ISSN: 0974-2115
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

Development and validation of a new stability indicating RP-HPLC method for the determination of Eprosartan and Hydrochlorothiazide

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ABSTRACT

A new stability indicating liquid chromatographic method has been developed for the determination of Eprosartan and Hydrochlorothiazide in pharmaceutical formulations (gradient mode; UV detection at 235 nm). Linearity was observed over the concentration range 1-300 µg/ml for Hydrochlorothiazide 19.2-750.3 µg/ml for Eprosartan respectively with regression equations y = 5980.5x+535.64 (R^2 = 0.9999) and y = 8199.2x + 565.86 (R^2 = 0.9998) respectively. The combined tablet formulation of Eprosartan and Hydrochlorothiazide was subjected to acidic, alkaline, oxidation, thermal, photolytic and humidity degradations and the method was validated as per ICH guidelines. The method was reported to be simple, specific, precise, accurate, robust and useful for the routine analysis of Eprosartan and Hydrochlorothiazide in pharmaceutical dosage forms.

KEY WORDS: Eprosartan, Hydrochlorothiazide, RP-HPLC, stability-indicating and validation.

1. INTRODUCTION

Eprosartan (Budavari, 2006) (CAS ID No. 133040-01-4) is an angiotensin II receptor antagonist used for the treatment of high blood pressure. Eprosartan (EPR) acts on the renin-angiotensin system in two ways to decrease total peripheral resistance. It blocks the binding of angiotensin II to AT1 receptors in vascular smooth muscle, causing vascular dilatation and inhibits sympathetic norepinephrine production, further reducing blood pressure. Hydrochlorothiazide (Bohm and Sachse, 2002) (CAS ID No. 58-93-5) is a first line diuretic drug of the thiazide class. Hydrochlorothiazide (HCTZ) acts by lowering blood pressure initially by increasing sodium and water excretion. This causes a decrease in extracellular volume, resulting in a decrease in cardiac output and renal blood flow. With long-term treatment, plasma volume approaches a normal value, but peripheral resistance decreases. The combination of EPR and hydrochlorothiazide can be effectively and safely used in patients (Kamila, 2008). From the literature survey it was found that Eprosartan was determined by ultraviolet spectrophotometry (Patel, 2010) and high-performance liquid chromatography (Ouyang, 1986) in pharmaceutical preparations and several analytical methods have been published for the determination of HCTZ also using flow injection (Bigley, 1986), spectrophotometric (Saglik, 2001; Ulvi, 1994; El Gindy, 2001), densitometric (Hertzog, 2002), HPLC (Erk, 2001; Luz et al., 2002), electrophoretic (Hillaert, 2001,) and polarographic (Martın, 1999) methods in tablets. The simultaneous determination of EPR and HCTZ was studied by HPTLC (Patel, 2009), HPLC and derivative spectrophotometric (Fatma, 2012) methods. In the present study a new stability indicating RP-HPLC method was proposed for the simultaneous determination of Hydrochlorothiazide and Eprosartan and validated (ICH guidelines 2005).

2. MATERIALS AND METHODS

Chemicals and reagents: Hydrochlorothiazide (Purity 99.8) and Eprosartan (Purity 99.4) were obtained from Ranbaxy Laboratories and Solvay (India). The combination of Hydrochlorothiazide and Eprosartan is available in as tablets with brand names TEVENTEN HCT, TEVETEN PLUS with label claim Hydrochlorothiazide: 12.5 mg and Eprosartan: 600 mg. Acetonitrile, formic acid, sodium hydroxide (AR), hydrochloric acid (AR) and hydrogen peroxide (AR) were procured from Merck (India) and all chemicals are of HPLC grade.

Instrumentation: Waters Model 2997 HPLC system with PDA detector and X Bridge Shield RP18 (150 x 3.0 mm, 3.5µm) column (Injection volume 5µL) was used for the chromatographic study. Gradient mode of elution was performed with column oven temperature 45°C.

