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Original Article

A RAPID RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTITATIVE ESTIMATION OF EPLERENONE IN TABLETS

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ABSTRACT

Objective: To develop a rapid, sensitive, accurate, precise, linear and rugged Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method and validate as per ICH guidelines for the quantitative estimation of Eplerenone in tablets.

Methods: The optimized method uses a reverse phase column, Waters Symmetry C18 (250 X 4.6 mm; 5µ), a mobile phase of triethylammonium phosphate buffer (pH 2.3):acetonitrile in the proportion of 40:60 v/v, flow rate of 1.0 ml/min and a detection wavelength of 240 nm using a UV detector.

Results: The developed method resulted in Eplerenone eluting at 3.63 min. Eplerenone exhibited linearity in the range 15-45µg/ml. The precision is exemplified by relative standard deviation of 0.34%. Percentage Mean recovery was found to be in the range of 98- 102, during accuracy studies. The limit of detection (LOD) and limit of quantitiation (LOQ) was found to be 39.16µg/ml and 118.66µg/ml respectively.

Conclusion: A sensitive, rapid, accurate, precise, linear and rugged RP-HPLC method was developed and validated for the quantitative estimation of Eplerenone in tablets as per ICH guidelines and hence it can be used for routine analysis in various pharmaceutical industries.

Keywords: RP-HPLC, Eplerenone, Method development, Validation.

INTRODUCTION

Eplerenone (fig. 1) is pregen-4-ene-7, 21-dicarboxylic acid, 9, 11-epoxy-17-hydroxy-3-oxo, γ-lactone, methyl ester (7α, 11α, 17α) [1, 3]. It has a molecular formula of $C_{24}H_{30}O_6$ and a molecular mass of 414.49. Eplerenone is the first highly selective aldosterone receptor antagonist (SARA) to effectively block aldosterone at receptor sites in body tissues, aldosterone being a component of rennin-angiotensin-aldosterone system [1-6]. Eplerenone is used for treatment of hypertension and heart failure [1-6]. Eplerenone is specifically indicated for the reduction of risk of cardiovascular death in people with heart failure and left ventricular dysfunction within 3–14 d of an acute myocardial infarction, in combination with standard therapies and as treatment against hypertension. It appears equivalent to spironolactone but is much more expensive [7]. It is marketed by Pfizer under the trade name Inspra.

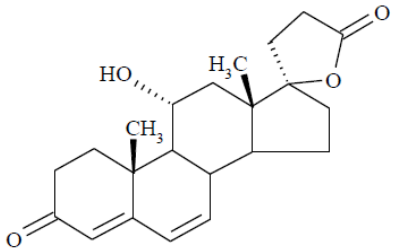

Fig. 1: Structure of Eplerenone

Eplerenone is a potassium-sparing diuretic, meaning that it helps the body get rid of water but still keep potassium. Few analytical methods have been reported for the determination of Eplerenone in biological fluids by LCMS [8-9], in bulk and formulations by UV Spectroscopy [10-11], TLC/Densitometry [1] and RP-HPLC [3, 12]. Literature reveals use of potassium dihydrogen orthophosphate as buffer (pH 3.2) and ammonium acetate buffer (pH 7.0) along with organic modifier as mobile phase for assay methods in formulations using RP-HPLC. As there is no literature reported on using triethyl ammonium phosphate as aqueous media along with organic modifier, we here report a new and a rapid RP-HPLC validated method for the quantitative estimation of Eplerenone in tablets using triethyl ammonium phosphate buffer (pH 2.3) along with acetonitrile as mobile phase.

MATERIALS AND METHODS

Chemicals and reagents

Analytically pure sample of Eplerenone with purities greater than 95% was obtained as the gift sample from Chandra labs, Hyderabad, India and tablet formulation [PLANEP] was procured from Apollo Pharmacy, Hyderabad, India with labelled amount 25 mg of Eplerenone. Acetonitrile (HPLC grade), water (HPLC grade), Triethylamine (AR Grade) and ortho phosphoric acid (AR Grade) were obtained from SD Fine chemicals (Hyderabad, India), 0.45 and 0.22µm Nylon membrane filters were obtained from Spincotech Private Limited, Hyderabad, India.

