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Simultaneous Determination of Eprosartan Mesylate and Hydrochlorthiazide in Pharmaceutical Dosage form by Reverse Phase High Performance Liquid Chromatography

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Abstract

A simple, rapid, sensitive and accurate reverse phase high performance liquid chromatographic (RP-HPLC) method has been developed and subsequently validated for the simultaneous determination of Eprosartan mesylate and hydrochlorthiazide in combination.Chromatographic separation of the two drugs was performed on a Purospher BDS C18 column (150 mm× 4.6 mm id, 5µm particle size). The mobile phase comprising of acetonitrile: methanol: 0.01M KH_2PO_4 buffer (40:40:10) was delivered at a flow rate of 1.0mL/min. The pH of the mobile phase is adjusted to 4 with ortho phosphoric acid .Detection was performed at 270nm.The total run time is 5 min and the retention time of Eprosartan mesylate is 3.56 min and hydrochlorthiazide is 4.62 min respectively.The described method is linear for the assay of Eprosartan mesylate and hydrochlorthiazide over a concentration range of 216-576µ g/mL and 9-24µg/mL respectively. Results of the analysis have been validated and by recovery studies.The excipients present in the formulations do not interfere with the assay procedure.The developed method was successfully applied to determine Eprosartan mesylate and hydrochlorthiazide in pharamaceutical formulations.

Keywords: Eprosartan mesylate; Hydrochlorthiazide; Validation; Pharmaceutical formulations

Introduction

The absolute risk of cardiovascular events is mainly determined by high blood pressure, although there are some other important contributors, such as age, race and presence of other cardio vascular risk factors. Hence, anti hypertensive therapy enables to reduce considerably the risk of developing cardiovascular complications that cause a high mortality rate in the industrialized countries [1,2]. Most hypertensive patients require more than one agent in order to achieve adequate blood pressure (BP) control [3]. Eprosartan mesylate[EPR] is chemically monomethanesulfonate of (*E*)-2-butyl-1-(p-carboxybenzyl)-α-2-thienylmethylimidazole-5-acrylicacid (Figure 1) is a new antihypertensive agent as an angiotension II receptor antagonist that is highly selective to elict a higher reduction in systolic blood pressure than other anti hypertensilve drugs [4,5]. Hydrochlorothiazide [HCT] is chemically 6-chloro-3,4-dihydro-2 H 1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide(Figure 1) is a thiazide diuretic. Thiazides affect the renal tubular mechanisms of electrolyte reabsorption, directly increasing excretion of sodium chloride in approximately equivalent amount.it is official in I.P,B.P,USP [6-8].

New tablet formulation in combination of Eprosartan mesylate 600mg and Hydrochlorthiazide 25 mg is commercially available in market (Teventen® HCT) for the treatment of edema and hypertension. Literature survey shows that various analytical methods have been reported for estimation of Eprosartam mesylate and hydrochlortiazide individually and combination with other drugs [9-19].Only one HPTLC [24] method is reported for its simultaneous estimation. No refernces were found for simultaneous determination of Eprosartan mesylate and Hydrochlorthiazide in pharmaceutical preparation by using RP-HPLC.In this work we introduce an simple, fast, isocratic RP-HPLC method for simultaneous determination of this combination in tablet formulation.The proposed method was developed, optimized, and validated according to International conference on Hormonization (ICH) guidelines [25].

Materials and Methods

HPLC grade acetonitrile, methanol and water were procured from E.Merck, Mumbai,India. Ortho phosphoricacid, potassium dihydrogen ortho phoshate AR grade were procured from Qualigens Fine Chemicals, Mumbai. A standard bulk drug sample hydrochlorthiazide was provided by Madras pharmaceuticals, Chennai and of Eposartan Mesylate was kindly supplied by Dishmann Pharmaceuticals Ltd, Ahamedabad, India. Teventen® HCT containing 600 mg of Eprosartan mesylate and 25 mg of Hydrochlorthiazide (Solvay pharmaceuticals, Mumbai, India) was purchased from the local market.

