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Original Article

A SIMPLE AND A CHEAP UV ASSAY METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF EPLERENONE IN TABLETS

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ABSTRACT

Objective: To develop a simple and a cheap UV spectrophotometric assay method for the estimation of Eplerenone in tablets and validate as per ICH guidelines.

Methods: The optimized method uses 100% potassium dihydrogen orthophosphate, pH 2.0 as a solvent for the estimation of assay of Eplerenone in tablets at a detection wavelength of 245 nm.

Results: The developed method resulted in Eplerenone exhibiting linearity in the range 5-15µg/ml. System precision and intra-day precision are exemplified by relative standard deviation of 1.17% and 0.94% respectively. Method was found to be rugged as precision was found to be 1.52%. Percentage Mean recovery was found to be in the range of 98-102 by percentage method during accuracy studies.

Conclusion: A simple and a cheap UV spectrophotometric assay method were developed and validated for the estimation of Eplerenone in tablets as per ICH guidelines and hence it can be used for routine analysis in various pharmaceutical industries.

Keywords: UV, 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. 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]. UV assay methods were reported [10-11] using methanol (30%) and methanol (70%) in water as solvent respectively. Both the UV methods are costlier due to use of organic solvent methanol. Hence, we here report a simple and a cheap UV spectrometric assay method for the estimation of Eplerenone in tablets using aqueous solution of potassium dihydrogen orthophosphate buffer (pH 2.0).

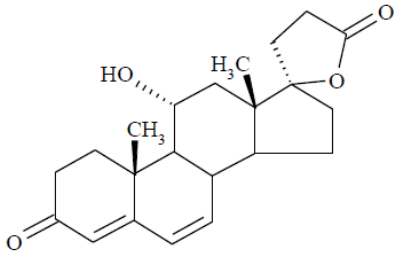

Fig. 1: Structure of Eplerenone

MATERIALS AND METHODS

Materials

Instrument

A double beam UV-visible spectrophotometer (Shimadzu, model 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) and a sonicator (sonica, model 2200 MH) were used in this study.

Chemicals and reagents

Analytically pure sample of Eplerenone with purities greater than 99% was obtained as gift sample from Chandra labs, Hyderabad, India and tablet formulation [PLANEP] was procured from Medplus pharmacy, Hyderabad, India with labelled claim of 25 mg. Potassium dihydrogen orthophosphate (AR Grade), Ortho phosphoric acid were obtained from SD Fine chemicals (Hyderabad, India).

Method

Preparation of buffer as solvent

1.36 g of potassium dihydrogen ortho phosphate was added to 500 ml of distilled water to get a clear solution whose pH was adjusted to 2.0 with ortho phosphoric acid.

Selection of suitable detection wavelength

Suitable wavelength for the total experiment was determined by recording UV spectrum in the range of 200-400 nm for Eplerenone and suitable wavelength selected was 245 nm (fig. 2).

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 80 ml of solvent and then the solution was made up to the mark using the solvent. This is considered as standard stock solution (100µg/ml). 1 ml of the stock solution was pipetted out and made up to 10 ml to get a concentration 10µg/ml, treated as the working standard, 100% target concentration.

Preparation of stock and working sample solution

Not less than 20 tablets were taken and converted in to powder using pestle and mortar. Test stock solution of 500µg/ml was prepared by transferring weight equivalent to 25 mg of Eplerenone to 40 ml of solvent which is sonicated and shaken intermittently for 15 min and later made up to 50 ml with solvent. This solution was filtered using 0.22micron syringe filter. From the above stock solution 0.2 ml was pipetted out and made up to 10 ml to get working sample solution concentration equivalent to 10µg/ml, 100% target concentration.

