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RESEARCH ARTICLE

Development and Validation of a New Stability-Indicating Liquid Chromatographic Method for the Simultaneous Determination of Eprosartan and Hydrchlorothiazide in Tablets

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Abstract: A validated stability-indicating high-performance liquid chromatographic method has been developed for the simultaneous determination of Eprosartan and hydrochlorothiazide in tablet dosage forms. Chromatographic separation was performed on HPLC system of waters Model 2997 using X Bridge Shield RP18 (150 x 3.0 mm i.d., 3.5 µm particle size) column with a mixture of 0.1% formic acid and acetonitrile as mobile phase with a flow rate of 0.8 mL/min (gradient mode) with UV detection at 235 nm. The combinationof drugs was subjected to stress conditions such as acidic, alkaline, oxidation photolytic and thermal degradations and the method was validated as per ICH guidelines.

Keywords: Eprosartan, Hydrochlorothiazide, RP-HPLC, Stability-indicating, ICH

Introduction

Eprosartan[1] (EPR) is an angiotensin II receptor antagonist used for the treatment of high blood pressure (Figure 1). It acts on the renin-angiotensin system in two ways to decrease total peripheral resistance. First, it blocks the binding of angiotensin II to AT1 receptors in vascular smooth muscle, causing vascular dilatation. Second, it inhibits sympathetic norepinephrine production, further reducing blood pressure.

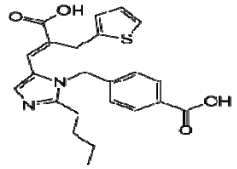

Figure 1. Chemical structure of Eprosartan (EPR)

Hydrochlorothiazide[2] (HCTZ) is a first line diuretic drug of the thiazide class (Figure 2). It 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 inpatients[3].

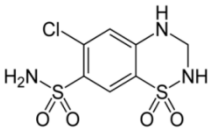

Figure 2. Chemical structures of hydrchlorothiazide (HCTZ)

Literature survey reveals that Eprosartan was determined by ultraviolet spectrophotometry[4] and high-performance liquid chromatography[5] in pharmaceutical preparations. Several analytical methods have been published for the determination of Hydrchlorothiazide in tablet susingflowinjection[6], spectrophotometric[7-9], densitometric[10], HPLC[7-13], electrophoretic[14-15] andpolarographic[16] methods. The simultaneous determination of EPR and HCTZ was studied by HPTLC[17], HPLC and derivative spectrophotmetry[18]. In the present study the authors have developed a simple, robust, precise and accurate RP-HPLC method has been developed for the simultaneous determination of hydrochlorothiazide and Eprosartan and validated as per ICH guidelines[19-20].

Instrumentation and chromatographic conditions

Liquid chromatographic separation was achieved by using a waters X Bridge shield RP-18 (150 mm × 3.0 mm, 3.5 µm) column and waters HPLC Model 2997 with Empower2 software and photodiode array detector, maintained at 45 ºC. Gradient mode elution was performed using Acetonitrile and 0.1% v/v formic acid. The overall run time was 20 min. and the flow rate of the mobile phase was 0.8 mL/min. The wavelength of the PDA detector was set at 235 nm. 5 µL of sample was injected into the HPLC system.

Experimental

Eprosartan standard (purity 99.4%) and was obtainedfrom Solvay, India) and hydrchlorothiazide standard (purity 99.8%) and was obtainedfrom Ranbaxy, India). Acetonitrile (HPLC grade), sodium hydroxide (Merck) and hydrochloric acid (Merck) and Hydrogen peroxide (Merck) and all other chemicals were of analytical grade.

The combination of Eprosartan and hydrochlorothiazide is available with brand names TEVENTEN HCT (Lupin) and TEVETEN PLUS (Torrent) (Label claim: 600 mg (EPR) and 12.5 mg (HCTZ).

Preparation of 0.1% formic acid solution

1.0 mL of Formic acid was added to a 1000 mL volumetric flask and made up to volume with HPLC grade water.

