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Original Article

METHOD DEVELOPMENT AND VALIDATION OF EPROSARTAN MESYLATE AND ITS IMPURITIES USING REVERSE PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Objective: Our main objective is to develop an accurate and precise RP-HPLC method for the determination of Eprosartan Mesylate and its impurities.

Methods: A Develosil ODS UG-5; (150 × 4.6) mm; 5 µm column was used for the Separation of drugs by a mobile phase consisting of Buffer and Acetonitrile mixture in the gradient proportion. The flow rate maintained was 0.8 ml/min and the wavelength used for detection was 235 nm.

Results: The linearity was observed in the range of 0.025-50µg/ml of spiked impurities in Eprosartan Mesylate, impurity 1 and impurity 2 with a correlation coefficient of 0.99927, 0.99910 and 0.99934 respectively. The mean percentage recoveries for LOQ, 50%, 80%, 100%, 150% and 200% accuracy were found to be 101.5±1.51, 107.0±1.7, 104.6±0.4, 102.8±0.36, 101.7±0.26 and 101.3±0.15 respectively for impurities in Eprosartan Mesylate, impurity 1 and impurity 2. Linearity, accuracy, precision and robustness parameters for the suggested method were estimated for validation.

Conclusion: The developed method is uncomplicated, accurate, sensitive and precise for the determination of related substances in the Eprosartan Mesylate. The satisfying % recoveries and low % RSD Values confirmed the suitability of the developed method for the usual analysis of Eprosartan mesylate in pharmaceuticals.

Keywords: Eprasartan impurities, HPLC, Method Development, Validation, Forced degradation studies

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INTRODUCTION

Eprosartan Mesylate (EM) Antagonizes the effect of angiotensin II (vasoconstriction and aldosterone secretion) by blocking the angiotensin II receptor (AT 1 receptor) in vascular smooth muscle and the adrenal gland, producing decreased Blood Pressure. C23H24N2O4S · CH4O3S and 520.62g/Mol are chemical formula and molecular weight of EM. The structural formula of EM is shown in fig. 1 (a). It is insoluble in water (0.00866 mg/ml) [1]. Impurity A is chemically known as alpha {2-butyl-1{{4-(methoxy carbonyl) phenyl) methyl}-1H-imidazol-5-yl} methylene}-2-thiophene propanoic acid ethyl ester. Impurity B is chemically known as 2-Carbethoxy-3-(2-thienyl) sodium propionate [2-4].

The development and validation of an analytical method is to ensure a specific, accurate and precise method for a particular analyte. The principal objective for that is to enhance the conditions and parameters, which should be observed in the evolution and establishment. From the literature review, it was found that there are few methods for the estimation of EM, Impurity A and Impurity B. but many methods for individual analysis of the drugs are present [5-14]. Hence it is aimed to acquire novel methods for the estimation of EM, impurity A and Impurity B using available analytical technique HPLC. Granting to the literature survey, few analytical methods such as UV-Visible (Vis) spectrophotometry [5-8], RP-HPLC [9-12], HPTLC [13] methods were covered for the estimation of EM individually and LC-MS and LCNMR method is for degradation products of EM [14]. The aim of the suggested method is to develop a simple and accurate methods for the determination of EM and its impurities A and B using the RP-HPLC technique in tablets.

MATERIALS AND METHODS

Chemicals and reagents

EM, Impurities A and B obtained from Fortune Laboratories (India) were of analytical grade (99.9% pure). Commercial samples of EM tablets were procured from the local medical store and applied within their shelf-life period.

Acetonitrile and Water of HPLC grade and KH_2PO_4 were obtained from Rankem R. F. C. L. Limited, Haryana, SD Fine Chem. Limited, Mumbai and MARCK Specialities private limited, Mumbai respectively.

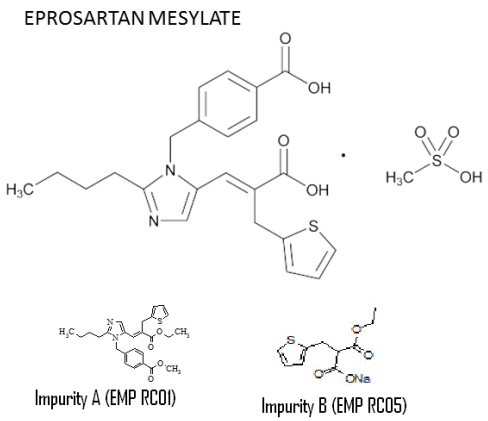

Fig. 1: Structure of eprosartan mesylate and its impurities

Chramatographic system

Quantitative HPLC was performed on PEAK chromatographic chemisorption version B.02.01 with Variable wavelength programmable UV detector VWD G1314A. Develosil ODS UG-5; (150 × 4.6) mm; packed with 5 µm particles) is utilized for the chromatographic separation.