Preparation of stock solution: Hydrochlorothiazide (2500 µg/ml) and Eprosartan (2400 µg/ml) stock solutions were prepared by accurately weighing 125 mg of HCTZ and 120 mg of EPR in a 50 mL volumetric flask with diluent. Standard solutions were prepared by further diluting 5mL of the stock solution to 50mL with diluent. Working standard solutions were prepared on daily basis from the stock solutions by dilution with mobile phase and the solutions were filtered through 0.45 µm membrane filter prior to injection.

Validation:

Linearity: A series of solutions were prepared from by diluting the stock solutions of Hydrochlorothiazide (1.0-300.0 µg/ml) and Eprosartan (19.2-750.3 µg/ml) with diluent. 5µL of these solutions were injected in to the system and the corresponding chromatograms were obtained. The peak area of Hydrochlorothiazide and Eprosartan were taken from the chromatograms and a calibration curve was drawn by taking the concentration of the drug solution

on the x-axis and the corresponding peak area value on the y-axis. The limit of quantification and limit of detection measured as described in ICH guidelines Q2 (R1) (ICH guidelines, 2005).

Precision: The intra-day precision of the assay method was evaluated by carrying out 6 independent assays of test samples of Eprosartan and Hydrochlorothiazide (Eprosartan 240 µg/ml and Hydrochlorothiazide 250 µg/ml) against a qualified reference standard and the % RSD was calculated. The inter-day precision study was performed on different days (n=6) on different system by different analyst (Eprosartan 240 µg/ml and Hydrochlorothiazide 250 µg/ml) and the % RSD was calculated.

Accuracy: The accuracy of the assay method was evaluated in triplicate by spiking individual standard solutions at three concentration levels (80, 100 and 120%), and the percentage recoveries were calculated. Standard addition and recovery experiments were conducted to determine the accuracy of the method for the quantification of Hydrochlorothiazide & Eprosartan respectively in the drug product and the % RSD was calculated.

Robustness: The robustness of the assay method was established by introducing small deliberate changes in the HPLC conditions which included flow rate (0.72 and 0.88 mL/min), percentage of acetonitrile in the mobile phase (absolute ±2% composition) and column oven temperature (±5°C) . Robustness of the method was studied using five replicates of Eprosartan (12000 µg/ml) and Hydrochlorothiazide (250 µg/ml)

Forced degradation studies: Forced degradation studies were intended to ensure the effective separation of Eprosartan and Hydrochlorothiazide and their degradation peaks of formulation ingredients at the retention time of Eprosartan and Hydrochlorothiazide respectively. Forced degradation studies were performed with 12000 µg/ml of Eprosartan and 250 µg/ml of Hydrochlorothiazide.

Acidic degradation: Initially degradation started with 0.1N HCl and acid concentration slowly increased to 1N HCl. The combined formulation of Eprosartan and of Hydrochlorothiazide was treated with 1N HCl and refluxed for 2 hours in thermostat maintained at 80°C.

Alkaline degradation: Initially alkaline degradation was studied with 0.1N NaOH and continued with 1N NaOH. The combined formulation of Eprosartan and Hydrochlorothiazide was treated with 1N NaOH and refluxed for 2 hours in thermostat maintained at 80°C.

Oxidative degradation: The combined formulation of Eprosartan and of Hydrochlorothiazide was treated with 1 % H_2O_2 and refluxed for 2 hours in thermostat maintained at 80°C.

Thermal degradation: The combined formulation of Eprosartan and of Hydrochlorothiazide was kept for thermal degradation at 105°C for 72 Hours in oven.

Photolytic degradation: The combined formulation of Eprosartan and of Hydrochlorothiazide was kept in photolytic chamber for photolytic degradation at 1289069 Lux Hours and 1024.2.66 Watt-Hour/m^2.

Humidity degradation: The combined formulation of Eprosartan and of Hydrochlorothiazide was kept in desiccator at 25°C, 95%RH for 120 hours.