Instruments and method

The HPLC system consisted of a separation module (Water Alliance 2695) and Photo Diode Array (PDA) detector from waters (Water's Corporation, (USA), Rheodyne injector with 20µl loop volume. Waters Empower software was applied for data collecting and processing. An isocratic elution was performed on a Hypersil BDS C_{18} column (150 mm x 4.6 mm, 5µ)

A mixture of acetonitrile, methanol and 0.01 M potassium di hydrogen orthophosphate buffer (50:40:10 v/v) was used as a mobile phase and pH 4 adjusted by using ortho phosphoric acid. It was filtered through 0.45µ membrane filter and degassed. Injection volume was

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20µl and run time was 10min and flow rate was 1.0ml mL/min. The column was maintained ambient temperature and the eluents were detected at 270nm.Quantification was achieved by peak-area-ratio method with reference to the standards.

Preparation of standard stock solutions and linearity solutions

The standard stock solutions of EPR and HCT (1 mg/mL) were prepared separately by dissolving 50 mg of each drug in 50 ml of methanol. Several aliquots of these standard stock solutions were taken in different 10 ml volumetric flask and diluted up to the mark with mobile phase such that the final linearity concentrations of EPR and HCT were 216-576µg/mL and 9-24µg/mL, respectively. The solutions were filtered through a 0.45µm membrane filter before injection in to the column.

Preparation of sample solution

Twenty tablets of each containing 600mg of EPR and 25mg of HCT were weighed and powdered. An amount of powder equivalent to 600mg of EPR and 25mg of HCT transferred to a 50mL of volumetric flask and extracted with a mixture of methanol and water (50:50). The mixture was sonicated for 20 min in an ultrasonic bath. The volume was adjusted with same solvent mixture and then filtered. From this solution, 1.5mL was pipetted and the volume was made up to 50mL, with mobile phase to get the concentration 360µg/mL of EPRand 15µg/mLof HCT. The solution was filtered through a 0.45µm membrane filter before injection in to the column.

Optimization of the method

The goal of this study was to develop a single isocratic phase HPLC method for the simultaneous determination of Eprosartan mesylate and hydrochlorthiazide. During optimizing the method some important parameters like pH of the mobile phase, concentration of the acid or buffer solution, percentage and type of the organic modifier, etc., were tested for a good chromatographic separation [26,27]. Trials showed that an acidic phase with reverse phase a Purospher BDS C18 column gives symmetric and sharp peaks. For this reason, 0.01 Mpotassium di hydrogen orthophosphate solution was preferred as an acidic buffer .Methanol and acetonitrile were chosen as organic buffer because it dissolves drugs very well. Mobile phase composition of acetonitrile, methanol and 0.01M potassium di hydrogen phosphate buffer (50:40:10 v/v/v) at flow rate of 1.0mL/min showed good resolution. When ortho phosphoric acid was used as modifier, resolution between EPR and HCT was much better than pH 4.0, with decrase in peak tailing. Retention time of the drugs obtained under these conditions were 3.56and 4.65 min for EPR and HCT respectively. For the quantitative analytical purposes the wave length was set at 270nm.The typical chromatogram of the standard and sample were shown in Figure 2 and Figure 3.

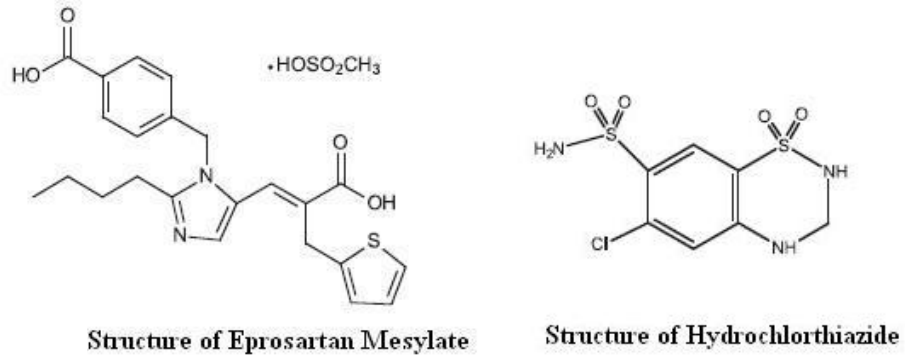

Figure 1: Structure of Eprosartan and Hydrochlorthiazide.