Chromatographic conditions

Manual injections (20 µl) were applied. The column was kept at ambient temperature. The wavelength was set at 235 nm for detection. To produce a suitable RP-HPLC method for the determination of EM, different mobile phases buffer, water and acetonitrile were used in different compositions at different flow rates. Lastly, the mobile phase Buffer(mobile phase A) and acetonitrile (Mobile phase B) mixture in the gradient proportion at a flow rate of 0.8 ml/min gave peaks with good resolution for EM, impurity A and B. EM, impurity A and B got eluted at retention times 7.76, 14.03 and 18.66 min respectively with symmetric peaks. The mobile phase was degassed and then filtered through 0.25 µm Microfiltration unit before it was pumped into the RP-HPLC system. By pumping the mobile phase through the column for at least 30 min before injecting the drug solution, equilibrium in the column was achieved. 21 min were the run time. Chromatogram showing the separated drugs is shown in fig. 2.

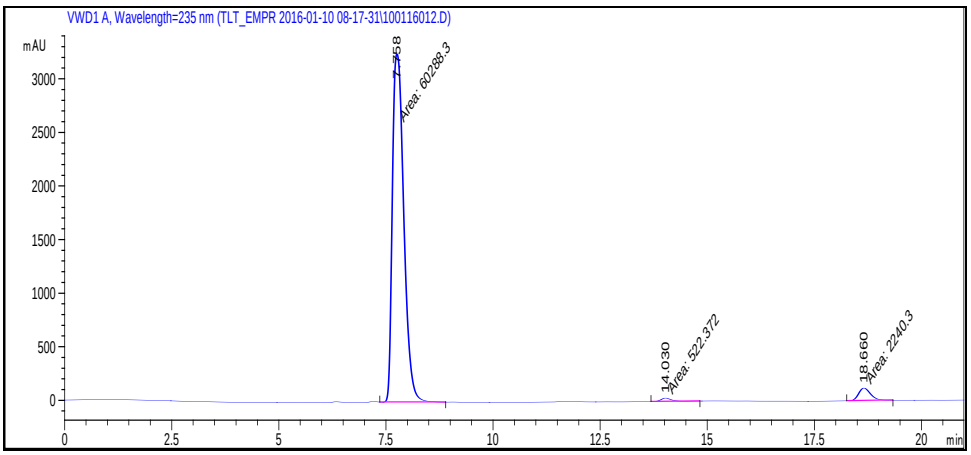

Fig. 2: Chromatogram of eprosartan mesylate and its impurity

Mobile phase preparation

Mobile phase A: 0.01M potassium dihydrogen orthophosphate was prepared and the pH was adjusted to 3. 0 with orthophosphoric acid.

Mobile phase B: Acetonitrile

Gradient elution mode was used for the development of chromatogram. In 0 minute A: B is 85:15; for 15 to 18 min, A: B is 75:25; and for 18 to 21 min A: B is 85:15.

Diluent

The mixture of Water and Acetonitrile in the ratio of 50:50v/v was prepared and used as diluents.

Impurities stock standard solutions

Each 5 mg of EMP RC01 (Impurity A) and EMP RC05 (Impurity B). impurity standards were weighed accurately and transferred into individual each 10 ml volumetric flasks. 5 ml of diluents was added into each flasks and sonicated to dissolve the mixtures. Finally, the solution was made up to the volume with diluent and mixed well

Diluted standard solution (Resolution solution)

From the impurities standard stock solution, each 2 ml of solution was transferred into 50 ml volumetric flask and made up the volume up to mark with diluent.

Sample preparation

10 mg of Eprosartan has weighed accurately about into 10 ml volumetric flasks. 5 ml of diluent

was added to the flasks and sonicated to dissolve the material. Finally, the solution was made up to the volume with diluents.

All final solutions were filtered using microfiltration unit of 0.45 µm.

