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Research Article

STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF EPROSARTAN MESYLATE AND HYDROCHLOROTHIAZIDE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

Objective: The objective of the method was to develop a simple, rapid, efficient, cost effective and reproducible stability indicating reverse phase high performance liquid chromatography method (RP-HPLC) for the simultaneous estimation of eprosartan mesylate and hydrochlorothiazide in active pharmaceutical ingredient and tablet dosage form

Methods: The RP-HPLC analysis was carried out on an enable C_{18} G column with a mobile phase of methanol-acetonitrile-potassium dihydrogen orthophosphate (0.01 M; pH adjusted to 3 with orthophosphoric acid) in the ratio of 20:28:52 (v/v/v). UV detection was performed at 240 nm.

Results: The method was found to be linear over a concentration range of 10-600 µg/ml and 0.4-25 µg/ml for eprosartan mesylate and hydrochlorothiazide respectively. The limit of detection was found to be as 3.1 µg/ml and 100 ng/ml for eprosartan mesylate and hydrochlorothiazide respectively. The limit of quantitation was found to be as 10 µg/ml and 100 ng/ml for eprosartan mesylate and hydrochlorothiazide. Recovery was found to be in the range 99-100% and precision less than 1%. Developed method was successfully applied for routine analysis of drug pharmaceutical formulation. Forced degradation studies were performed for eprosartan mesylate and hydrochlorothiazide. The drugs were degraded in acidic, basic and oxidative conditions. The peaks of degraded products were well resolved from the actual drug.

Conclusion: The developed method was simple, rapid, accurate, precise and stability indicating for the simultaneous estimation of eprosartan mesylate and hydrochlorothiazide in active pharmaceutical ingredient and tablet dosage form

Keywords: Eprosartan mesylate; Hydrochlorothiazide; RP-HPLC, Degradation, Validation

INTRODUCTION

Eprosartan,4-({2-butyl-5-[2-carboxy-2-(thiophene-2-ylmethyl)eth-1-ene-yl]-1H-imidazol-1-yl}methyl)benzoic acid is an antihypertensive, used in the treatment of hypertension. The drug acts on the rennin-angiotensin system by two ways to decrease the total peripheral resistance. First it blocks the binding of angiotensin II to AT_1 receptor in vascular smooth muscle causing vascular dialation. Second it inhibits sympathetic norepinephrine production further reducing blood pressure. Hydrochlorothiazide, 6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide is a diuretic used in combination with eprosartan to treat hypertension [1,2]. New marketed formulation in combination of eprosartan mesylate 600 mg and hydrochlorthiazide 25 mg is commercially available as tablet (Teventen® HCT) for the treatment of edema and hypertension.

Stability indicating methods have become an important aspect of analytical method validation and a part of US FDA requirements [3]. The stability of a drug product or a drug substance is a critical parameter which may affect purity, potency and safety. Changes in drug stability can risk patient safety by formation of a toxic degradation product(s) or deliver a low dose than expected. Hence it is essential to know the purity profile and behavior of a drug substance under various environmental conditions [4,5]. Very few methods were published for the simultaneous estimation of eprosartan and hydrochlorothiazide involving HPLC techniques [6-8], spcetrophotometric methods [9-11]. All these methods were expensive, time consuming, complex in nature and not stability indicating. Consequently, there was still a need to develop a simple, less time consuming and stability indicating method for the simultaneous estimation of eprosartan and hydrochlorothiazide. Therefore, we attempted to develop a stability indicating, rapid and reproducible RP-HPLC method for the simultaneous estimation of eprosartan and hydrochlorothiazide in API and tablet dosage form.

MATERIALS AND METHODS

Chemicals

Eprosartan mesylate Fig. 1(a) and hydrochlorothiazide Fig. 1(b) were obtained as gift samples from Mylan laboratories (Hydereabad). Methanol, acetonitrile of HPLC grade were obtained from Sigma Aldrich. Analytical grade potassium dihydrogen orthophosphate, orthophosphoricacid purchased from Finar chemicals limited. Teventen® HCT of label claim 600 mg (Eprosartan mesylate) and 25 mg (Hydrochlorthiazide) manufactured by Solvay pharmaceuticals, Mumbai, India was purchased from the local market.