Preparation of stock solutions

Hydrochlorothiazide (2500 µg/mL) and Eprosartan (2400 µg/mL) stock solutions were prepared by accurately transferring 125 mg of HCTZ and 120 mg of EPR in a 50 mL volumetric flask with diluent. Standard solutions were prepared by further diluting 5 mL of the stock solution to 50 mL 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.

Method Validation

Linearity

A series of solutions were prepared from the stock solutions of Hydrochlorothiazide (1.0-300.0 µg/mL) and Eprosartan (19.2-750.3 µg/mL) using the diluents and 5 µL of each solution was injected in to the HPLC system and the peak area of the chromatogram was noted. A graph was drawn by taking the concentration of the drug solutions on the x-axis and the corresponding peak area on the y-axis.

Precision

The intra-day and inter-day precision of the assay method was evaluated by carrying out six independent assays of test samples of Eprosartan (540 µg/mL) and hydrochlorothiazide (250 µg/mL) against a qualified reference standard 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 and Eprosartan respectively 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).

Limit of quantification (LOQ) and limit of detection (LOD)

The LOQ and LOD were determined as described in International Conference on Harmonization guidelines Q2 (R1).

Analysis of commercial formulations

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

Forced degradation studies

Forced degradation studies[15] were intended to ensure the effective separation of Eprosartan and hydrochlorothiazide and their degradation peaks. Forced degradation studies were performed with the combined formulation containing 12000 µg/mL of Eprosartan and 250 µg/mL of Hydrochlorothiazide and diluted as per the requirement before injecting in to the system.

Acidic and alkaline degradation studies

Acid decomposition was carried out by refluxing the combined formulation of Eprosartan and of hydrochlorothiazide solution with 1 N HCl in a thermostat maintained at 80 ºC for 2 h and then the stressed sample was cooled, neutralized and diluted with mobile phase. Similarly alkaline degradation was conducted using 1 N NaOH for 2 h in thermostat maintained at 80 ºC. After cooling the solution was neutralized and diluted with mobile phase as per the requirement and 5 µL was injected in to the system.

Oxidation degradation studies

Solutions for oxidative stress studies were prepared by refluxing the combined formulation of Eprosartan and of hydrochlorothiazide solution with 1% H_2O_2 and after refluxation for 2 h at 80 ºC in the thermostat the drug solution was cooled and diluted accordingly with the mobile phase.

Thermal degradation studies

For thermal stress testing, the combined formulation of Eprosartan and of hydrochlorothiazide was heated in an oven at 105 °C for 72 h, cooled and then injected in to the HPLC system.

Photolytic degradation studies

The combined formulation of Eprosartan and of hydrochlorothiazide was kept in photolytic chamber at 1289069 Lux Hours and 1024.2.66 Watt-Hour/m^2 and analyzed.

Humidity degradation

The combined formulation of Eprosartan and of Hydrochlorothiazide was kept in desiccator at 25 °C, 95% RH for 120 h. The sample solutions were prepared with the stressed sample with diluent as per the requirement and filtered through 0.45 µm filter. 5 µL of this solution was injected into the HPLC system and analysed.

Results and Discussion

A reversed-phase liquid chromatographic technique was developed to quantitate eprosartan and hydrochlorothiazide in pharmaceutical dosage forms (Tablets) and validated as per ICH guidelines. No stability indicating liquid chromatographic method has not yet been reported earlier.

Method development and optimization

For selection of column, a spiked sample of Eprosartan and hydrochlorothiazide was prepared and injected into HPLC system on different columns. The required system suitability criterion was obtained using X Bridge Shield RP-18 (150x3.0 mm), 3.5 µm column (Table 1). The optimized chromatographic conditions were shown in Table 2 and the chromatogram obtained for the blank was shown in Figure 3.