Analysis of commercial formulations: Twenty tablets of two different brands containing Eprosartan and Hydrochlorothiazide were procured from the local pharmacy store and analyzed as per the method. The percentage recovery was calculated (from the linear regression equation using the mean peak area obtained from the respective chromatograms.

3. RESULTS AND DISCUSSION

The authors have developed a validated stability indicating liquid chromatographic method (gradient mode) for the determination of Eprosartan and of Hydrochlorothiazide. Mobile phase containing a mixture of 0.1 % formic acid and acetonitrile with flow rate 0.8 mL/min has been used in the present study. A flow rate of 0.8 ml/min and UV detection at 235 nm with column oven temperature 45°C were maintained. A mixture of water: acetonitrile (50:50 v/v) was used as diluent. Two sharp peaks were observed at 7.210 min and 2.998 mins for Eprosartan and Hydrochlorothiazide respectively. The representative chromatogram of blank as well as Hydrochlorothiazide and Eprosartan were shown in Figure 2 along with the corresponding peak purity plots were shown in Figure.3. Hydrochlorothiazide and Eprosartan obey Beer-Lambert's law over the concentration range 1.0-300.3 µg/ml and 19.2-750.3 µg/ml respectively with regression equations y = 5980.5x+535.64 (R^2 = 0.9999) (HCTZ) and y = 8199.2x + 565.86 (R^2 = 0.9998) (EPR) respectively. The LOQ and LOD were determined as described in International Conference on Harmonization guidelines Q2 (R1). The LOQ and LOD for Eprosartan were found to be 2.305 µg/ml and 0.761 µg/ml respectively whereas the LOQ and LOD for Hydrochlorothiazide were found to be 0.921 µg/ml and 0.304 µg/ml respectively. The method is more precise as the percentage relative standardization (% RSD) was found to be 0.16-0.34 and 0.15-0.34 for intra-day and inter-day precision studies respectively for HCTZ and the % RSD was found to be 0.14-0.36 and 0.18-0.33 for intra-day and inter-day precision studies respectively for EPR (RSD < 2). The % RSD in accuracy studies was found to be 0.27-0.36 (RSD < 2) with percentage recovery 99.33-99.84 for HCTZ and 0.25-1.43 (RSD < 2) with percentage recovery 99.75-100.19 for EPR (Table.1). The method is more robust as the % RSD was found to be 0.15-0.54 and 0.13-0.78 for HCTZ and EPR respectively (Table.2). The

proposed method was applied for the determination of Hydrochlorothiazide and Eprosartan tablets and the percentage recovery was found to be 99.40-99.44 and 99.23-99.44 respectively (Table.3).

Table.1. Accuracy study of Eprosartan and Hydrochlorothiazide

Component	Level	Mean	% RSD
Eprosartan	80%	100.12	1.43
	100%	100.19	1.00
	120%	99.75	0.25
Hydrochlorothiazide	80%	99.84	0.27
	100%	99.33	0.36
	120%	99.78	0.32

Table.2. Robustness Study of Eprosartan and Hydrochlorothiazide

Conditions	Parameter	System suitability					
		Hydrochlorothiazide			Eprosartan		
		Tailing	Theoretical plates	% RSD	Tailing	Theoretical plates	% RSD
Flow rate (± 0.08, mL/min)	0.72 mL/min	1.12	2986	0.19	1.11	89456	0.13
	0.88 mL/min	1.08	3085	0.54	1.04	90456	0.52
ACN: formic acid (± 2%, v/v)	58:42	1.13	2688	0.19	1.10	96415	0.16
	62:38	1.09	2860	0.54	1.09	98954	0.36
Column oven temperature (± 5°C)	40°C	1.10	2895	0.15	1.11	78056	0.44
	50°C	1.10	3046	0.36	1.06	90146	0.78