Instrument

HPLC analysis was performed on Shimadzu LC-20AD Prominence Liquid Chromatograph comprising a LC-20AD pump, Shimadzu SPD-20A Prominence UV-VISIBLE detector and a reverse phase C18 column, Waters Symmetry (250 X 4.6 mm; 5µ). A manually operating Rheodyne injector with 20 µL sample loop was equipped with the HPLC system. The HPLC system was controlled with "Lab solutions lite" software. A double beam UV-visible spectrophotometer (Shimadzu, model UV-1800) having two matched quartz cells with 1 cm light path and loaded with UV probe software (version 2.41) was used for recording of spectra and measuring absorbance. An electronic analytical weighing balance (0.1 mg sensitivity, Shimadzu AY 220), digital pH meter (DELUX model 101), a sonicator (sonica, model 2200 MH).

Method

Selection of wavelength

Suitable wavelength for the HPLC analysis was determined by recording UV spectrum in the range of 200-400 nm for Eplerenone. Suitable wavelength selected was 240 nm (fig. 2).

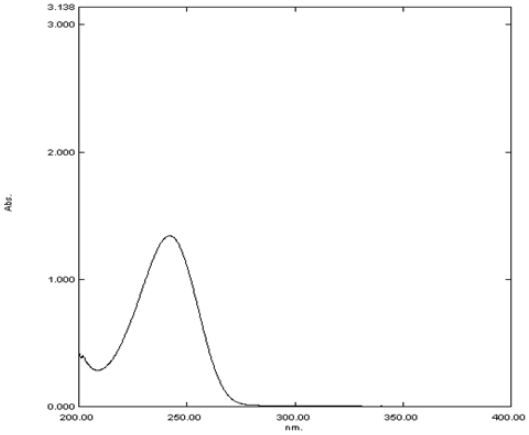

Fig. 2: UV spectrum of eplerenone

Chromatographic conditions

The developed method uses a reverse phase C18 column, Waters Symmetry C18 (250 X 4.6 mm; 5µ), mobile phase of triethylammonium phosphate buffer (pH 2.3): acetonitrile in the proportion of 40:60 v/v. The mobile phase was set at a flow rate of 1.0 ml/min and the volume injected was 20µl for every injection. The detection wavelength was set at 240 nm.

Buffer preparation

The buffer solution was prepared by adding 5 ml of triethylamine to 1000 ml of HPLC grade water and later pH was adjusted to 2.3 using 30% v/v of ortho phosphoric acid in water. The buffer was then filtered through 0.45 µm nylon membrane filter.

Mobile phase preparation

The mobile phase was prepared by mixing buffer and acetonitrile in the ratio of 40:60 v/v and later it was sonicated for 10 min for the removal of air bubbles.

Preparation of stock and working standard solution

10 mg of Eplerenone was accurately weighed and taken in 100 ml clean and dry volumetric flask containing 50 ml of diluent (same as mobile phase) and then sonicated for 2 min to dissolve. Later the solution was made up to the mark using the mobile phase. This is considered as the stock standard solution (100µg/ml). From the stock solution, 3 ml was pipetted out and to 10 ml using the mobile phase to get a concentration of 30µg/ml, treated as 100% target concentration.

Preparation of stock and working sample solution

Not less than 20 tablets were weighed and taken into a mortar, crushed and then uniformly mixed. Test stock solution of Eplerenone (500µg/ml) was prepared by transferring weight equivalent to 25 mg of Eplerenone to 40 ml of a mobile phase which is sonicated for 4 min and later made up to 50 ml with the mobile phase. This solution was filtered using 0.22micron syringe filter. From the above stock solution 0.6 ml was pipetted out and made up to 10 ml to get working sample solution equivalent to a concentration of 30µg/ml for Eplerenone, concentration equal to 100% target concentration.