Validation

The proposed method was validated for the analysis of EM, EMP RC01 and EMP RC05 using following parameters. System-suitability studies are an intact part of method development and are practiced to ensure satisfactory performance of the chromatographic system. For five replicate injections of the drugs and impurities, Number of theoretical plates (N) and tailing factor (T) were assessed. Linearity was established by plotting a graph between concentration versus peak area and the correlation coefficient was determined. A series of solutions of Eprosartan impurities with concentrations ranging from LOQ% to 200% of the target concentration (0.5%) prepared and injected into the HPLC system. To obtain proportionality, the slope and intercept of the regression line and correlation coefficient were calculated statistically from the calibration curve of the EM and its impurities. To find out variations in the test methods precision was studied for EM and its impurities of spiked test preparation with Eprosartan impurities blend solution to get 0.5% of each impurity with respect to test concentration and analyzed as per test method when analysis carried out by Analyst to Analyst, System to System and Column to Column Variation (ruggedness). The mentioned solution was injected six times and the area was measured for all six injections in HPLC. The % relative standard deviation (%RSD) and % content results were used for assessment of precision and ruggedness. The accuracy of the method was demonstrated by analyzing EM and its impurities of spiked test preparation with LOQ, 50%, 80%, 100%, 150% and 200% (0.025, 12.5, 20, 25, 40 and 50 µg/ml) of target concentration (i.e., 0.5% of each impurity) of Eprosartan impurities. After injection, recovery values for individual drugs were estimated. Specificity is the ability of a method to differentiate the analyte(s) of interest from other components in the sample. Placebo was prepared as per the marketed product formulas of drugs. Placebo interference from excipients was studied. Robustness of the method was determined by varying flow rate, and filtration. Bench top stability (25 °C and 60 % RH) and Refrigerator (8 °C and 55%RH) stability were determined on the 1st and 2nd day. Forced degradation study was conducted to demonstrate the effective separation of degradants from EM. EM was exposed to the following stress conditions such as refluxed with 0.1N HCl solution for about 2 h at 60 °C (Acid). Refluxed with 0.1N NaOH solution for about 2 h 30 min at 60 °C (Base). Treated with 1% Hydrogen peroxide (H_2O_2) for 2 h at 25 °C (Peroxide). Refluxed with purified water for about 6 h at 60 °C. (Aqueous). Exposed to Sun-Light for about 1.2 Million. Lux. Hours. Exposed to UV-Light for about 200 Watts/m2. Dry heat at 105 ° C for about 24 h. Exposed to humidity at 25 °C, 90% RH for about 7 d to induce degradation. Limit of detection and limit of quantitation were determined by signal to noise ratio. The precision of Eprosartan impurities at about Limit of

Quantitation level was conducted. Six test preparations having impurities at the concentration level of about Limit of Quantitation in the presence of placebo were prepared and injected into HPLC system.

RESULTS

Gradient reverse–phase HPLC procedure was suggested as a suitable method for the analysis of EM and known impurities. Buffer and acetonitrile mixture in the proportion of above-mentioned proportion based on time at a flow rate of 0.8 ml/min was found to be a suitable mobile phase for complete and rapid separation of analytes. 7.76, 14.03 and 18.66 min were the retention times for EM, EMP RC01 and EMP RC05 respectively.

System suitability parameters

System suitability studies for the EM, EMP RC01 and EMP RC05 reported that the % relative standard deviation values of five replicate injections of different solutions of EMP RC01 and EMP RC05 compare with EM were found to be 1.81 and 2.4. The theoretical plates for the EM, EMP RC01 and EMP RC05 were found to be 5758, 6154 and 5199 respectively. The related data were presented in table 1.

Table 1: System suitability studies data

System suitability parameters	Observed value			Acceptance criteria
	Eprosartan mesylate	Impurity A (EMP RC01)	Impurity B (EMP RC05)	
Percentage relative standard deviation	0.1	0.6	0.1	% RSD should not be more than 2.0
Theoretical plates	5758	6154	5199	Not less than 3500

Linearity

A linear calibration curve was obtained over the concentration range from 0.025 to 50 µg/ml of spiked impurities into the test and impurities solution for quantitative application purpose. The correlation coefficient for EM, EMP RC01 and EMP RC05 were 0.9999, 0.9999 and 0.9997 respectively. The calibration curve of EM, EMP RC01 and EMP RC05 was present in fig. 3. The regression equation of EM was found to be y=39765x+4764 with a coefficient of correlation 0.9999. The regression equation of EMP RCO1 was found to be y=58316x-4377 with a coefficient of correlation 0.9999, the regression equation of EMP RCO5 was found to be y=62627x-25360 with a coefficient of correlation 0.9997 where x is concentration and y is absorbance. The curve fittings of EM, EMP RCO1 and EMP RCO5 were found to be 99.99%, 99.99 and 99.97% respectively. The related linearity data of EM, EMP RCO1 and EMP RCO5 was present in table 2.