Table 1. Selection of column in method optimization

Column conditions	Remark
Luna C-18(2) (150x4.6 mm), 5 µm	HCTZ peak eluting in the void.
YMC pro pack C-18 (150x4.6 mm), 5 µm, 45 °C	HCTZ peak eluting in the void.
Kromasil C-18 (150x 4.6 mm), 3.5 µm	HCTZ peak eluting in the void.
Flow rate 1.1 mL/min, Gradient2	Eprosartan peak co-eluting with the blank peak.
X Bridge RP-18 (150x4.6 mm) 3.5 µm	Eprosartan peak co-eluting with the blank peak
X Bridge Shield RP-18 (150x3.0 mm), 3.5 µm	Eprosartan peak separated from blank peak
X Bridge Shield RP-18 (150x3.0 mm), 3.5 µm, different gradient	Eprosartan peak separated from blank peak

Method validation

The typical chromatogram of hydrochlorothiazide and Eprosartan was shown in Figure 4 and the corresponding peak purity plots were shown in Figure 5. Beer-Lambert's law was obeyed over the concentration range 1-300 µg/mL for hydrochlorothiazide and 19.2-750.3 µg/mL for Eprosartan respectively (Table 3) with regression equations y = 5980.5 x +535.64 (R^2 = 0.9999) (Figure 6 A) and y = 8199.2x + 565.86 (R^2 = 0.9998) (Figure 6 B) respectively.

Table 2. Optimized chromatographic conditions

Column	: X Bridge Shield RP18 (150x3.0) mm, 3.5 µm		
Injection volume	: 5µL		
Mobile phase	: 0.1% Formic acid in water and Acetonitrile Gradient mode		
Flow rate	: 0.8 mL/min		
	Time, min	% (0.1% formic acid)	% Acetonitrile (%v/v)
	0	90	10
	2	90	10
	4	80	20
	16	10	90
	17	10	90
	17.5	90	10
	20	90	10
Detection wavelength	: 235 nm		
Column oven Temp.	: 45°C		

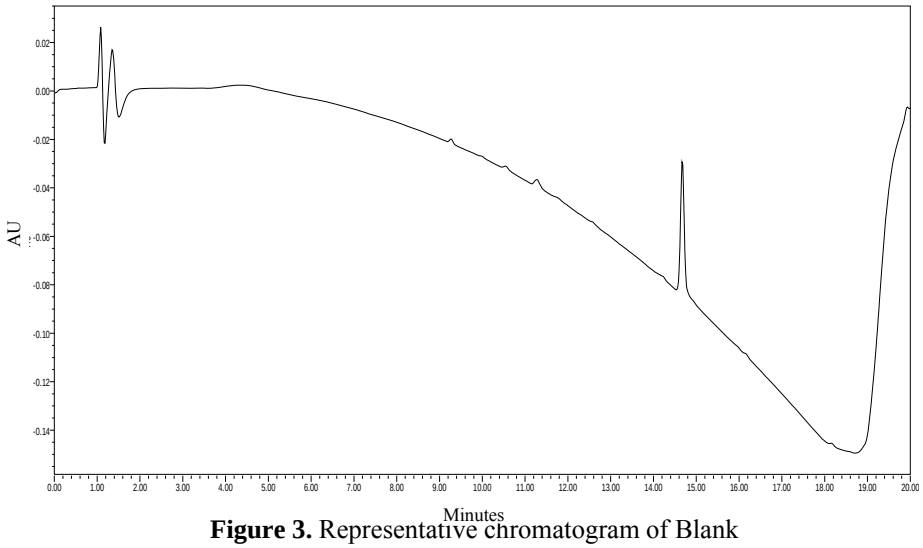

Figure 3. Representative chromatogram of Blank

Table 3. Linearity of Hydrochlorothiazide and Eprosartan

Hydrochlorothiazide		Eprosartan	
Conc. µg/mL	*Mean peak area	Conc. µg/mL	*Mean peak area
1.001	8946	19.208	156894
10.010	97196	24.010	201649
20.020	195989	48.021	401246
30.030	294899	144.062	1180596
60.060	589665	240.104	1960469
100.100	983495	420.182	3446658
137.638	1362983	480.208	3934697
175.175	1749458	540.234	4406879
200.200	1966459	600.260	4895216
250.250	2458536	660.286	5498466
300.300	2960249	750.325	6120964

**Mean of three replicates*

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.