* Mean of three replicates

Table.3. Analysis of commercial formulation (Tablets)

Brand name	Labeled amount (mg)		*Amount found (mg)		Recovery (%)	
	EPR	HCTZ	EPR	HCTZ	EPR	HCTZ
Brand I	600	12.5	595.76	12.43	99.23	99.44
Brand II	600	25	596.64	24.85	99.44	99.40

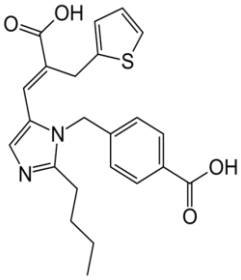
* Mean of three replicates

The system suitability tests were performed to ensure that the complete testing system was suitable for the intended application. The tailing factor was 1.11 (HCTZ) and 1.04 (EPR) which is <1.5–2 or <2 and the theoretical plates were found to be 3082 for HCTZ and 97156 for EPR which is >2000. The specificity of the method can be defined from the forced degradation studies. The typical chromatograms of the stressed samples were shown in Figure 5a-Figure 10a and their peak purity plots were shown in Figure 5b-Figure 10b. Hydrochlorothiazide and Eprosartan has shown a very slight decomposition i.e. less than 4% during acidic, alkaline, oxidative, thermal, photolytic and humidity degradation studies indicating that the two drugs are very much resistant towards all degradations (Table.4). The purity angle is less than the purity threshold in all the studies. As degradant peaks were not observed during the forced degradation studies LC-MS studies were not performed. The present stability-indicating liquid chromatographic method is specific because the drug peaks were well separated and the method can be used in industries for the determination of Hydrochlorothiazide and Eprosartan in pharmaceutical formulations.

Table. 4. Forced degradation studies of Hydrochlorothiazide and Eprosartan

Stress conditions	Drug Recovered (%)		Drug decomposed (%)		Purity angle		Purity threshold	
	HCTZ	EPR	HCTZ	EPR	HCTZ	EPR	HCTZ	EPR
Untreated	100	100	-	-	0.532	0.790	1.851	1.029
Acidic degradation	100.13	98.96	-	1.04	0.349	0.083	1.051	1.030
Alkaline degradation	99.34	98.83	0.66	1.17	0.678	0.079	2.125	1.030
Oxidative degradation	96.48	96.40	3.52	3.60	0.762	0.080	2.023	1.030
Thermal degradation	98.78	97.35	1.22	2.65	0.045	0.107	1.060	1.079
Photolytic degradation	100.48	97.99	-	2.01	0.048	0.110	1.061	1.082
Humidity degradation	99.90	98.73	0.10	1.27	0.048	0.107	1.063	1.07

* Mean of three replicates



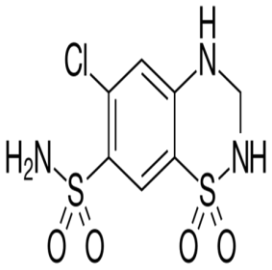
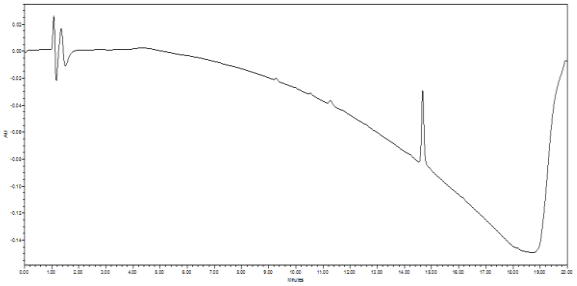


