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STABILITY INDICATING RP-HPLC METHOD FOR DETERMINATION OF EPROSARTAN IN PURE AND PHARMACEUTICAL FORMULATION

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Abstract

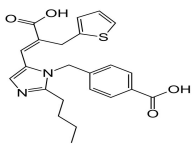
A simple, sensitive and specific RP-HPLC method was developed for the determination of Eprosartan in pure and tablet forms. The method showed a linear response for concentrations in the range of 20-120 µg/mL using Methanol: Acetonitrile: Buffer solution (Dissolve 0.02 M potassium di-hydrogen orthophosphate in water. Adjust pH of solution to 6.85 with orthophosphoric acid) in the ratio (45:35:20) as the mobile phase with detection at 232 nm using photodiode array (PDA) detector and a flow rate of 1 mL/min and retention time 7.1 min. The value of correlation coefficient, slope and intercept were, 0.9998, 1661.8 and 114.82, respectively. The method was validated as per ICH guidelines for precision, recovery, ruggedness and robustness. The specificity of the method was investigated under different stress conditions including acidic, basic, photochemical and thermal as recommended by ICH guidelines. The drug undergoes degradation under acidic, basic, photochemical and thermal degradation conditions. All the peaks of degraded product were resolved from the active pharmaceutical ingredient with significantly different retention time. As the method could effectively separate the drug from its degradation product, it can be employed as a stability-indicating one.

Keywords*:* Eprosartan, RP-HPLC, Degradation studies.

1. Introduction

Stability indicating methods have become an important aspect of any analytical method validation and a part of US FDA requirements[1]. Chemically, Eprosartan is designated as 4-({2-butyl-5-[2-carboxy-2-(thiophen-2-ylmethyl) eth-1-en-1-yl]-1*H*-imidazol-1-yl} methyl) benzoic acid (Fig. 1) is a new antihypertensive drug, the drug acts on the renin-angiotensin system in two ways to decrease total peripheral resistance. First, it blocks the binding of angiotensin II to AT_1 receptors in vascular smooth muscle, causing vascular dilatation. Second, it inhibits sympathetic norepinephrine production, further reducing blood pressure[2,3]. Very few methods appeared in the literature in the spectrophotometric determination of Eprosartan[4,5,6]. Therefore, we have made an attempt to develop a new, simple, accurate stability indicating RP-HPLC method for the determination of Eprosartan in pure and tablet forms. The proposed method was validated as per ICH guidelines Q2A[7].

Fig.1 chemical structure of Eprosartan.



2. Experimental

2.1 Chemicals and Instrumentation: Eprosartan was supplied by ABS LIFE SCIENCE LTD. and tablets (Label Claim: 400 mg per tablet, Product Name: EPROZAR and Manufacturer: INTAS Pharmaceuticals was procured from the market. Methanol, Acetonitrile and Potassium dihydrogen orthophosphate AR Grade, Orthophosphoric acid LR Grade were purchased from RFCL Ltd., New Delhi, India. High purity water was prepared by using Millipore Milli-Q plus water purification system.

The HPLC used was a shimazdu HPLC LC-20AT series with SPD-20A UV photodiode array detector and LCsolution software, Japan was used for all the experiments. The column used was XTerra® RP18, 250 x 4.6 mm, 5 µ (water, Ireland) and Luna C_8 (Octylsilane), 250 x 4.6 mm, 5 µ (Phenomenax, USA). Thermal Stability studies were performed in a dry air oven (Thermo labs, India). Micrositer syringer- 50 µL (Hamilton Company, USA).

2.2 Chromatographic conditions: Chromatographic separation was achieved at ambient temperature on a reversed phase column using a mobile-phase consisting of a mixture of Methanol: Acetonitrile: Buffer solution (Dissolve 0.02 M potassium di-hydrogen orthophosphate in water. Adjust pH of solution to 6.85 ± 0.05 with orthophosphoric acid) in the ratio (45:45:10). The mobile phase so prepared was filtered through 0.22 µm nylon membrane filter and degassed by sonication. Flow rate of 1 mL / min was maintained. Detection was carried out at 232 nm. The injection volume was 20 µL for assay and degradation level.