Table 2: Linearity results

Spiked samples	Eprosartan Mesylate		Impurity A (EMP RC01)		Impurity B (EMP RC05)	
	Concentration	Response	Concentration	Response	Concentration	Response
	0.02	823	0.03	1812	0.03	1916
	12.4	504749	12.422	741508	12.502	753741
	19.84	794211	19.876	1167607	20.004	1222085
	24.8	992360	24.845	1441049	25.005	1515836
	37.199	1478074	37.267	2171077	37.507	2295992
	49.599	1978813	49.69	2908244	50.01	3142757
Slope	39765		58316		62627	
intercept	4764		-4377		-25360	
Correlation coefficient	0.9999		0.9999		0.9997	

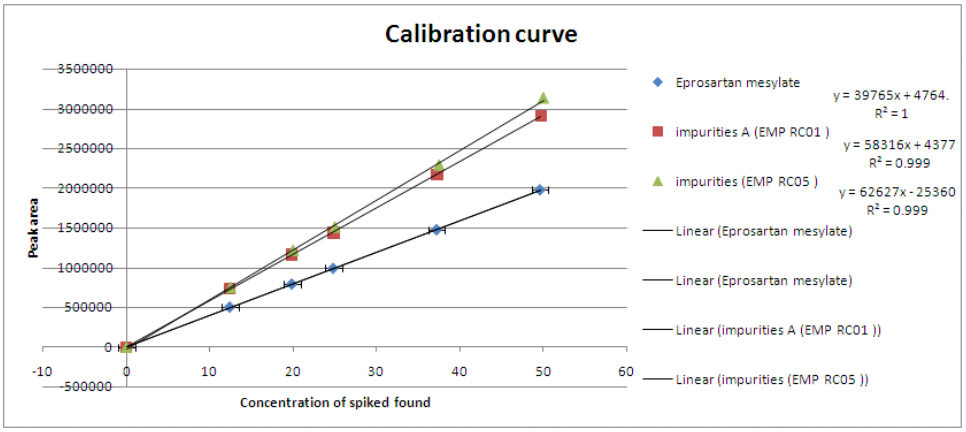

Fig. 3: Calibration curve of eprosartan mesylate and its impurities

Precision

The drugs got eluted giving single symmetrical peaks, well removed from the solvent front. The % relative standard deviation (%RSD) of the peak areas for five injections of the standard solution of EM, EMP RC01 and EMP RC05 was used for determination of the precision of the HPLC system. %RSD for the EM, EMP RC01 and EMP RC05 were found to be 1.3%, 0.9% and 1.2% respectively for method precison and 0.1%, 0.1% and 0.1% respectively for system precision. The %RSD of drugs and impurities under this method was not more than 2.

Accuracy studies

Recovery studies were carried out by analyzing mixtures of spikied test preparation with LOQ, 50%, 80%, 100%, 150% and 200% of target concentration (i.e., 0.5% of each impurity) of Eprosartan

impurities. The recoveries of EM, EMP RC01 and EMP RC05 were evaluated. The mean percentage recoveries for LOQ, 50%, 80%, 100%, 150% and 200% accuracy were found to be 101.5%±1.5, 107%±1.6, 104.6%±0.4, 102.8%±0.4, 101.7%±0.3 and 101.3%±0.2 respectively for EM; 103.9%±1.2, 96%±1.4, 96.2%±0.7, 97.5%±0.2, 98.1%±0.3 and 97.8%±0.2 respectively for the EMP RC01; and 98.6%±1.1, 103%±2.9, 99%±2.0, 96.8%±0.8, 96.2%±1.3 and 101.7%±0.6 respectively for the EMP RC05. The results of percentage recovery data were within the limit.