Figure.1. Chemical structure of (A) Eprosartan and (B) Hydrochlorthiazide



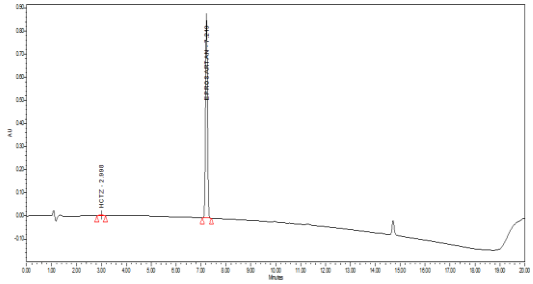
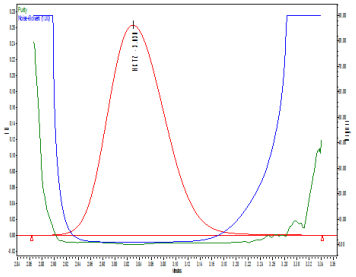


Figure.2a. Representative chromatogram of blank b) Representative chromatogram of Hydrochlorothiazide (5 µg/ml) and Eprosartan (240 µg/ml)



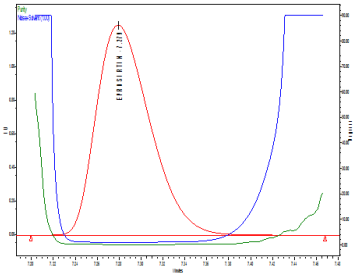
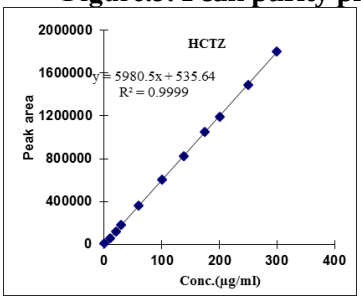


Figure.3. Peak purity plots of Hydrochlorothiazide and Eprosartan



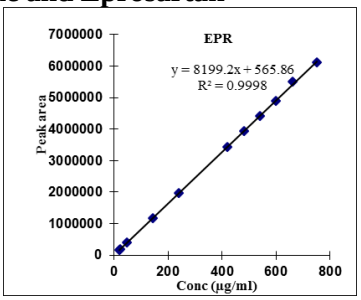


Figure. 4. Calibration curves of and Eprosartan and Hydrochlorothiazide

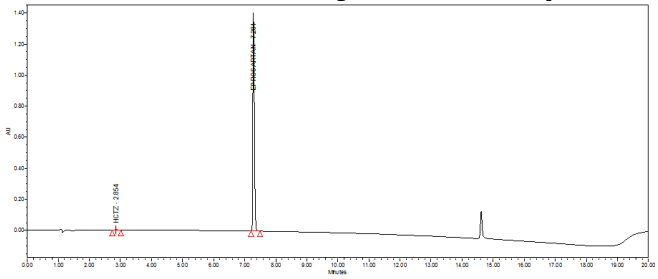


Figure.5a. Representative chromatogram of Hydrochlorothiazide (5 µg/ml) and Eprosartan (240 µg/ml) (Acidic degradation)

Figure. 5b. Peak purity plots of Hydrochlorothiazide and Eprosartan (Acidic degradation)

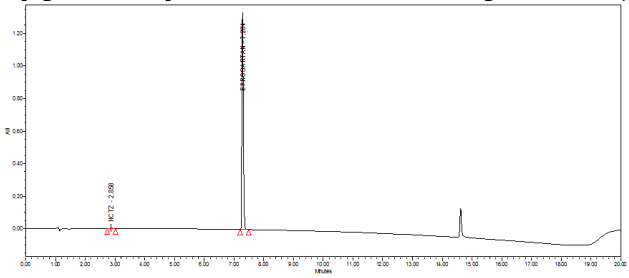


Figure.6a. Typical chromatogram of Hydrochlorothiazide (5 µg/ml) and Eprosartan (240 µg/ml) (Alkaline degradation)