2.3 Standard preparation: 100 mg of Eprosartan working standard was accurately weighed and transferred to a 100 mL volumetric flask. Solution was sonicated and diluted up to the mark with mobile phase. A series of standard solutions in the concentration range of 20, 40, 60, 80, 100 and

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120µg/ml were prepared followed by a suitable dilution of stock solution with the mobile phase.

2.4 Sample preparation: 20 tablets were weighed and finely powdered. Blend equivalent to 50 mg of Eprosartan was transferred to a 100 mL volumetric flask. About 60 mL of mobile phase was added and the solution was sonicated for 15 min and make up to the mark with mobile phase. The resulting solution was filtered through 0.22µm nylon membrane filter. The solution was mixed well and centrifuged at 2500 RPM for 10 min.

2.5 Preparation of calibration graph: The linearity of response for Eprosartan assay method was determined by preparing and injecting solutions with concentrations of about 2, 4, 6, 8, 10 and 12µg/ml of Eprosartan.

2.6 Method validation:

2.6.1 Precision: Precision was measured in terms of repeatability of application and measurement. Repeatability of standard application was carried out using six replicates of the same standard concentration (6 µg / mL for standard application). Repeatability of sample measurement was carried out in six different sample preparations from same homogenous blend of marketed sample (6 µg /mL for sample application). It showed very low % relative standard deviation (% RSD) of peak area of Eprosartan.

2.6.2 Accuracy: Accuracy (Recovery) study was performed by spiking 30, 50 and 70% of Eprosartan working standard to a preanalysed sample. The preanalysed sample was weighed in such a way that final concentration is half or 50% of the sample preparation before spiking. The percentage sum level of preanalysed sample and spiked amount of drug should be 80, 100 and 120% of simulated dosages nominal or target concentration of sample preparation. The accuracy of the analytical method was established in duplicate across its range according to the assay procedure.

$$\% \text{ Recovery} = \frac{\%Amount\,\text{Recovered}}{\%SumLevel} \times 100$$

2.6.3 Ruggedness and robustness: Method robustness and ruggedness was determined by analysing same sample at normal operating conditions and also by changing some operating analytical conditions such as column make, mobile phase composition, flow rate, instrument and analyst. The robustness and ruggedness of the method was established as the % deviation from mean assay value obtain from precision study is less than ±2.0%.

2.7 Analysis of marketed formulation: 20 tablets were weighed and finely powdered. Transfer blend equivalent to 100 mg of Eprosartan to a 100 mL volumetric flask. Add about 60 mL of mobile phase and sonicate for 15 min and make up volume with mobile phase. Mix well and centrifuge the solution at 2500 RPM for 10 min. Dilute the solution up to the desired concentration and inject it into the HPLC system.

2.8 Forced Degradation Studies:

2.8.1 Preparation of acid and based- induced degradation product: Tablet powder equivalent to 100 mg of Eprosartan was transferred to 100 mL volumetric flask. To it, 10 mL of mobile phase was added and sonicated for 15 min with intermittent shaking. To it 5 mL of 1 N HCl was added and 5 mL of 1 N NaOH were added separately. The sample was heated on a boiling water bath for 30 min, cooled to room temperature and diluted to volume with mobile phase, mixed well. The acidic forced degradation and the alkaline forced degradation was performed in dark in order to exclude the possible degradative effect of light. This solution was centrifuged at 2500 rpm for 10 min and 5 mL of supernatant liquid was transferred to 25 mL volumetric flask, diluted to volume with mobile phase, mixed well and injected into the HPLC system.