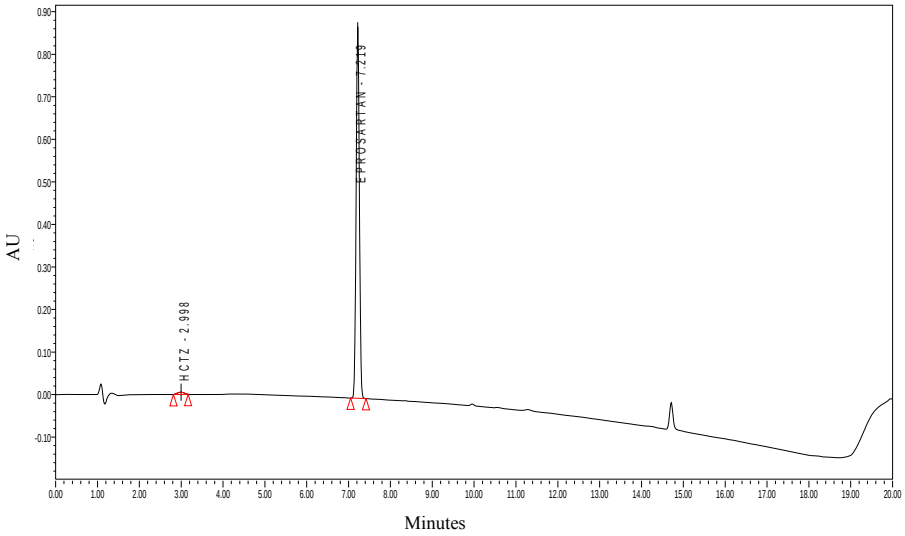

Figure 4. Typical chromatogram of hydrochlorothiazide (5 µg/mL) and Eprosartan (240 µg/mL)

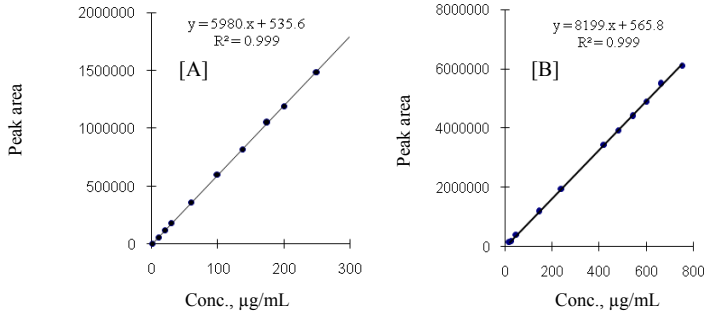

Figure 5. Calibration curves of Eprosartan [A] and Hydrochlorothiazide [B]

The % RSD for intra-day and inter-day precision study was found to be 0.14-0.36 and 0.18-0.33 for EPR (Table 5) where as the RSD for intra-day and inter-day precision study for HCTZ was found to be 0.16-0.34 and 0.15-0.34 (Table 4) respectively which is less than 2 % indicating that the method is precise.

Table 4. Precision study of Hydrochlorothiazide

Hydrochlorothiazide (HCTZ)			
Intra-day		Inter-day	
*Mean peak area ± SD	RSD %	*Mean peak area ± SD	RSD %
2499456±3999.130	0.16	2498745±3997.992	0.16
2516423±5536.131	0.22	2503369±3755.054	0.15
2498456±8494.750	0.34	2488942±8462.403	0.34
2498997±4498.195	0.18	2498512±6246.280	0.25
2516846±7298.853	0.29	2496349±8237.952	0.33
2509781±6525.431	0.26	2489994±4730.989	0.19

**Mean of three replicates*

The % RSD accuracy study was found to be 0.25-1.43 for EPR and 0.27-0.36 for HCTZ (Table 6) respectively. The % RSD for robustness study was found to be 0.13-0.78 for EPR and 0.15-0.54 for HCTZ (Table 7) respectively.