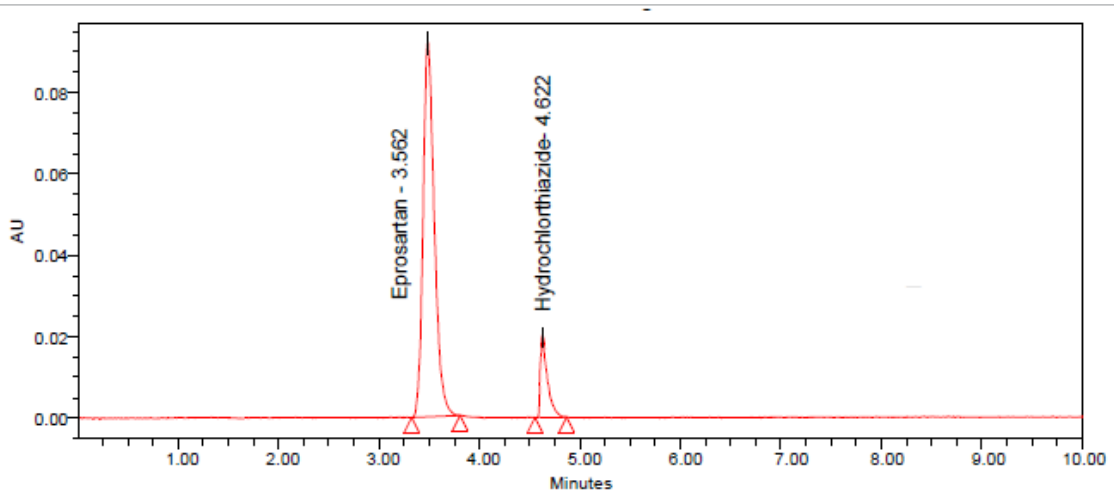

Figure 2: Chromatogram of standard.

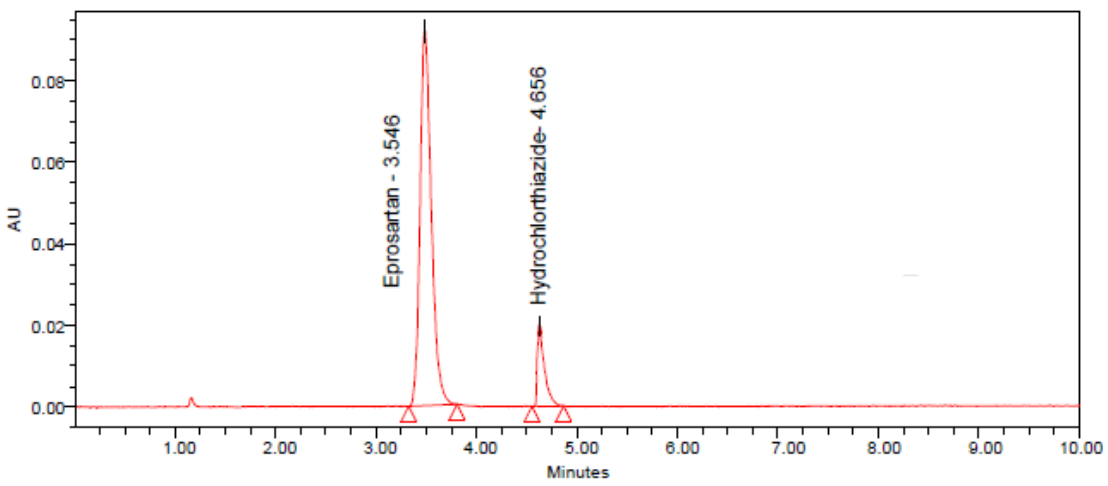

Figure 3: Chromatogram of sample.

S.NO	Parameters	Eprosartan Mesylate	Hydrochlorthiazide	Acceptance criteria
1	Retention time	3.56	4.65	
2	RSD of replicate injections	0.145	0.324	Not more than 2%
3	Asymmetric factor	0.76	0.52	Not more than 2
4	Theoretical plates	5678	4590	Not more than 3000
5	Resolution factor		3.86	More than 2

Table1: system suitability.