2.8.2 Preparation of hydrogen peroxide - induced degradation product: Tablet powder equivalent to 100 mg of Eprosartan was transferred to 100 mL volumetric flask. To it, 10 mL of mobile phase was added and sonicated for 15 min with intermittent shaking. To it 5 mL of 3.0% H_2O_2 was added. The sample was heated on a boiling water bath for 30 min, cooled to room temperature and diluted to volume with mobile phase, mixed well. This solution was centrifuged at 2500 rpm for 10 min and 5 mL of supernatant liquid was transferred to 25 mL volumetric flask, diluted to volume with mobile phase, mixed well and injected into the HPLC system.

2.8.3 Photo-degradation product: Tablet powder equivalent to 100 mg of Eprosartan (previously kept in UV light for 24 hr) was transferred to 100 mL volumetric flask. To it, 10 mL of mobile phase was added and sonicated for 15 min with intermittent shaking and diluted up to the mark with mobile phase. This solution was centrifuged at 2500 rpm for 10 min and 5 mL of supernatant liquid was transferred to 25 mL volumetric flask, diluted to volume with mobile phase, mixed well and injected into the HPLC system.

2.8.4 Thermal degradation product: Tablet powder equivalent to 100 mg of Eprosartan was transferred to 100 mL volumetric flask. To it, 10 mL of mobile phase was added and sonicated for 15 min with intermittent shaking. The sample was heated on a boiling water bath for 30 min, cooled to room temperature and diluted to volume with mobile phase, mixed well. This solution was centrifuged at 2500 rpm for 10 min and 5 mL of supernatant liquid was transferred to 25 mL volumetric flask, diluted to volume with mobile phase, mixed well and injected into the HPLC. The specificity degradation study data for the determination of Eprosartan and its degradants in pharmaceutical dosage form is given in Table 5 & Table 6. The no stress treatment sample (as control) has been evaluated relative to the standard concentration where as rest of the stressed condition samples (Figs. 2-8) is evaluated relative to the control sample with respect to the % assay and %

degradation. The percentage degradation results are calculated by area normalization method.

Fig. 2 Chromatogram of standard Eprosartan.

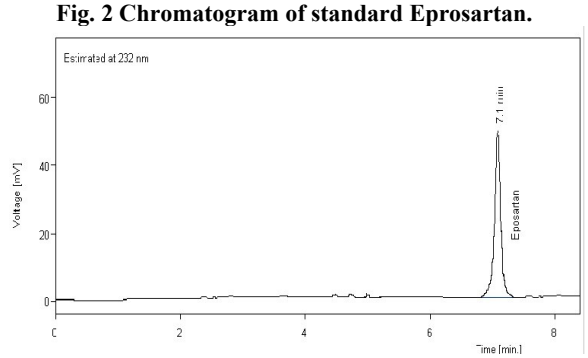


Fig. 3 Chromatogram of test Eprosartan.

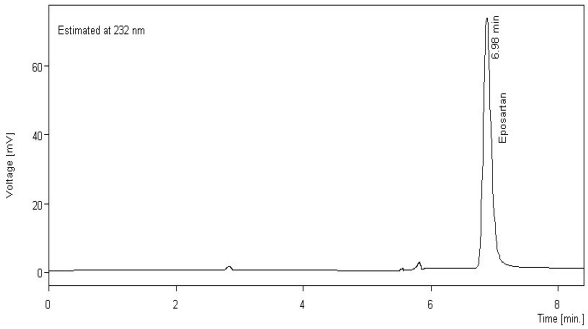


Fig. 4 Chromatogram of acid degraded sample.