Table 5. Precision study of Eprosartan

Intra-day		Inter-day	
*Mean peak area ± SD	RSD %	*Mean peak area ± SD	RSD %
4260552 ± 9373.214	0.22	4365664 ± 13970.125	0.32
4261864 ± 15342.710	0.36	4264977 ± 8956.452	0.21
4256489 ± 5959.085	0.14	4263498 ± 14069.543	0.33
4259974 ± 6815.958	0.16	4264784 ± 10661.960	0.25
4260646 ± 13634.067	0.32	4268264 ± 7682.875	0.18
4261169 ± 14061.858	0.33	4359874 ± 9591.723	0.22

**Mean of three replicates*

Table 6. Accuracy study of Eprosartan and Hydrochlorothiazide

Drug	Level	% Recovery*	%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

**Mean of three replicates*

Table 7. Robustness study of eprosartan and hydrochlorothiazide

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

Analysis of commercial formulations

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 8) and no interference was observed with the excipients.

Table 8. 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

Forced degradation studies/specificity

During acidic degradation, the chromatogram shows peaks at 2.854 min and 7.281 min indicating the elution of Hydrochlorothiazide and Eprosartan respectively (Figure 6). On acidic degradation HCTZ has not at all undergone degradation where as 1.04% of decomposition was observed with EPR. The purity angle (0.083) was less than the purity threshold (1.030) indicating that EPR does not interference with degradants and similarly the purity angle (0.349) was less than the purity threshold (1.051) for HCTZ. Therefore it can be concluded that the method is specific and selective.

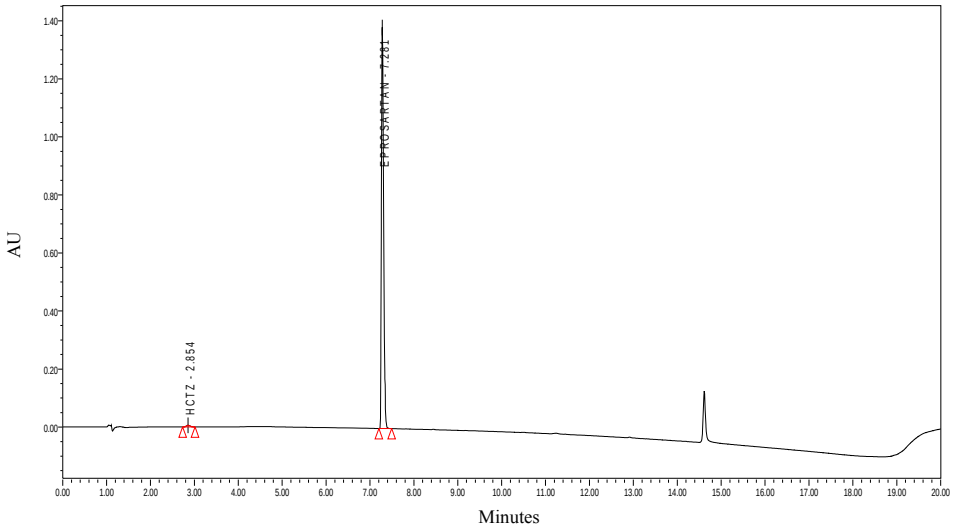

Figure 6. Typical chromatogram of Hydrochlorothiazide (5 µg/mL) and Eprosartan (240 µg/mL) (Acidic degradation)

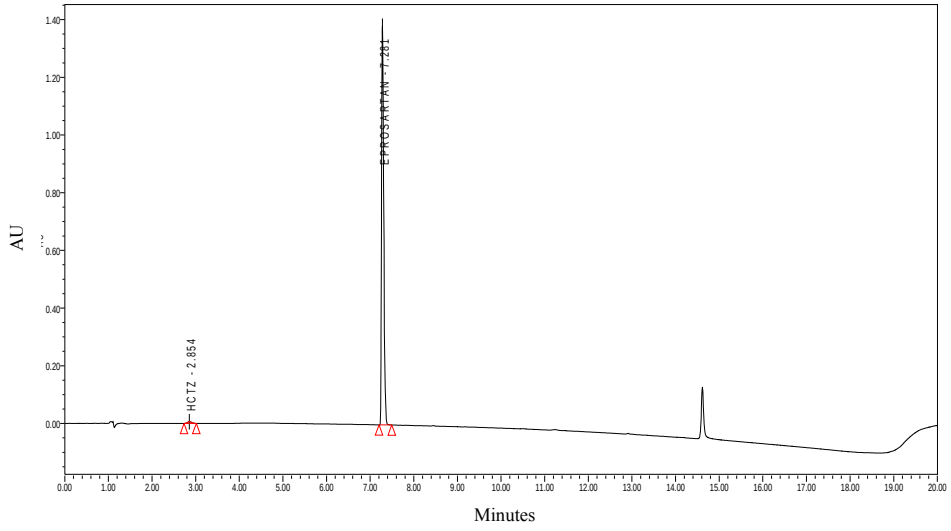

Figure 7. Typical chromatogram of Hydrochlorothiazide (5 µg/mL) and Eprosartan (240 µg/mL) (Acidic degradation)