Instrumentation and chromatographic conditions:

The HPLC system used was LC 20AT Shimadzu system with LC Solutions software. The system was equipped with LC 20AT prominence pump and UV detector.
The chromatographic separation was carried out on enable C_{18} G column (150×4.6 mm, 5 µ). Elution was performed with a mobile phase containing methanol-acetonitrile-potassium dihydrogen orthophosphate (pH-3; 0.01M) (20:28:52, v/v/v). The pH of the buffer was adjusted with orthophosphoric acid. The mobile phase was freshly prepared, filtered through 0.45 µm membrane filter and degassed prior to use.

Preparation of standard stock solutions

Standard stock solutions were prepared by transferring 100 mg of eprosartan and 100 mg of hydrochlorothiazide separately into 100 ml volumetric flasks. To the flasks methanol was added and sonicated to dissolve the sample and the volume was made up to the mark using methanol. From these stock solutions working standards were prepared such that a single sample contains both the drugs over a concentration range of 5-600 µg/ml of eprosartan mesylate and 0.2-25 µg/ml of hydrochlorothiazide

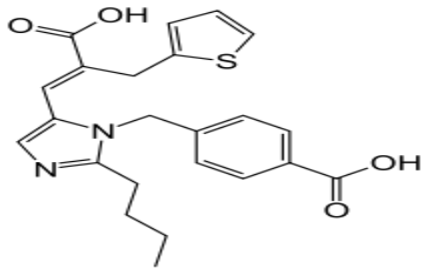

Fig. 1(a): Eprosartan mesylate

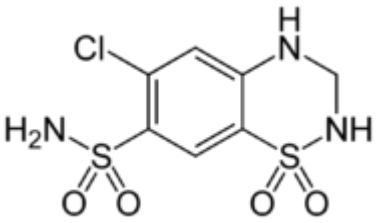

Fig. 1(b): Hydrochlorothiazide

Preparation of sample solution

Twenty tablets were weighed and powdered. Tablet powder equivalent to 600 mg of eprosartan mesylate and 25 mg of hydrochlorothiazide was accurately weighed and transferred into 100 ml volumetric flask and dissolved in 20 ml of mobile phase and sonicated for five minutes. The volume was made upto the mark using mobile phase. This is then filtered through 0.45 µ membrane filter to obtain clear solution. This stock sample solution was diluted quantitatively with mobile phase to obtain the suitable working sample solutions for chromatographic measurements.

Method validation

The developed method was validated according to ICH guidelines in terms of specificity, sensitivity, linearity, LOD, LOQ, accuracy, Precision and robustness.

Specificity

Specificity was established by complete separation of analyte in the presence of tablet excipients and without interferences at the retention time of eprosartan mesylate and hydrochlorothiazide.

Linearity

Linearity was established by least squares linear regression analysis of calibration curve. Linearity was determined in the range of 5-600 µg/ml of eprosartan mesylate and 0.2-25 µg/ml of hydrochlorothiazide.

LOD and LOQ

LOD and LOQ were determined by using the following formulae,

$$LOD = \frac{3.3 \times SD}{slope} \quad ; \quad LOD = \frac{10 \times SD}{slope}$$

Where SD is the standard deviation of response

Precision and accuracy

Precision and accuracy of the method was monitored for three days. Accuracy of the method was calculated by recovery studies at three levels by standard addition method. Precision of the method was determined by performing intraday and interday precision. Intraday precision was determined by three replicate analyses of three concentrations (10, 75 and 600 µg/ml of eprosartan mesylate, 0.4, 3.1, 25 µg/ml of hydrochlorothiazide) within a day. Interday precision was performed for three days by three replicate injections of three samples in a day.

Robustness

Robustness of the method was investigated under a variety of conditions like change in pH of flow rate (± 0.2 ml), composition of mobile phase (±2 ml) and wavelength (±2 nm). In each variation analysis was made in three replicates and %RSD of peak areas were determined.

Forced degradation studies

In order to establish whether the developed method is stability indicating both the drugs were stressed under various conditions (acid, base, oxidation and thermal) to perform forced degradation studies.

Acid degradation studies

Eprosartan and hydrochlrothiazide standard solutions of 100 µg/ml were prepared with mobile phase and treated with 5 ml of 1N HCl. The resultant solution was analysed for every 24 h after prior dilution.