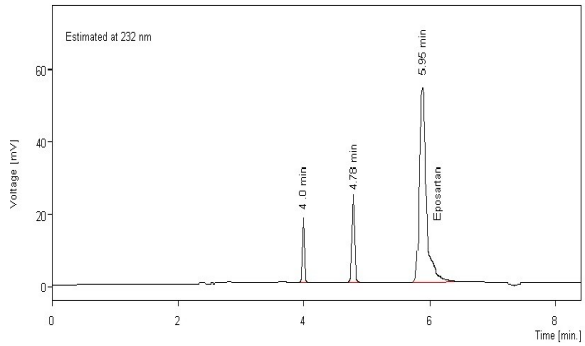


Fig.5 Chromatogram of alkali degraded sample.

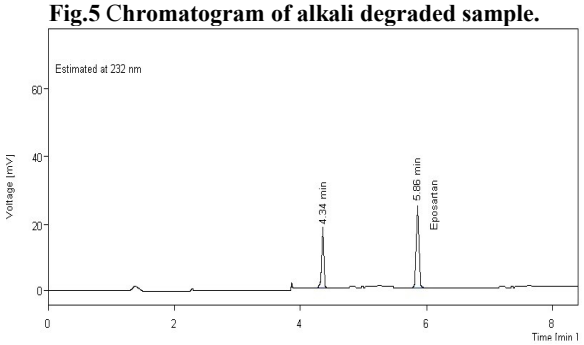


Fig. 6 Chromatogram of Hydrogen Peroxide degraded sample.

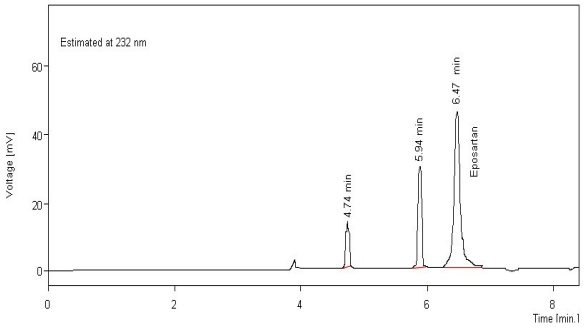


Fig. 7 Chromatogram of UV degraded sample.

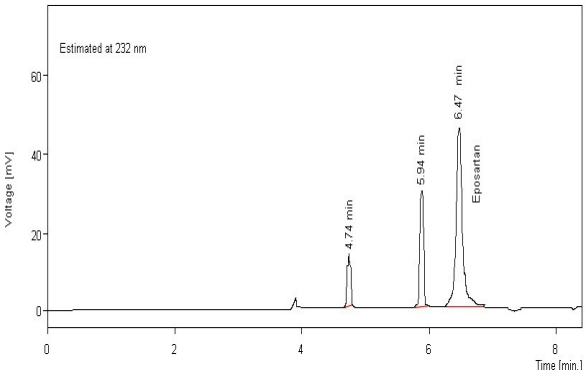
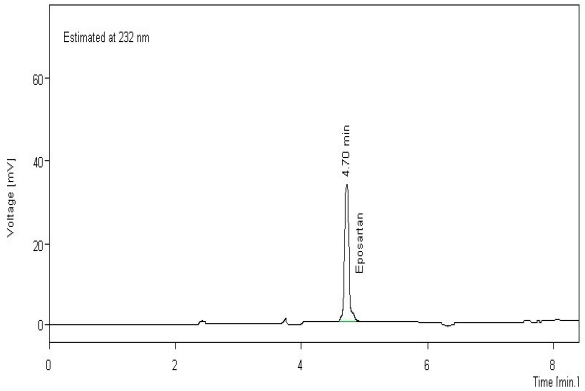


Fig. 8 Chromatogram of Thermal degraded sample.



2.9 Detection of the related impurities: Weigh and finely powder not less than 20 tablets. Transfer blend equivalent to 50 mg of Eprosartan to a 50 mL volumetric flask. Add about 30 mL of mobile phase and sonicate for 20 min and make up volume with mobile phase. Mix well and centrifuge the solution at 2500 rpm for 10 min. The clear supernatant solution was injected into the HPLC system.