During alkaline degradation, the chromatogram shows peaks at 2.858 min and 7.284 min indicating the elution of Hydrochlorothiazide and Eprosartan respectively. 1.17 % of EPR has undergone alkaline degradation and only 0.66 % of HCTZ was decomposed (Figure 7). The purity angle (0.079) for EPR was less than the purity threshold (1.030) indicating that no interference of degradants and similarly the purity angle (0.0678) was less than the purity threshold (2.125) for HCTZ.

During oxidative degradation, the chromatogram shows peaks at 2.856 min and 7.290 min indicating the elution of Hydrochlorothiazide and Eprosartan respectively. During oxidative degradation about 3.60 % of EPR and 3.52 % of HCTZ have undergone decomposition without degradants (Figure 8). The purity angle (0.080) was less than the purity threshold (1.030) indicating that EPR peak was well separated and similarly the purity angle (0.762) was less than the purity threshold (2.023) for HCTZ.

During thermal degradation the chromatogram shows peaks at 2.867 min and 7.326 min indicating the elution of Hydrochlorothiazide and Eprosartan respectively. HCTZ has undergone 1.22 % degradation where as 2.65 % of decomposition was observed with EPR (Figure 9). The purity angle (0.107) was less than the purity threshold (1.079) indicating that EPR indicating that there is no interference. Similarly the purity angle (0.045) was less than the purity threshold (1.060) for HCTZ.

During photolytic degradation the chromatogram shows peaks at 2.868 min and 7.332 min indicating the elution of Hydrochlorothiazide and Eprosartan respectively (Figure 10). HCTZ has not at all undergone degradation where as 2.01% of decomposition was observed with EPR. The purity angle (0.110) was less than the purity threshold (1.082) indicating that EPR indicating that there is no interference and similarly the purity angle (0.048) was less than the purity threshold (1.061) for HCTZ. Therefore it can be concluded that the method is specific and selective. As no new degradants were observed mass spectral analysis was not performed.

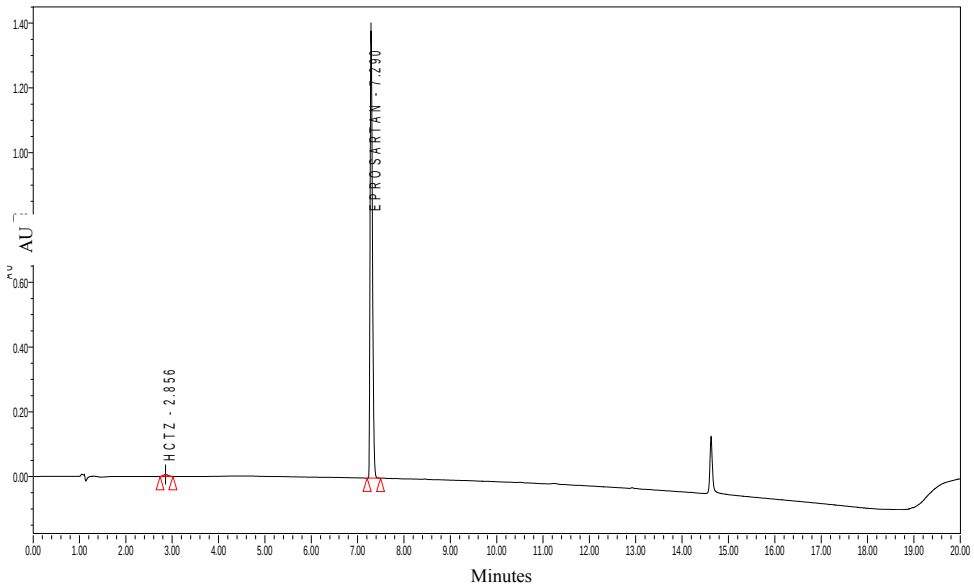

Figure 8. Typical chromatogram of Hydrochlorothiazide (5 µg/mL) and Eprosartan (240 µg/mL) (Oxidative degradation)