Alkaline degradation studies

Eprosartan and hydrochlrothiazide standard solutions of 100 µg/ml were prepared with mobile phase and treated with 5 ml of 1N NaOH. The resultant solution was analysed for every 24 h after prior dilution.

Oxidation studies

Eprosartan and hydrochlrothiazide standard solutions of 100 µg/ml were prepared with mobile phase and treated with 5 ml of 3% H_2O_2. The resultant solution was analysed for every 24 h after prior dilution.

Thermal stress studies

Accurately weighed sample of about 100 mg of eprosartan and hydrochlorothiazide were transferred into separate vials. These vials are kept in hot air oven at 60^0c for about seven days. These samples are analysed for every 24h for seven days.

RESULTS AND DISCUSSION

Method development:

Various methods have been developed for the quantitation of eprosartan and hydrochlorothiazide [6-11] in pharmaceutical formulations and biological fluids; here we report a simple, efficient and less time consuming RP-HPLC method for the simultaneous estimation of eprosartan and hydrochlorothiazide in API and tablet dosage form. Some chromatographic parameters such as column type, mobile phase, wave length were investigated to obtain suitable peaks for eprosartan and hydrochlorothiazide with good resolution. Chromatographic method development began with the selection of wavelength and optimization of mobile phase composition.

Selection of wavelength

The samples of eprosartan mesylate and hydrochlorothiazide were prepared in the concentration of 10 µg/ml. These samples were scanned in the range of 200-400 nm using UV-visible spectrophotometer. From the overlain spectra Fig 2 the detection wavelength was selected as 240 nm.

Optimization of mobile phase

The feasibility of several mixtures of solvent such as methanol and acetonitrile using different buffers such as formic acid, potassium dihydrogen ortho phosphate with variable pH range 3-5 was tested for complete resolution of eprosartan mesylate and hydrochlorothiazide. Enable C_{18} G column with mobile phase methanol, acetonitrile and potassium dihydrogen orthophosphate at pH 3.0 provided very good resolution and peak shape for eprosartan mesylate and hydrochlorothiazide

Chromatographic conditions were optimized with respect to specificitry, reproducibility and time of analysis.after the method was developed and optimized it was validated.Representative chromatogram of eprosartan mesylate and hydrochlorothiazide in APT is given in Fig. 3

