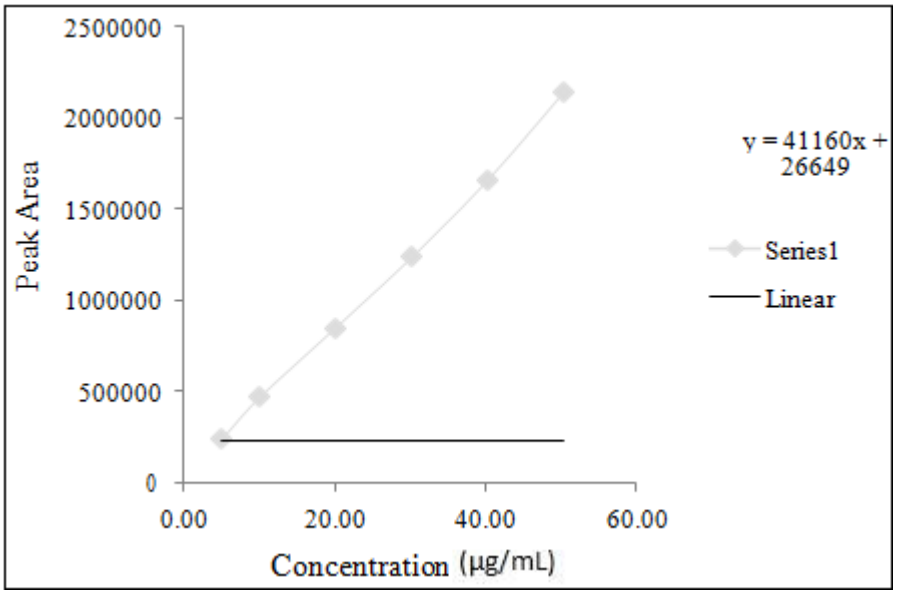

Figure 4: Linearity curve of Eprosartan

Table 2: Results of Inter and Intra-dayAccuracy & Precision (% CV) for Hydrochlorothiazide

Sample ID	LQC	MQC	HQC
Nominal Concentration (µg/mL)	12.72	25.44	37.50
DAY 1			
Mean Concentration (µg/mL)	12.62	25.12	37.37
SD	0.22	0.23	0.19
% CV	1.72	0.92	0.51
DAY 2			
Mean Concentration (µg/mL)	12.58	25.32	37.25
SD	0.21	0.25	0.49
% CV	1.67	0.99	1.32
DAY 3			
Mean Concentration (µg/mL)	13.12	24.89	37.56
SD	0.25	0.35	0.56
% CV	1.91	1.41	1.49

Table 3: Results of Inter and Intra-day Accuracy & Precision (% CV) for Eprosartan

Sample ID	LQC	MQC	HQC
Nominal Concentration (µg/mL)	12.57	25.16	37.73
DAY 1			
Mean Concentration (µg/mL)	12.83	25.02	38.12
SD	0.15	0.23	0.2
% CV	1.16	0.94	0.53
DAY 2			
Mean Concentration (µg/mL)	12.71	25.18	37.25
SD	0.10	0.18	0.26
% CV	0.8	0.71	0.7
DAY 3			
Mean Concentration (µg/mL)	12.55	25.34	38.81
SD	0.18	0.26	0.31
% CV	1.43	1.03	0.8

Table 4: LOD and LOQ results of Hydrochlorothiazide

LOD

	Hydrochlorothiazide	
Sr. No.	**Retention Time**	**Peak Area**
1	2.58	6543
2	2.58	6425
3	2.58	6632
Mean	**2.58**	**6533.3**
Std. Dev.	**0.00**	**103.83**
% CV	**0.00**	**1.58**

LOQ

	Hydrochlorothiazide	
Sr. No.	**Retention Time**	**Peak Area**
1	2.58	10386
2	2.57	10081
3	2.58	10224
Mean	**2.57**	**10230.3**
Std. Dev.	**0.0057**	**152.59**
% CV	**0.22**	**1.49**

Table 5: LOD and LOQ results of Eprosartan

LOD

	Eprosartan	
Sr. No.	Retention Time	Peak Area
1	4.41	6653
2	4.41	6627
3	4.39	6515
Mean	**4.4**	**6598.3**
Std. Dev.	**0.011**	**73.33**
% CV	**0.26**	**1.11**

LOQ

	Eprosartan	
Sr. No.	Retention Time	Peak Area
1	4.39	10471
2	4.39	10236
3	4.37	10119
Mean	**4.38**	**10275.33**
Std. Dev.	**0.011**	**179.26**
% CV	**0.25**	**1.74**

Table 6: System suitability results of Hydrochlorothiazide and Eprosartan

	Hydrochlorothiazide		Eprosartan	
Sample ID.	Peak Retention Time	Peak Area	Peak Retention Time	Peak Area
1	2.53	1187607	4.18	1023391
2	2.56	1223586	4.25	1044164
3	2.57	1226642	4.35	1002354
4	2.57	1232197	4.35	1006607
5	2.57	1197494	4.36	977339
6	2.57	1234386	4.36	1004001
Mean	**2.562**	**1216985.3**	**4.308**	**1009642.7**
Std. Dev.	**0.0160**	**19565.26**	**0.0757**	**22442.97**
% CV	**0.63**	**1.61**	**0.82**	**1.33**

Table 7: Stress degradation studies

Samples	Stress degradation	Oxidative stress	Acid stress	Alkaline stress	Photolytic stress
Hydrochlorothiazide	101.73%	99.62%	98.86	--	101.70
Eprosartan	98.67	101.10	99.06	--	100.12

Table 8: Assay results for Hydrochlorothiazide and Eprosartan

Brand Name	% Assay
TEVETEN	% Assay for Hydrochlorothiazide = 99.32
	% Assay for Eprosartan = 100.75

3. Discussion

By using run time of 6 minutes with the mobile phase of methanoladn 0.1 % orthophosphoric acid at a flow rate of 0.8ml/min, using Phenomenex C18 column with 5 Micro meter particle size at a detection of wavelength of 232nm 99.32% and 100.75% was found to be highly sensitive, accurate and specific method found to be more validated for simultaneous estimation of hydrochlorthiazide and eprosartan. The results obtained from the analysis are good enough by changing the flow rate and mobile phase composition separately and analysis was performed by different analysts. Hence the method is said to be robust and rugged. The drugs are tested for the stress conditions like Photostability, acid stability, alkaline and oxidation conditions for 24 hours. The drugs are stable and did not show any signs of degradation under stress conditions except in alkaline medium. Hence the proposed method is said to be stable and it is successful in the identification of the medium that in which selected drug is going to degrade. This method also succeeded in the clear separation of degradation peak from the respective drug peak.

4. Conclusion

Results of the combined dose form by established approach showed good agreement. The proposed method was thus determined to be suitable for the regular analysis of hydrochlorothiazide and eprosartan in combined dosage form and to be a good approach for achieving trustworthy results.

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