On humidity degradation the chromatogram shows peaks at 2.864 min and 7.326 min indicating the elution of Hydrochlorothiazide and Eprosartan respectively (Figure 11). On humidity exposure to 95%RH at 25°C for 120 hours, HCTZ has not at all undergone degradation where as 1.27 % of decomposition was observed with EPR . The purity angle (0.107) was less than the purity threshold (1.070) indicating that EPR indicating that there is no interference and similarly the purity angle (0.048) was less than the purity threshold (1.063) for HCTZ. Therefore it can be concluded that the method is specific and selective.

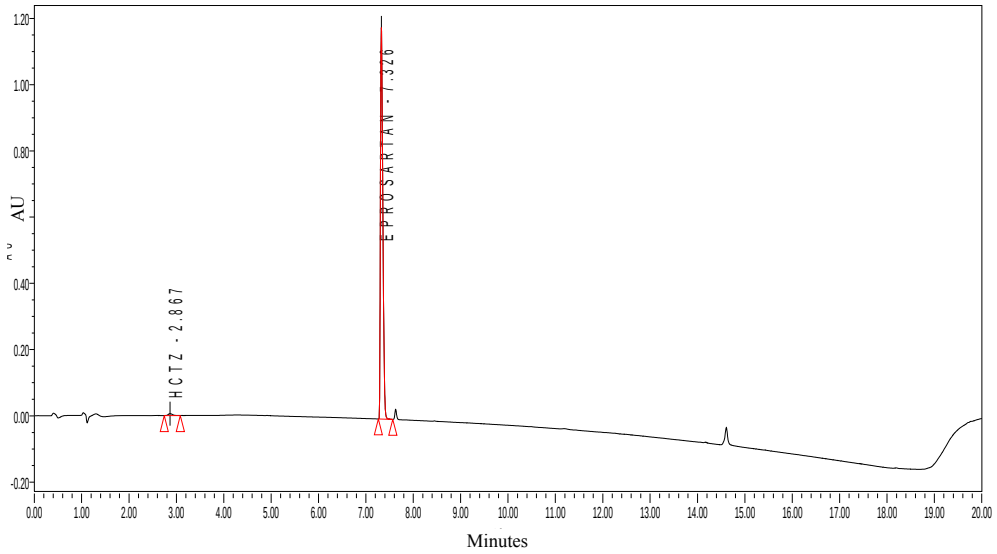

Figure 9. Typical Representative chromatogram of Hydrochlorothiazide (5 µg/mL) and Eprosartan (240 µg/mL) (Thermal degradation)