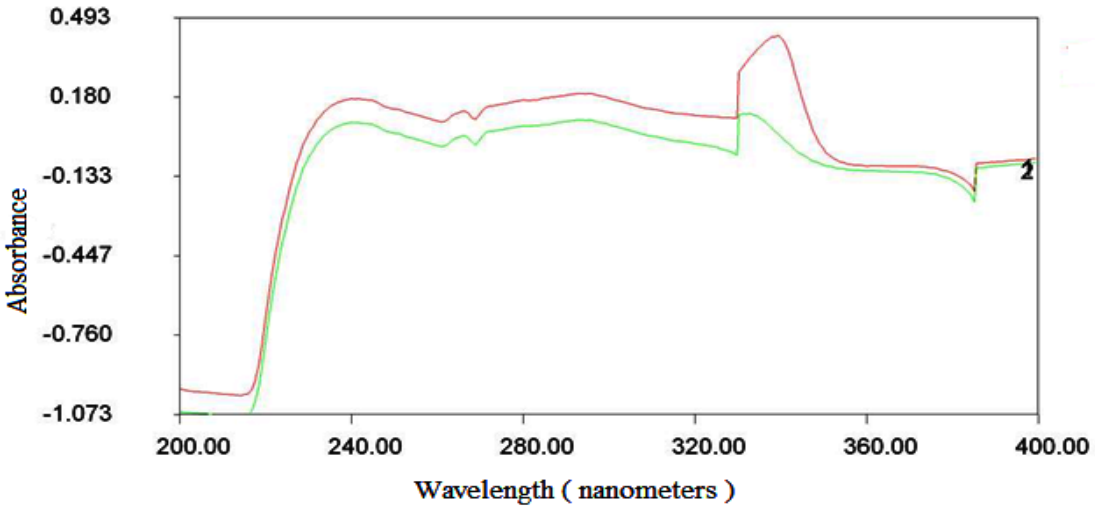

Fig. 2: Overlain spectra of eprosartan mesylate and hydrochlorothiazide

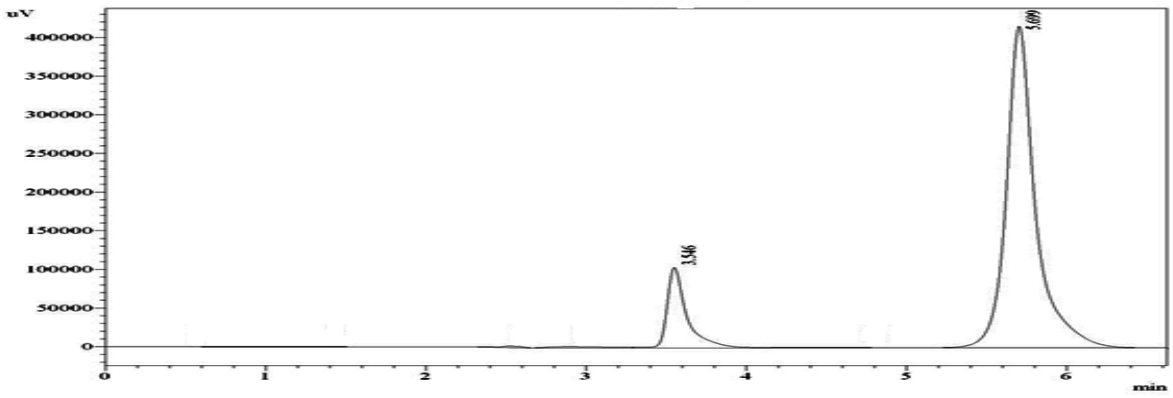

Fig. 3: Chromatogram showing simultaneous estimation of eprosartan mesylate (Rt 5.69) and hydrochlorothiazide (Rt 3.54)

Method validation

After establishing the optimal conditions for separation, linearity, precision, accuracy, LOD, LOQ were determined. The validation was conducted according the ICH guidelines.

Specificity and Sensitivity

Chromatograms of sample solution (tablet sample) and blank were analysed and no interferences were found at the retention time of eprosartan mesylate and hydrochlorothiazide.

The correlation coefficients for both the drugs were found to be 0.998 and 0.999. Table 1 represents the regression data including, correlation coefficient, slope, linearity range

Precision

Precision of the method was determined by performing intraday and interday precision. The % RSD obtained for intraday and interday precision was less than 2%. Intraday and interday precision values are presented in Table 2. The % RSD values were within 2 and the method was found to be precise.

Accuracy

Accuracy of the method was determined by recovery studies (standard addition method). Recovery was performed at three levels i.e. 50%, 100%, 150% of those expected by spiking a previously analysed sample solution with standard drug solution.

The samples were analysed for three repeated times and the peak areas were recorded. Percentage recovery values were in the accepted range according to ICH guidelines. Percentage recovery values were presented in Table 2.

Representative chromatograms of sample were shown in Fig. 4. LOD and LOQ values are determined and reported in Table 1. The limit of detection was found to be as 3.1 µg/ml and 0.1 µg/ml for eprosartan mesylate and hydrochlorothiazide respectively. The limit of quantitation was found to be as 10 µg/ml and 0.1 µg/ml for eprosartan mesylate and hydrochlorothiazide.

Chromatogram of sample solution(marketed formulation)

Linearity

Calibration standards were prepared in the concentration of 5-600 µg/ml of eprosartan and 0.2-25 µg/ml of hydrochlorothiazide. The calibration curve for eprosartan mesylate Fig. 5(a) was linear over the concentration range of 10-600 µg/ml and that for hydrochlorothiazide Fig. 5(b) was linear over the range of 0.4-25 µg/ml.

Robustness

Robustness of the method is determined by making deliberate changes in the parameters like flow rate, wavelength, mobile phase ratio. The samples are analysed by changing the parameters and the peak areas are recorded. Samples were analysed in triplicates and %RSD was calculated from peak areas. The % RSD obtained for robustnes were less than 2%. Results of robustness are summarized in Table 3.