Method validation

The chromatographic conditions were validated by evaluating linearity, recovery, method and system precision, accuracy, system suitability, solution stability, limit of detection (LOD), Limit of Quantification (LOQ), robustness, ruggedness studies in accordance with ICH guideline Q2(R1).

System suitability: The column efficiency, resolution and peak symmetry were calculated for the standard solutions (Table 1). The values obtained demonstrated the suitability of the system for the analysis of this drug combination and the system suitability parameters fall with in± 2% standard deviation range during performance of the method. Here asymmetric factor for peaks of EPR and HCT was less than 2% and resolution was satisfactory. The peaks obtained for EPR and HCT were sharp and have clear base line separation.

Solution stability: In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 hr at room temperature. The results indicated that both the solutions, retention time and peak area of EPR and HCT did not show much variation (%RSD less than 2.0). There was no significant degradation with in the indicated period. Hence, it was concluded that both the solutions were stable for 24 hr at room temperature.

Linearity study

The peak areas of EPR and HCT were linear with respect to the concentrations over the range of 216-576µg/mL and 9-24µg/mL respectively. The slope and intercept value for calibration curve Y =15665X+20062 (R^2= 0.999) for EPR and 4259.4X—185.5(R^2= 0.999) for HCT. The results showed that excellent correlation exists between peak area and concentration of the drugs with in the concentration range indicated previously. The data was analyzed by "linear regression least squares fit", and the parameters are listed in Table 2.

Limit of detection and Limit of quantification

The linearity for EPR was performed from216-576µg/mL and that for HCT from 9-24µg/mL. Lineaity graph was plotted and the correlation coefficient (R^2) determined. The limit if detection (LOD) was calculated from the linearity curve using the formula

LOD= 3.3X {Residual Standard deviation/Slope}.

The LOD for EPR was confirmed to be 3µg/mL and for HCT it was confirmed to be0.5µg/mL.

The Limit of quantification (LOQ) was calculated from the linearity curve using the formula.

LOQ= 10X {Residual Standard deviation/Slope}

The LOQ for EPR was confirmed to be10µg/mL and for HCT it was confirmed to be2µg/mL.

Accuracy and precision: The accuracy of the method was determined by recovery experiments. It was confirmed by studying the recovery at three different concentrations, 75%, 100%, and 125% of those expected by spiking a previously analyzed test solution with additional drug standard solutions, the analysis being done in replicate. The % RSD and % relative error in all cases were within the acceptable limit (≤ 2%). It is evident from the results of accuracy study, reported

Drug	Range µg/mL	Slope	Intercept	R^2	LOD µg/mL	LOQ µg/mL
Eprosartan Mesylate	216-576	15665	+20062	0.999	3	10
Hydrochlorthiazide	9-24	4259.4	—185.5	0.999	0.5	2

Table 2: Linearity study.

in Table 3 that the proposed method enables very accurate quantitative simultaneous estimation of EPR and HCT.

Precision of this method was determined by injecting the standard solution of the three analytes six times. The R.S.D of the peak area of six replicates was found to be less than 1.0%. Intermediate precision of the method was also evaluated by analyzing five samples of the three analytes at different days (6 days). Results which are represented in Table 4 shows good intermediate precision of the method (average percentage of EPR for the 6 days is 101.5% with R.S.D of 0.7%,while it is 99.3%for HCT with a R.S.D of 0.62%. From the data obtained, the developed RP-HPLC method was found to be precise.

Ruggedness and robustness

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC (LC 10 AT), Water Alliance 2695 by different operators using different columns. Robustness of the method was determined by subjecting the method to slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP- HPLC method developed is rugged and robust.