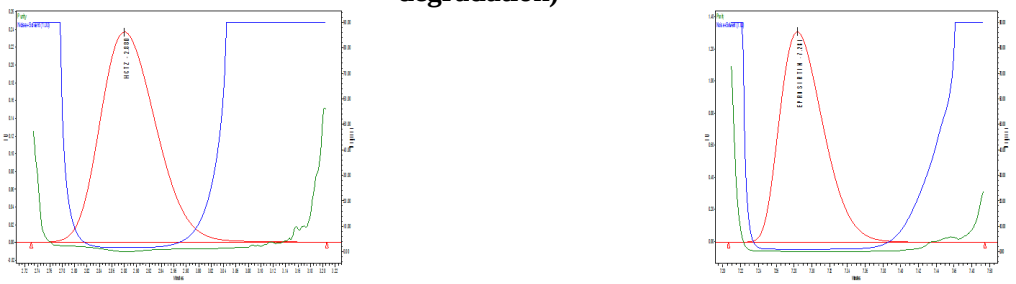


Figure.6b. Peak purity plots of Hydrochlorothiazide and Eprosartan (Alkaline degradation)

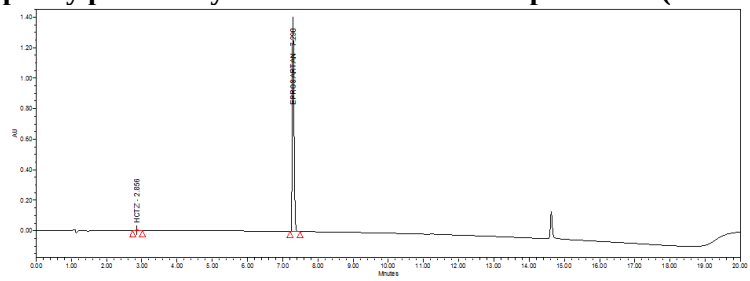


Figure.7a. Representative chromatogram of Hydrochlorothiazide (5 µg/ml) and Eprosartan (240 µg/ml) (Oxidative degradation)

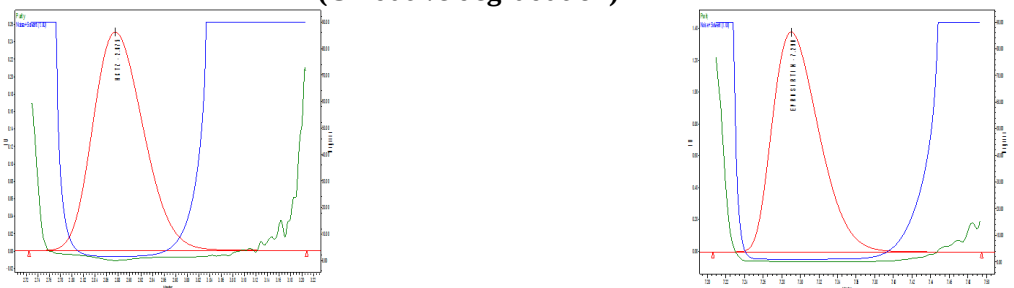


Figure.7b. Peak purity plots of Hydrochlorothiazide and Eprosartan (Oxidative degradation)

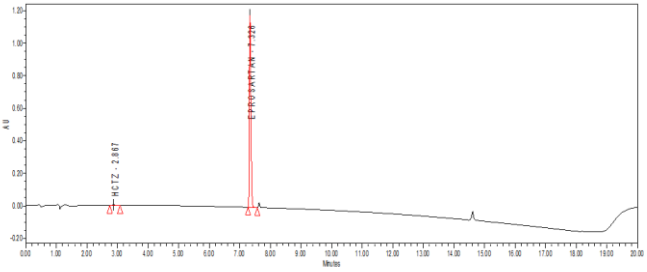


Figure.8a. Representative chromatogram of Hydrochlorothiazide (5 µg/ml) and Eprosartan (240 µg/ml) (Thermal degradation)