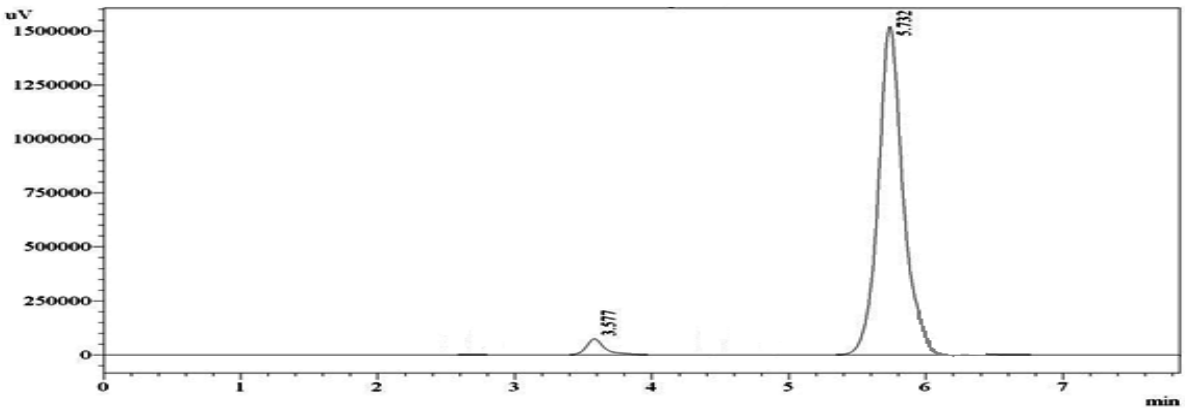

Fig. 4:

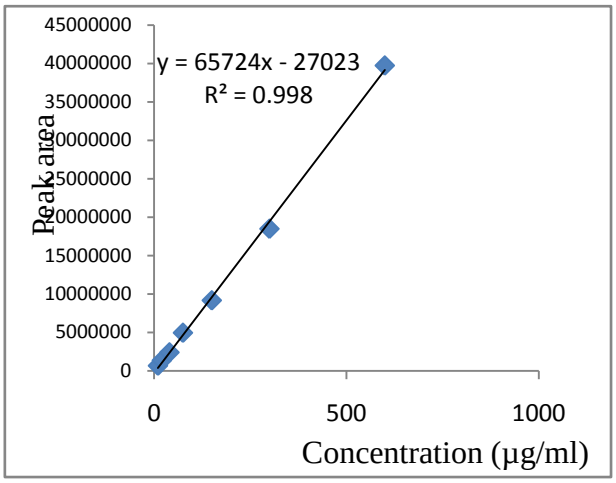

Fig. 5a: Calibration curve of hydrochlorothiazide

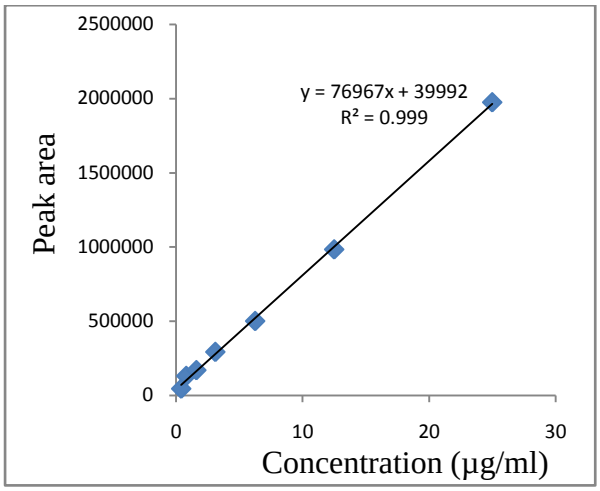

Fig. 5b: Calibration curve of eprosartan mesylate

Table 1: Analytical performance parameters for linearity

Parameter	Eprosartan mesylate	Hydrochlorothiazide
Linear range	10 – 600 µg/ml	0.4 – 25 µg/ml
Slope	65724	76702
R^2 value	0.998	0.999
LOQ	10 µg/ml	0.4 µg/ml
LOD	3.1 µg/ml	0.1 µg/ml

Table 2: Intraday, inter day precision and accuracy for eprosartan mesylate and hydrochlorothiazide