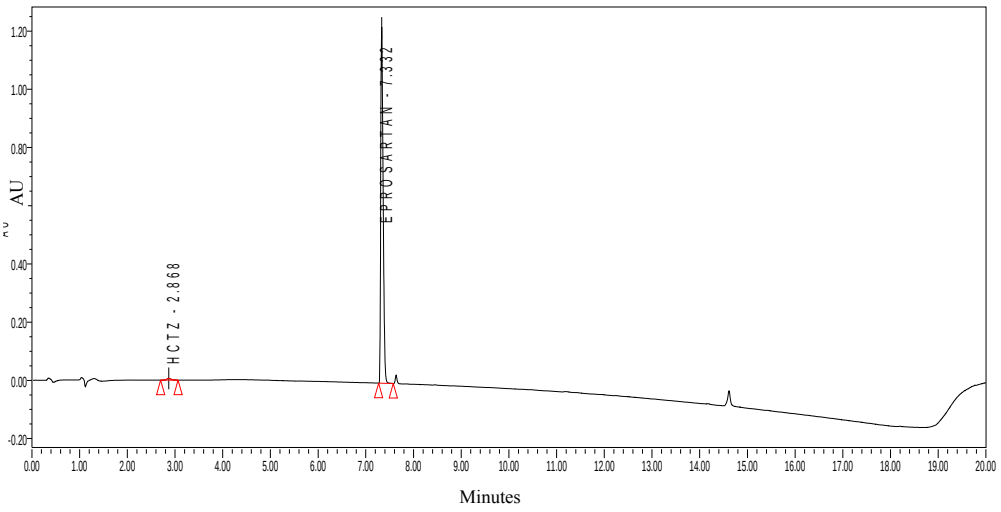

Figure 10. Typical chromatogram of Hydrochlorothiazide (5 µg/mL) and Eprosartan (240 µg/mL) (Photolytic degradation)

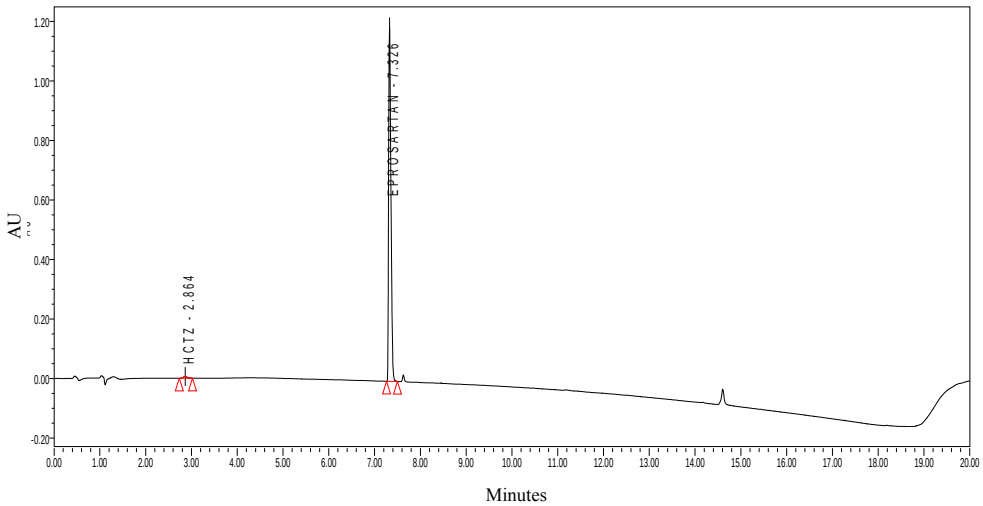

Figure 11. Typical chromatogram of Hydrochlorothiazide (5 µg/mL) and Eprosartan (240 µg/mL) (Humidity degradation)

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 (HCTZ) and 97156 (EPR) which is >2000. A brief summary of forced degradation studies of Eprosartan and Hydrochlorothiazide was given in Table 9.

Table 9. Forced degradation studies of Hydrochlorothiazide and Eprosartan

Stress conditions	Mean peak area		Drug recovered %		Drug decomposed %		Purity angle		Purity threshold	
	HCTZ	EPR	HCTZ	EPR	HCTZ	EPR	HCTZ	EPR	HCTZ	EPR
Untreated	2468469	1966496	100	100	-	-	0.532	0.790	1.851	1.029
Acidic degradation	2471650	1946123	100.13	98.96	-	1.04	0.349	0.083	1.051	1.030
Alkaline degradation	2452295	1943454	99.34	98.83	0.66	1.17	0.678	0.079	2.125	1.030
Oxidative degradation	2381563	1895769	96.48	96.40	3.52	3.60	0.762	0.080	2.023	1.030
Thermal degradation	2438272	1914341	98.78	97.35	1.22	2.65	0.045	0.107	1.060	1.079
Photolytic degradation	2480377	1926923	100.48	97.99	-	2.01	0.048	0.110	1.061	1.082
Humidity degradation	2466101	1941465	99.90	98.73	0.10	1.27	0.048	0.107	1.063	1.070

Conclusion

The present developed RP-HPLC method was stability indicating simple, specific, precise, accurate and robust. It can be applied for the determination of Eprosartan and Hydrochlorothiazide in pharmaceutical dosage forms as well as for pharmacokinetic studies.

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