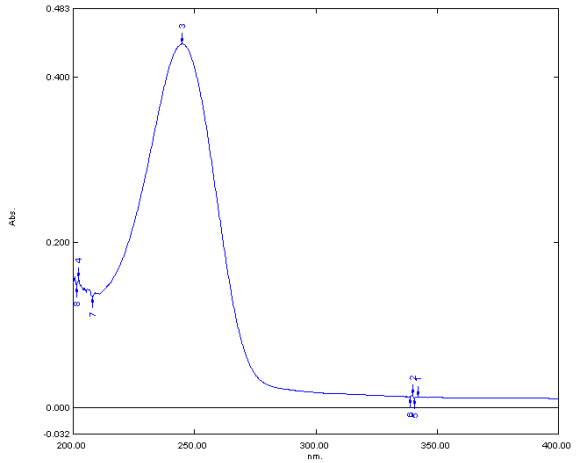

Fig. 2: UV spectrum of standard

RESULTS AND DISCUSSION

Method development

Various solvents were explored including water, potassium dihydrogen orthophosphate solution at various pH's, Hydrochloric acid at 0.1N and 0.05N and sodium hydroxide at 0.1N and 0.05N. Eplerenone was found to be soluble and stable for minimum of 1 hour at room temperature using potassium dihydrogen ortho phosphate solution at pH 2.0 and hence this solvent was initiated for the determination of suitable detection wavelength and working standard. In order to test the applicability of the developed method to a commercial formulation, assay of PLANEP tablets were studied at working concentration. Assay for working concentration of sample at 245 nm was in acceptance limits (95-105%) using the solvent via intermittent shaking and sonication method for 15 min fig. 3 illustrates UV spectrum for the sample. Hence the method is optimized.

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. UV spectrophotometric 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 linearity, accuracy, and system precision, intra-day precision and inter-day precision / intermediate precision/ruggedness.

Precision

System precision

Six replicate recording of absorbance at 245 nm of standard solution at working concentration showed % RSD (Relative Standard Deviation) less than 2, which indicates acceptable reproducibility and thereby the precision of the system. System precision results are tabulated in table 1.

Method precision

Method precision was determined by performing assay of sample under the tests of (i) repeatability (Intraday precision) and (ii) Intermediate precision (Inter day precision or ruggedness) performed during 2 consecutive days by two different analysts, at working concentration.

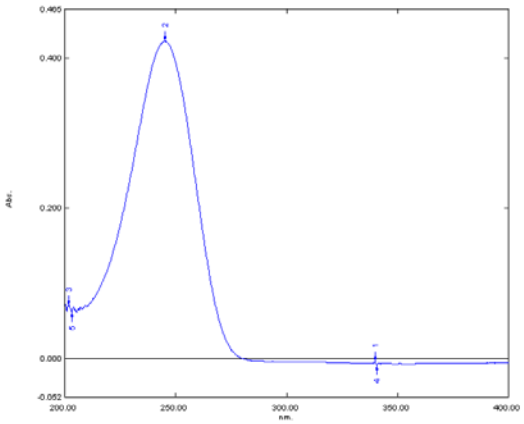

Fig. 3: UV spectrum of sample

Table 1: System precision results of Eplerenone

n	Absorbance
1	0.430
2	0.430
3	0.423
4	0.430
5	0.431
6	0.418
Average	0.427
SD	0.005
%RSD	1.17

Repeatability (Intraday precision)

Six consecutive recording of absorbance at 245 nm of the sample from the same homogeneous mixture at working concentration showed % RSD less than 2 concerning % assay, 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 2).

Table 2: Intraday precision results of eplerenone

n	Sample absorbance	% Assay
1	0.427	99
2	0.429	99.46
3	0.429	99.46
4	0.433	101.40
5	0.435	100.85
6	0.432	100.15
Average		100.05
SD		0.94
% RSD		0.94

Intermediate precision (Inter day precision/Ruggedness)

Assay precision between two consecutive days performed by different analysts of the sample showed % RSD less than 2, which indicate the method developed is inter day precise/rugged (table 3).