RESULTS AND DISCUSSION

Method development

A Reverse phase HPLC method was developed keeping in mind the system suitability parameters i.e. tailing factor (T), number of theoretical plates (N), runtime and the cost effectiveness. The optimized method developed resulted in the elution of Eplerenone at 3.63 min. Fig. 3 and 4 represent chromatograms of blank solution and the standard solution (30µg/ml) respectively. The total run time is 6 min. System suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (RT), number of theoretical plates (*N*) and peak Tailing factor (T) were evaluated for injection of the standard at the working concentration. The results are given in table 1.

In order to test the applicability of the developed method to a commercial formulation, PLANEP was chromatographed at working concentration (30µg/ml) and it is shown in fig. 5. The sample peak was identified by comparing the retention time with the standard drug fig. 4. System suitability parameters were within the acceptance limits, ideal for the chromatographed sample. Integration of separated peak area was done and drug concentration was determined by using the peak area concentration relationship obtained in the standardization step. The protocol affords reproducible assay of the drug in the sample ranging between 98 and 102%, which is the standard level in any pharmaceutical quality control.

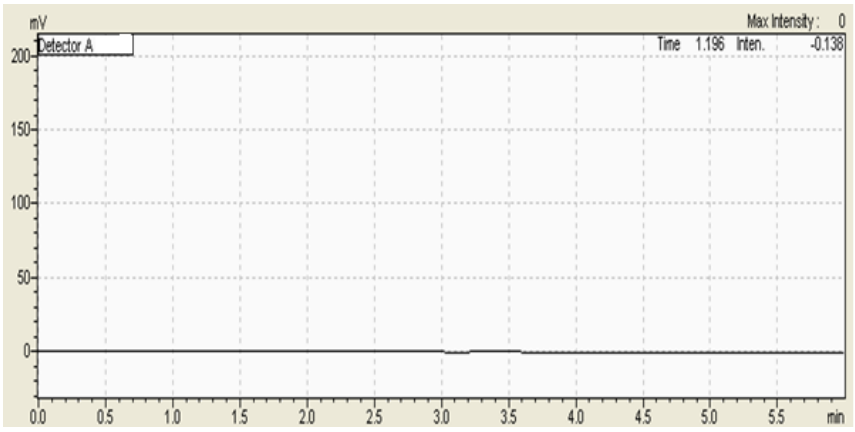

Fig. 3: Typical chromatogram of blank solution

Table 1: System suitabilitystudies results

Parameters	Eplerenone
Retention time (min)	3.63
Number Of Theoretical plates (N)	5954
Tailing factor (T)	1.82