Sample	Concentration (µg/ml)	Precision (%RSD)		Accuracy (% recovery)
		Intraday	Interday	
Eprosartan mesylate	10	0.4	0.8	99.75
	75	0.9	0.3	99.6
	600	0.6	0.3	100.06
Hydrochlorothiazide	0.4	0.3	0.6	99.4
	3.1	0.1	0.4	99.2
	25	0.5	0.9	99.89

Table 3: Robustness values of eprosartan mesylate and hydrochlorothiazide

Parameter		Eprosartan mesylate (% RSD)	Hydrochlorothiazide (% RSD)
Flow rate (ml/min)	0.9	0.5%	0.8%
	1.1	0.62%	0.9%
Mobile phase (MET:ACN:BUFFER)	20:30:50	1.2%	1.42%
	20:26:54	1.27%	1.47%
Wavelength (nm)	238	0.2%	0.82%
	242	0.4%	0.85%

Assay

The validated method was applied to the determination of eprosartan mesylate and hydrochlorothiazide in commercially available Teventen® HCT tablets. The percentage assay was found to be 99.52% for eprosartan mesylate and 99.67% for hydrochlorothiazide respectively. The results of assay indicate that the developed method is selective without interference from excipients of tablet.

Stability studies:

In order to establish whether the developed method is stability indicating both the drugs were stressed under various conditions (acid, base, oxidation and thermal) to perform forced degradation studies. Eprosartan mesylate was stable in acid but decomposed in alkaline, oxidative degradation studies. Hydrochlorothiazide was stable in alkaline and decomposed in acid, oxidative degradation studies. No decomposition was seen on exposure of solid drugs to dry heat. The peaks of degraded products were well separated from the analyte peak with good resolution Fig. 6 (a, b, c, d) which indicates that the developed method is stability indicating. The forced degradation studies data are summarized in Table 4

CONCLUSION

A simple, rapid, accurate, precise, low cost and stability indicating RP-HPLC method for the simultaneous estimation of eprosartan and hydrochlorothiazide in API and tablet dosage form has been developed and validated. The intra-run and inter-run variability and accuracy results were found in acceptable limit.

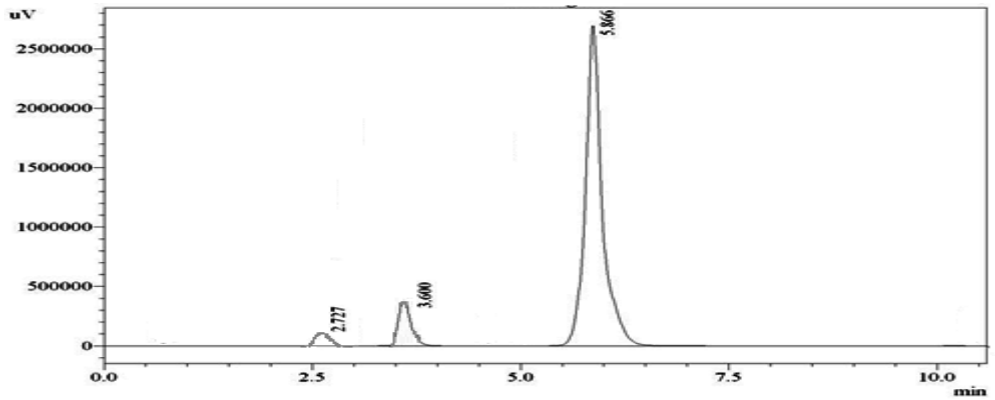

Fig. 6a: Chromatogram showing acid degradation of eprosartan mesylate and hydrochlorothiazide

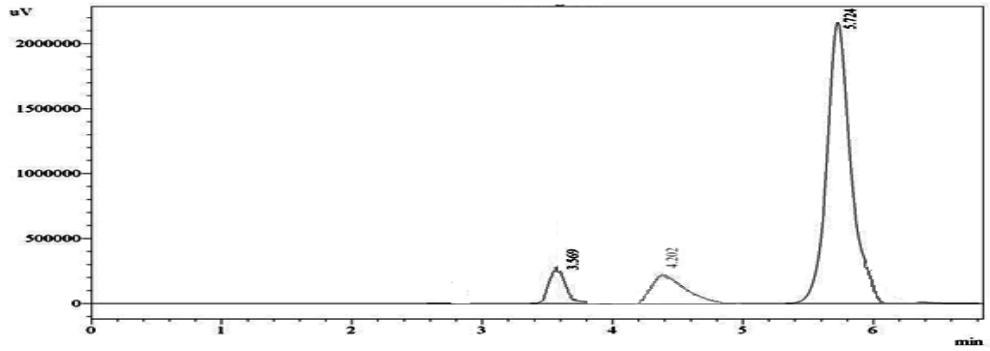

Fig. 6b: Chromatogram showing base degradation of eprosartan mesylate and hydrochlorothiazide