Linearity

Standard solutions of Eplerenone at different concentrations level (50%, 75%, 100%, 125% and 150%) were prepared. Calibration curve (fig. 4) was constructed by plotting the concentration level of drug versus absorbance at 245 nm. The results show an excellent correlation between absorbance and concentration level of drug

within the concentration range (5-15µg/ml) for the drug (table 4). The correlation coefficient was greater than 0.995, which meet the method validation acceptance criteria and hence the method is said to be linear in the range of 5-15µg/ml.

Table 3: Inter day precision results

n	Analyst 1	Analyst 2
1	99	98.05
2	99.46	96.91
3	99.46	97.37
4	101.46	100.85
5	100.85	99.31
6	100.15	99.69
Average	100.05	98.69
SD	0.94	1.5
%RSD	0.94	1.52

Table 4: Calibration data for Eplerenone

% Level	Concentration (µg/ml)	Absorbance
50	5	0.244
75	7.5	0.318
100	10	0.399
125	12.5	0.467
150	15	0.544

Regression equation: y=0.299x+0.0948, Regression coefficient: 0.999

Accuracy

Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of sample by percentage method 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. Table 6 summarizes the validation parameters.

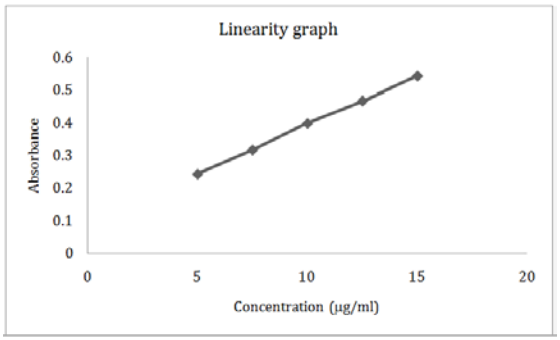

Fig. 4: Linearity graph of Eplereone

Table 5: Results of accuracy studies for eplerenone

% Level	Absorbance	% Recovery	% Mean recovery	%RSD
50	0.225	100.22		
50	0.228	101.26	100.7	0.225
50	0.224	100.88		
100	0.436	97.99		
100	0.441	100.3	99.09	0.439
100	0.441	98.98		
150	0.647	96.98		
150	0.653	98.07	98.44	0.655
150	0.667	100.28		

Table 6: Optical characteristics and validation parameters of Eplerenone

Parameters	Results
Detection wavelength (nm)	245
Beer's Law limits (µg/ml)	5-15
Regression equation (y = mx+c)	y=0.299x+0.0948
Correlation coefficient (r^2)	0.999
Slope (m)	0.299
Intercept (c)	0.0948
% Relative Standard Deviation (% RSD) System precision	1.17
(% RSD) Intra-day precision	0.94
(% RSD) Inter-day precision	≤ 2
Accuracy (% Mean Recovery)	
50 % Level	100.7
100 % Level	99.09
150 % Level	98.44

CONCLUSION

A simple UV spectrophotometric method was developed and validated for the quantitative estimation of Eplerenone in capsules as per ICH guidelines. The optimized method uses 100% potassium dihydrogen orthophosphate, pH 2.0 as a solvent for the estimation of assay of Eplerenone in tablets at a detection wavelength of 245 nm. The developed method resulted in Eplerenone exhibiting linearity in the range 5-15µg/ml.

System precision and intra-day precision are exemplified by relative standard deviation of 1.17% and 0.94% respectively. Method was found to be rugged as precision was found to be 1.52%. Percentage Mean recovery was found to be in the range of 98-102 by percentage method during accuracy studies. Accordingly it is concluded that the developed UV spectrophotometric assay method is simple, accurate, precise, linear and rugged and therefore the method can be used for the routine analysis of Eplerenone in tablets in various pharmaceutical industries.

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CONFLICT OF INTERESTS

Declared None

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