Limit of detection and limit of quantitation

Limit of Detection and Limit of Quantitation were established based on the signal to noise ratio. Limit of detection by signal to noise ratio of EM, EMP RC01 and EMP RC05 were found to be 3.5, 3.6 and 3.0 respectively. Limit of quantitation by signal to noise ratio of EM, EMP RC01 and EMP RC05 were found to be be 9.8, 10.1 and 9.3 respectively. % recovery of LOQ level of EM, EMP RC01 and EMP RC05 were found to be 102.8%±2.1, 103.9%±1.31, and 99.5%±1.53 respectively. %RSD value of LOQ level of EM, EMP RC01 and EMP RC05 were found to be 2.1, 1.3 and 1.5 respectively.

Ruggedness

Ruggedness for EM, EMP RC01 and EMP RC05 determined by varying analysts, system and column carrying out the procedure. Totally 2 analysts, systems and columns carried out the procedure and the results were within the limits.

Robustness

Robustness of the method was determined by varying flow rate and filter. The optimized method flow rate was 0.8 ml/min and robustness was varied to 0.7 ml/min and 0.9 ml/min. The optimized filtration is 0.45 µm PVDF and it was varied to unfiltered and 0.45 µm Nylon. The variation in flow rate and filter membrane for filtration was not shown any deviation from the true value and %RSD of all variations were within the limit.

Stability studies

Stability studies (Refrigerator stability and Benchtop stability) have reported the percentage deviation from the true value within the limit for EM, EMP RC01 and EMP RC05. Stability data were presented in table 3.

Forced degradation studies

Forced degradation studies reports shown little deviation in EM. Percentage deviation of forced degradation studies was mentioned in table 4.

Table 3: Stability and robustness data

Bench top stability data (Stability of test preparation at ambient temperature about (25 °C))												
Time in hours	**Eprosartan mesylate**				**Impurity A (EMP RC01)**				**Impurity B (EMP RC05)**			
	% Imp		**Difference from Initial**		**% Imp**		**Difference from Initial**		**% Imp**		**Difference from Initial**	
	Spl-1	**Spl-2**			**Spl-1**	**Spl-2**			**Spl-1**	**Spl-2**		
Initial	0.496	0.516	NA		0.486	0.503	NA		0.491	0.499	NA	
24	0.525	0.545	0.03	0.03	0.485	0.495	0.00	0.01	0.483	0.507	0.01	0.01
Stability studies at refrigerator temperature about (8 °C)												
Time in hours	Eprosartan mesylate				Impurity A (EMP RC01)				Impurity B (EMP RC05)			
	% Imp		Difference		% Imp		Difference from Initial		% Imp		Difference	
	Spl-1	Spl-2	from Initial		Spl-1	Spl-2			Spl-1	Spl-2	from Initial	
Initial	0.496	0.516	NA		0.486	0.503	NA		0.491	0.499	NA	
24	0.522	0.528	0.03	0.01	0.500	0.502	0.01	0.00	0.517	0.499	0.03	0.00

Table 4: Forced degradation data

Eprosartan mesylate	**Area**	**% Degradation**
Unstressed	42630	0
Acid stressed	41613	2.39
Base stressed	41160	3.45
H2O2 stressed	40185	5.74
Thermal stressed	41232	3.28
Humidity	41025	3.76

DISCUSSION

The developed method can be used for routine analysis because the linearity found in EM, EMP RC01 and EMP RC05 is nearing 1 that is 0.9999, 0.9999 and 0.9997 respectively which shows the good regression for linearity. Maximum recovery is obtained by this developed method and the mean percentage recovery for each component is nearing 100%. Therefore this method can be used for the routine analysis and one most important reason is that the developed method does not involve the use of expensive reagents. The spectrophotometric assay methods employed in our study indicated less interference from excipients used in the formulation by the percent recoveries values. Most of the existing methods consumed expensive reagents for individual drug analysis. But the method we developed involves chemicals like acetonitrile and buffer, which are easily available. Also, our proposed method requires less time for the determination of known impurities of EM simultaneously compared to other methods.

CONCLUSION

The developed method is uncomplicated, accurate, sensitive and precise for the determination of related substances in the Eprosartan Mesylate. The satisfying % recoveries and low % RSD Values confirmed the suitability of the developed method for the usual analysis of Eprosartan mesylate in pharmaceuticals.

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CONFLICT OF INTERESTS

Declare none

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