Selectivity

Selectivity of the current method was demonstrated by good separation of the two active ingredients (Eprosatan mesylate and Hydrochlorthiazide). It was checked for the interference of impurities in the analysis of a blank solution (without any sample) and then a drug solution of 20µg/ml was injected into the column, under optimized chromatographic conditions, (Figure 4) to demonstrate the separation of both EPR and HCT from any of the impurities, if present. Further

Amount(%) ofdrug added	Theoretical content(µg/ml)	Conc.found (µg/ml)±SD*	Recovery (%)	SEM	RE(%)	RSD (%)
EPROSARTAN MESYLATE						
0	360	361.12±0.382	100.30	0.224	0.71	1.54
80	648	647.16±0.482	99.84	0.341	0.76	1.14
100	720	721.21±0.261	100.13	0.201	0.78	0.87
120	792	791.19±0.167	99.87	0.128	1.31	0.56
HYDROCHLORTHIAZIDE						
0	15	15.39±0.216	102.65	0.118	0.45	0.96
80	27	27.42±0.314	101.51	0.147	0.69	0.36
100	30	30.51±0.169	101.77	0.318	1.24	0.61
120	33	32.88±0.145	99.61	0.452	1.32	0.57

*SD= standard deviation(n=3),SEM= Standard Error of Mean,*RSD=SD/Mean×100, RE(%)=%Relative Error =(Mean assayed concentration-Added Concentration/ Added Conentration×100)

Table 3: Accuracy of the Method.

Day	Eprosartan Mesylate	Hydrochlorthiazide
1	102.1±0.4	99.4±0.8
2	101.1±1.3	99.9±1.8
3	101.2±0.9	99.0±0.6
4	100.5±0.6	99.1±1.2
5	101.4±1.5	99.2±1.0
6	101.1±1.1	99.1±1.5

Table 4: Intermediate precision of the method (% of the three active ingredients during 6 days).

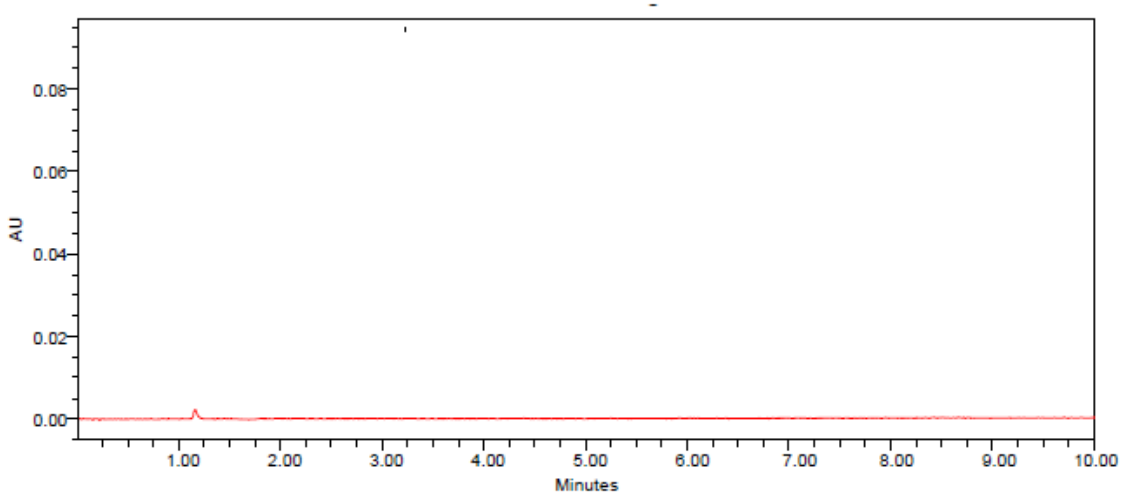

Figure 4: Chromatogram of placebo.

Drug	Labelled Amount (mg)	Amount of mg/ Tablet found*	% of Label claim	%RSD
Eprosartan Mesylate	600	599.6	99.93	0.421
Hydrochlorthiazide	15	15.12	100.6	0.356

*Average of six determinations

Table 5: Analysis of Formulation.

more, matrix components, e.g. excipients, do not interfere with the two analytes as they have no absorbance.

Tablet studies

The proposed method was successfully applied to the analysis of marketed products and the results obtained are given in Table 5.

Conclusion

The method represents a fast analytical procedure for the simultaneous quantitation of Eprosartan mesyate and hydrochlorthiazide. The sample preparation is simple, the analysis time is short and the elution is isocratic. The method is amenable to the large number of samples with excellent precision and accuracy.

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