3. Results and discussion

3.1 Method of development: The chromatographic conditions were optimized with a view to develop a stability- indicating assay method. Two different columns were tried as under chromatographic conditions namely, XTerra® RP18, 250 x 4.6 mm, 5 µ (water, Ireland) and Luna C_8 (Octylsilane), 250 x 4.6 mm, 5 µ (Phenomenax, USA). Luna C8 gave good peak shape but a lower retention with low peak purity. XTerra® RP18 column had given a good peak shape with response at affordable retention time with peak purity of Eprosartan on higher side. The chromatographic conditions finally comprised of a mobile-phase in the ratio of Methanol: Acetonitrile: Buffer solution (Dissolve 0.02 M potassium dihydrogen orthophosphate in water. Adjust pH of solution to 6.85 ± 0.05 with ortho-phosphoric acid) in the ratio (45:45:10) at a flow rate of 1 mL / min using XTerra® RP18 column; 250 x 4.6 mm; 5 µ (G. L. Sciences, Japan) at 232 nm.

3.2 Calibration curve: These results indicate that the response is linear over the range of 20, 40, 60, 80, 100 and 120µg/ml of Eprosartan with coefficient of regression, R^2, value as 0.9998 as shown in Table 1. The value of correlation coefficient, slope and

intercept were 0.9998, 1661.8, and 114.82 respectively.

3.3 Method validation :

3.3.1 Precision: The %RSD for repeatability of sample preparation is 0.95%. This shows that precision of the method is satisfactory as % relative standard deviation is not more than ± 2.0%. Table 2 represent the precision of method.

3.3.2 Ruggedness and Robustness: Method robustness and ruggedness was determined by analysing same sample at normal operating conditions and also by changing some operating analytical conditions such as column make, mobile phase composition, flow rate, instrument and analyst. The deliberate aforementioned changes in parameters alter the result of Eprosartan 0.03% to method precision study, which is not a significant change. The robustness and ruggedness of the method is established as the % deviation from mean assay value obtain from precision study is less than ±2.0%. Table 3 represent the ruggedness and robustness of the method.

3.3.3 Accuracy: The accuracy of the method was established by recovery studies. Results indicate that the individual recovery of Eprosartan ranges from 100.41% to 101.54% with mean recovery of 100.75% and % relative standard deviation of 0.41%. The recovery of Eprosartan by proposed method is satisfactory as % relative standard deviation is not more than ± 2.0% and mean recovery between 98.0 - 102.0%. Table 4 represent the accuracy of method.

3.4 Analysis of the marketed formulation: The drug content was found to be 98.02% with a % RSD of 0.95%. It was noted that no degradation of Eprosartan had occurred in the marketed formulation that were analysed by this method. The low RSD value indicated the suitability of this method for routine analysis of Eprosartan in pharmaceutical dosage form.

3.5 Stability- Indicating property: The stress studies were conducted and the data were depicted in (Table 5). The chromatogram of no stress treatment of control and sample showed no additional peaks (Figs. 2,3). The chromatogram of acid degraded sample showed additional peaks at retention time (RT) of 4.00, 4.78 and 5.95 min, respectively Fig.4. The chromatogram of alkali degraded sample showed additional peaks at RT of 4.34 min and 5.86 min, respectively Fig. 5. The chromatogram of hydrogen peroxide degraded sample showed additional peaks at RT of 3.32, 4.83 and 5.96 min respectively Fig. 6. The chromatogram of UV degraded sample showed additional peaks at RT of 4.74, 5.94 and 6.47 min, respectively Fig. 7. The chromatogram of thermal degraded sample showed additional peak at RT of 4.7 min Fig. 8. Rest of the peaks, if any, were from its blank or placebo in each of these specified conditions. In each forced degradation samples where additional peaks were observed, the response of the drug was changing from the initial control sample. This indicates that the drug is susceptible to acid-base hydrolysis degradation, hydrogen peroxide degradation, UV degradation and thermal degradation. The lower RT of the degraded component indicated that they were more polar than the analyte itself.