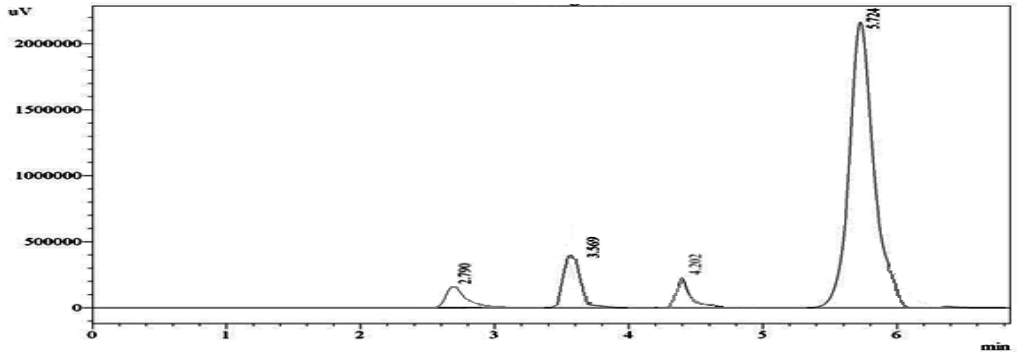

Fig. 6c: Chromatogram showing oxidative degradation of eprosartan mesylate and hydrochlorothiazide

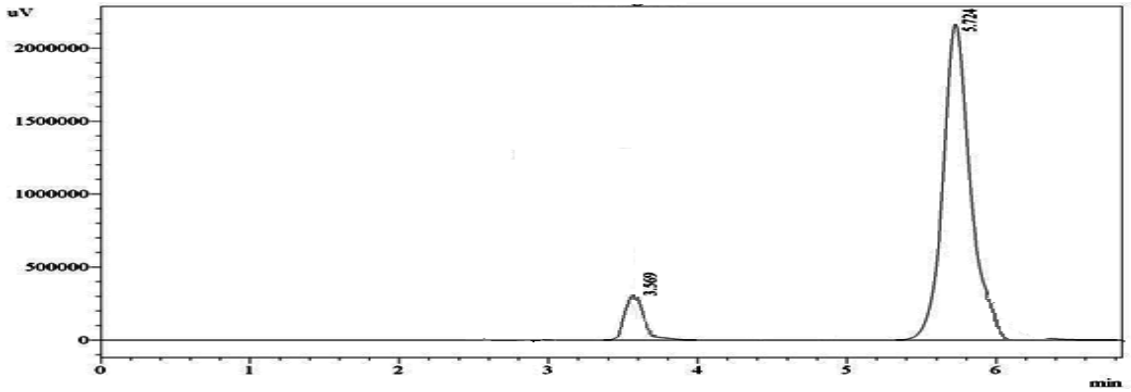

Fig. 6d: Chromatogram showing thermal degradation of eprosartan mesylate and hydrochlorothiazide (both the drugs are stable in thermal conditions).

Table 4: Degradation details of eprosartan mesylate and hydrochlorothiazide

S. No	Degradation Studies	Percentage Degradation	
		Eprosartan mesylate	Hydrochlorothiazide
1	Acid	Stable	11%
2	Base	13%	Stable
3	Oxidation	11.5%	12%
4	Thermal	Stable	Stable

The results of forced degradation studies reveal that the method is stability indicating. The proposed method has the capability to separate the analyte from their degradation products obtained during forced degradation studies and excipients found in tablets. Simplicity, stability and economical nature makes the method superior to the other reported HPLC methods. The method can be employed for the routine analysis of eprosartan mesylate and hydrochlorothiazide.

ACKNOWLEDGEMENT

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