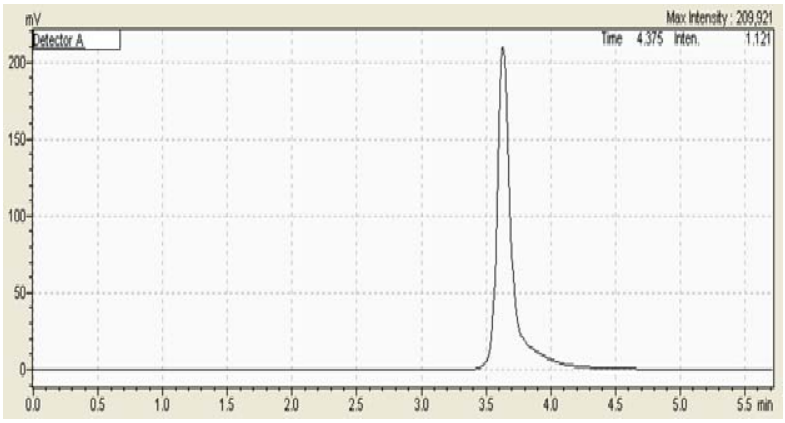

Fig. 4: Typical chromatogram of the standard solution

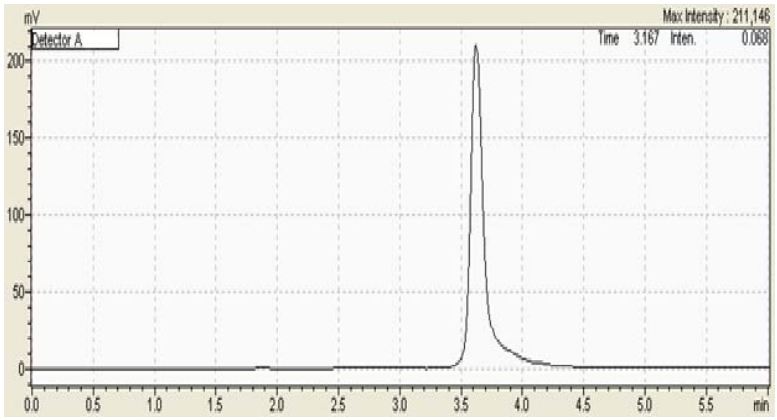

Fig. 5: Typical chromatogram for the tablet formulation.

Method validation

Validation of the analytical method is the process that establishes by laboratory studies in which the performance characteristics of the method meet the requirements for the intended analytical application. RP-HPLC method developed was validated according to International Conference on Harmonization (ICH) guidelines [13] for validation of analytical procedures. The method was validated for the parameters like system suitability, specificity, linearity, accuracy, precision, and ruggedness, limit of detection (LOD) and limit of quantitiation (LOQ).

Specificity

Fig. 3-5 for blank, standard drug solution and sample chromatogram reveal that the peaks obtained in the standard solution and the sample solution at working concentrations are only because of the drug as blank has no peak at the retention time of Eplerenone. Accordingly it can be concluded that, the method developed is said to be specific.

Precision

System precision

Six replicate injections of the standard solution at the working concentration showed % RSD (Relative Standard Deviation) less than 2 concerning peak area for the drug, which indicates the acceptable reproducibility and thereby the precision of the system. System precision results are tabulated in table 2.

Method precision

Method precision was determined by performing assay of sample under the tests of repeatability (Intraday precision) at working concentration.

Repeatability (Intraday precision)

Six consecutive injections of the sample from the same homogeneous mixture at working concentration showed % RSD less than 2 concerning % assay for the drug which indicate that the method developed is method precise by the test of repeatability and hence can be understood that the method gives consistently reproducible results (table 3).

Linearity

Standard solutions of Eplerenone at different concentrations level (50%, 75%, 100%, 125% and 150%) were prepared. Calibration curve was constructed by plotting the concentration level of drug versus the corresponding peak area. The results show an excellent correlation between peak area and concentration level of drug within the concentration range (15-45µg/ml) for the drug and the results are given in table 4 and fig. 6. The correlation coefficient of Eplerenone is 0.998 and hence the method is said to be linear.

Accuracy

Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of sample at three different levels (50-150%). At each level, three determinations were performed. Percent mean recovery was calculated as shown in table 5**.** The accepted limits of recovery are 98%-102% and all observed data are within the required range which indicates good recovery values and hence the accuracy of the method developed.

Sensitivity

The sensitivity of measurement of Eplerenone by use of the proposed method was estimated in terms of the limit of quantitation (LOQ) and the limit of detection (LOD). LOQ and LOD were calculated by the use of the equations LOD = $3.3\sigma/S$ and LOQ = $10\sigma/S$ where σ is the standard deviation of response of calibration plot and S is the slope of the corresponding calibration plot. The limit of detection (LOD) and limit of quantitiation (LOQ) was found to be 39.16µg/ml and 118.66µg/ml respectively.