3.6 Detection of the related impurities: The sample solution showed no additional peak other than principal peak. Hence, related impurities are not present in the market sample.

Table 1 : Regression characteristics of the proposed HPLC method

S. No	Drug	Eprosartan
1	Range (µg/ml)	20-120µg/ml
2	Detection wavelength (l max)	232 nm
3	Mean 'R²' value	0.9998
4	Slope (m)	1661.8
5	Intercept (c)	114.82
6	Run time	10 min
7	Retention Time (min)	7.1
8	Theoretical Plates (N)	3276
9	Tailing Factor	1.02

Table 2 : Method precision of Eprosartan

Sample Preparation	% Assay Eprosartan	% Deviation From Mean Assay value Eprosartan
1	99.79	1.77
2	97.86	-0.16
3	98.04	0.02
4	97.26	-0.76
5	97.26	-0.76
6	97.92	-0.10
Mean ±SD	98.02 ±0.93	
%RSD	0.95	

Table 3 : Ruggedness and robustness of Eprosartan

Parameter	Normal (Original)	Changed conditions
Column make	X Terra® RP18 column; 250 x 4.6 mm; 5 µ	Luna C8 (Octylsilane), 250 x 4.6 mm; 5 µ
Flow rate	1 mL/min	1.2 mL/min
Mobile phase Composition	(pH 6.85) Methanol: Acetonitrile: Buffer (KH_2PO_4) (45:35:20)	(pH 6.50) Methanol: Acetonitrile: Buffer (KH_2PO_4) (40:40:20)
Pump	Jasco PU-2080 plus series	Shimazdu LC-20AT
Detector	Jasco UV-2075 plus series	Shimazdu UV-VIS detector
% assay of Eprosartan	98.02%	98.05%

Table 4 : Recovery studies of Eprosartan

Sample preparation	% simulated dosage normal	% sum level	% amount recovered	% recovery
Preanalysed sample				99.02
1	80	80.46	81.18	100.90
2	80	79.98	81.19	101.52
1	100	100.90	101.52	100.61
2	100	101.08	101.50	100.41
1	120	121.24	121.85	100.50
2	120	121.04	121.70	100.54
Mean				100.75
± Standard deviation				0.41
% Relative standard deviation				0.41

Table 5 : Stressed Study Data of Eprosartan.

S. No	Condition	% assay Eprosartan	Retention time of drug	% Degradation
1	No stress treatment	98.02	6.984	Nil
2	Acid	99.19	4, 4.78, 5.95	0.09
3	Alkali	99.77	4.34, 5.86	0.07
4	H_20_2	98.32	3.32, 4.83, 5.96	0.05
5	UV	99.22	4.74, 5.94, 6.47	0.49
6	Thermal	99.07	4.70	0.03

Table 6 : Summaries of Forced Degradation Results:

S. No	Stress condition	Time	% Assay of active substance	Mass balance (%assay + % degradation products)	Remarks
1	Acid degradation (1 N HCl)	1/2 hr	99.76	99.4	No degradation products formed
2	Alkali degradation (1 N NaOH)	1/2 hr	99.88	99.3	No degradation products formed
3	H_2O_2 degradation (3%)	1/2 hr	97.64	98.1	Mild degradation formed
4	UV degradation	24 hr	99.44	99.2	No degradation products formed
5	Thermal degradation (60 ^{0}C)	1/2 hr	99.45	99.5	No degradation products formed

4. Conclusions

The developed HPLC technique is precise, specific, accurate and stability indicating. Statistical analysis proves that the method is reproducible and selective for the analysis of Eprosartan in pharmaceutical dosage form. The method can be used to determine the purity of the drug available from various sources. As the method separates the drug from its degradation products, it can be employed as a stability indicating one.

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