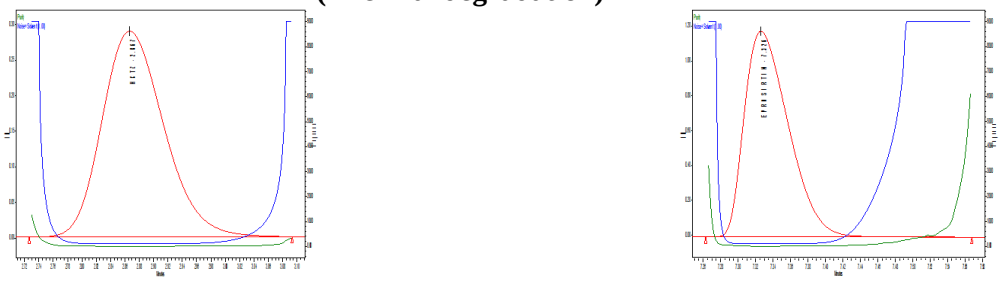


Figure.8b. Peak purity plots of Hydrochlorothiazide and Eprosartan (Thermal degradation)

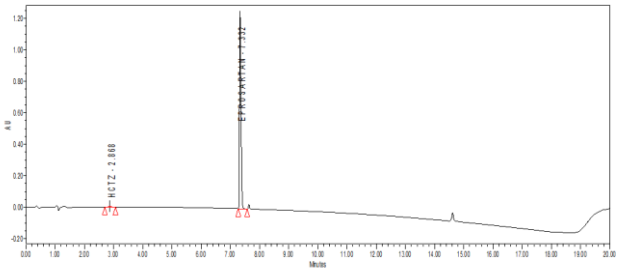


Figure.9a. Representative chromatogram of Hydrochlorothiazide (5 µg/ml) and Eprosartan (240 µg/ml) (Photolytic degradation)

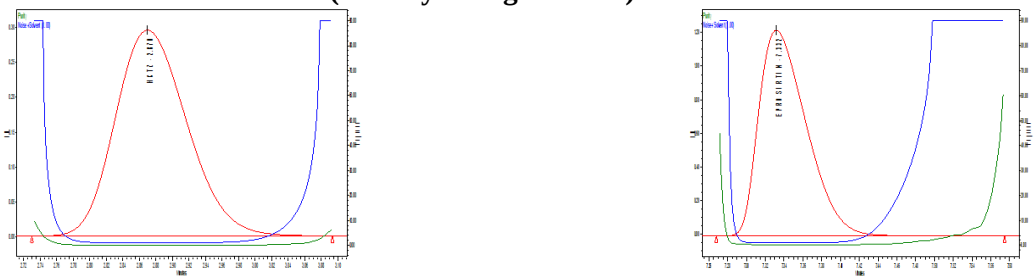


Figure.9b. Peak purity plots of Hydrochlorothiazide and Eprosartan (Photolytic degradation)

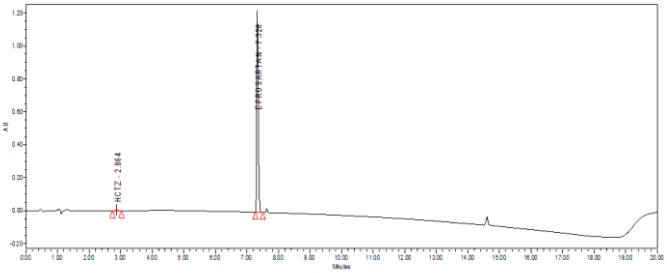


Figure.10a. Representative chromatogram of Hydrochlorothiazide (5 µg/ml) and Eprosartan (240 µg/ml) (Humidity degradation)

Figure.10b. Peak purity plots of Hydrochlorothiazide and Eprosartan (Humidity degradation)

4. CONCLUSION

The proposed stability-indicating liquid chromatographic method was validated and it is simple and specific. The combination of Hydrochlorothiazide and Eprosartan is highly resistant towards all forced degradation studies.

5. ACKNOWLEDGEMENT

The authors are grateful to to M/s GITAM University, Visakhapatnam for providing the research facilities. There is no conflict of interest.

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