Table 2: System precision results of Eplerenone

Injection no. (n)	RT	Peak Area
1	3.631	1728619
2	3.642	1712266
3	3.634	1724394
4	3.634	1722646
5	3.648	1720596
Average	3.637	1721704
Standard Deviation	0.007	6046.5
%RSD	0.192	0.351

Table 3: Intraday precision results of Eplerenone

n	RT	Peak area	%Assay
1	3.632	1746464	101.43
2	3.624	1751581	101.73
3	3.628	1743313	101.25
4	3.632	1755407	101.95
5	3.631	1752102	101.76
6	3.628	1739400	101.02
Average	3.628	1755378	101.52
Standard Deviation	0.0028	12136.2	0.35
%RSD	0.077	0.691	0.344

Table 4: Calibration data for Eplerenone

% Level	Concentration (µg/ml)	Peak area
50	15	838943
75	22.5	1221643
100	30	1631265
125	37.5	2059254
150	45	2525331
Regression coefficient		0.998
Regression equation		y=56138.5x-28868

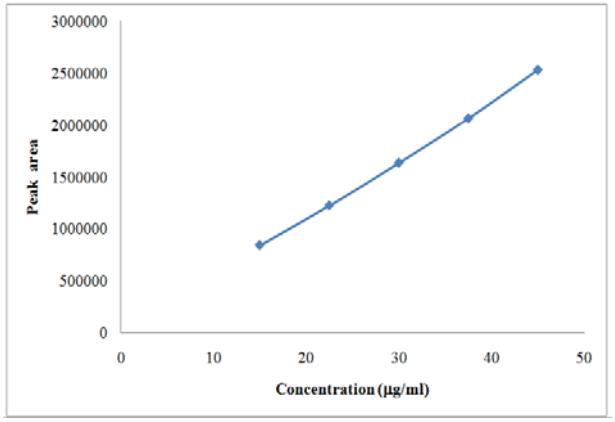

Fig. 6: Linearity graph of Eplerenone

Table 5: Results of Accuracy studies for Eplerenone

% Level	Peak area	% Recovery	% Mean recovery	%RSD
50	873273	101.443	100.92	**0.66**
	862248	100.162		
	870870	101.164		
100	1751581	101.735	101.649	**0.35**
	1743313	101.255		
	1755407	101.958		
150	2538435	98.281	98.51	**0.31**
	2553430	98.862		
	2541449	98.398		

Table 6: Ruggedness results of Eplerenone

n	RT	Peak area	%Assay
1	3.627	1740565	101.095
2	3.629	1736765	100.875
3	3.637	1745625	101.389
4	3.641	1735499	100.801
5	3.641	1740967	101.119
6	3.641	1750437	101.669
Average	3.636	1741643	101.15
Standard deviation	0.005	5104.79	0.322
%RSD	0.161	0.293	0.318

Ruggedness

Ruggedness was evaluated by performing assay of the formulations by different analyst by injecting six consecutive injections of the sample at the working concentration from the same homogeneous mixture of tablets. This study showed % RSD less than 2 concerning % assay for both the drugs which indicate that the method developed is rugged and hence can be understood that the method gives reproducible results irrespective of analyst (table 6).

CONCLUSION

A reverse phase HPLC isocratic method developed has been validated as per ICH guidelines in terms of specificity, accuracy, precision, linearity, and ruggedness, limit of detection and limit of quantitation, for the quantitative estimation of Eplerenone in tablets. The precision is exemplified by relative standard deviation of 0.34%. A good linear relationship was observed for the drug between concentration ranges of 15 and 45µg/ml. Accuracy studies revealed that mean recoveries were between 98 and 102%, an indicative of accurate method. The limit of detection (LOD) and limit of quantitiation (LOQ) was found to be 39.16µg/ml and 118.66µg/ml respectively. Accordingly it can be concluded that the developed reverse phase isocratic HPLC method is sensitive, accurate, precise, linear and rugged and therefore the method can be used for the routine analysis of Eplerenone in tablets.

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