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ORIGINAL ARTICLE

Method Development and Validation of RP-HPLC Method for Estimation of Eplerenone in Bulk and Pharmaceutical Formulations

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ABSTRACT

Objective: This method focused on development of simple, selective and precise liquid chromatographic method for determination of Eplerenone in API (Active Pharmaceutical Ingredient) and pharmaceutical formulations.

Methods: This chromatographic method employed a C18 column with Isocratic mobile phase consisting of Acetonitrile and Phosphoric acid 0.1% (50:50, v/v). The UV detection was performed at 241 nm. This was followed with validation of developed procedure for as per ICH guidelines.

Results: The validation parameters of linearity, accuracy, precision, and specificity were found to be distinct able. There was no interference detected with neither excipients nor degradation products in the determination of active pharmaceutical ingredient and tablet formulation. The results presented a linear response over a range from 2 to 20 µg/ml with good accuracy and intra- and inter-day precision. The recovered values from pharmaceutical dosage form ranged from 98 to 102%. Moreover, stability of the procedure was evaluated by subjecting solutions of Eplerenone and its drug products to four different stress conditions: acidic, oxidative, reductive and ultraviolet degradation. Moreover, forced degradation study was also performed under thermal stress and photolytic conditions.

Conclusions: Overall, the method developed was found to be suitable for routine quantitative determination of the active in both bulk and pharmaceutical tablet dosage form.

INTRODUCTION

Eplerenone is a novel selective aldosterone blocker belonging to the spironolactone group of steroidal mineralocorticoids. Chemically it is pregen-4-ene-7,21-dicarboxylic acid, 9,11-epoxy-17-hydroxy-3-oxo, γ-lactone, methyl ester (7α,11α,17α) [1,3] (Figure **1**). It has a molecular formula of $C_{24306}HO$ and a molecular mass of 414.49 [1].

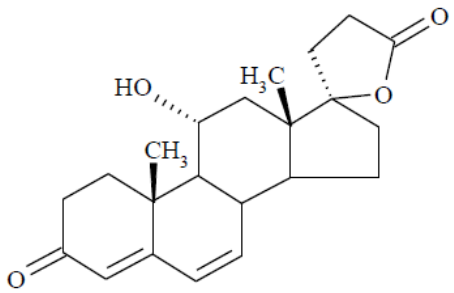

Figure 1. Structure of Eplerenone.

Eplerenone is used widely in the treatment of hypertension and heart failure due to its cardioprotective and renal protective effects. The drug is well tolerated in dosage range from 50 to 400 mg once daily. The drug is recommended in patients with mild to moderate hypertension either as monotherapy as well as in combination with other antihypertensive agents [2,3] for LVH regression and blood pressure control [4, 5].

The clinical efficacy is evident from its use in a wide range of other heart conditions as well as its use in combination with other therapies: angiotensin-receptor blockers, calcium channel blockers, or beta-blockers; in different ethnic groups [6] Although It acts through the same mechanism of Mineralocorticoid Receptor (MR) blockade but is highly comparable to aldosterone for its cardio and renal protective efficiency [7]. This also marks its use in elderly patients on ACEIs or ARBs providing them significant benefits in terms BP control, fibrinolysis, and cardiovascular protection. Moreover, It is often prescribed with other medical therapies and has been found to reduce the rate of death with AMI (acute myocardial infarction) patients intricated by systolic dysfunction of left ventricle and heart failure [8,9] or other cardiovascular conditions in hospitalized patients.

In literature, various methods are available for determination of eplerenone by UV Spectroscopy [1,10-12] and RP-HPLC Method for Eplerenone determination in human plasma and urine [13] but hardly for both bulk and pharmaceutical dosage forms [14]. This requires simple, precise, accurate and robust method for routine drug analysis of Eplerenone.

METHODOLOGY

Instrumentation and chromatographic conditions

The Instruments included weighing Balance Mettler Toledo AB-20 4S, Ultrasonic water Bath, Magnetic stirrer, Whatman filter and HPLC LC-20 with software version LC 2.0 and UV Visible detector. The column was specified as C18, 250 x 4.6mm, 5 microns. The flow rate was set at 1.0 ml per minute with injection volume of 20 microliters.

Reagents and chemicals

Working standards of pharmaceutical grade (%, w/w) and product samples were obtained from Central Drug Laboratory Govt of Pakistan. All chemicals and reagents employed in the testing were of analytical grade.

Preparation of mobile phase

Preparation of phosphoric acid 0.1% v/v

An amount of 1 ml of phosphoric acid (85 %) was transferred to a flask calibrated to 1000 ml containing about 900 ml of distilled water. Volume was makeup with distilled water and mixed.

Prepared mixture of acetonitrile and phosphoric acid 0.1% v/v was mixed in the ratio of 50: 50 and filtered through 0.45-micron filter and degassed.

Preparation of diluents

Acetonitrile was used as diluents.

Preparation of reference solution

Weighed 50mg of Eplerenone working standard and transferred accurately to a 100 ml volumetric flask followed by addition of 70 ml of diluent. Dissolved by sonicating on ultrasonic water bath for 5 minutes followed by stirring on magnetic stirrer for 5 minutes. The solution was cooled to room temperature and volume make up with diluent and mixed.

Then 5 ml of the above solution was taken to a 100 ml volumetric flask and make up the volume with diluent and mixed. The solution was filtered through 0.45-micron filter and the filtrate was used as reference solution.

Preparation of test solution

The 10 tablets of Eplerenone were crushed in mortar and pestle to fine powder. Weighed powder about the weight of one tablet (equivalent to the 50 mg of eplerenone) was transferred accurately to a volumetric flask of 100ml. Then 70 ml of diluent was added and

dissolved by sonicating on ultrasonic water bath for 5 minutes followed by stirring on magnetic stirrer for 15 minutes. The solution was then cooled to room temperature and make up the volume with diluent and mixed.
Then 5 ml of the above solution was transferred to a volumetric flask of 100 ml and make up the volume with diluent and mix. The solution was filtered through Whatman filter. The filtrate was again filtered through 0.45-micron filter and used as test solution.

Testing procedure

Inject 20µl of standard (five Replicates) and sample solution (two Replicates) into the chromatograph. The chromatograms were recorded and the concentration of Eplerenone calculated using peak area.

RESULTS

Method validation

The developed method was then validated as per recommended guidelines of ICH [15].

System suitability tests

The standard preparation chromatographed and the peak areas were recorded. The symmetry factor for the Eplerenone is not more than 2.0, theoretical plates not less than 1000 and the relative standard deviations for 5 replicate injections of the standard preparation was not more than 2.0 (Table **1**).

Table 1. Eplerenone system suitability parameters.

Parameters	Eplerenone
Linearity range	2 to 20 µg/ml
Regression equation	
Y = mx +c	3E+07x-22238
Theoretical plates	1000
Asymmetric factor	2
RSD %	1.3
LOD (mg/l)	0.0578
LOQ (mg/l)	0.01156

Selectivity and specificity

This was established by injecting the diluent (used for preparing test and reference solutions), mobile phase and the dilution of excipient. Chromatograms were recorded.
The chromatograms of the diluent showed no peaks that interfere with the peak of analyte excipient obtained with reference solution (Figure **2**). Thus, the procedure shows to be selective for detection and quantitation of the analyte.

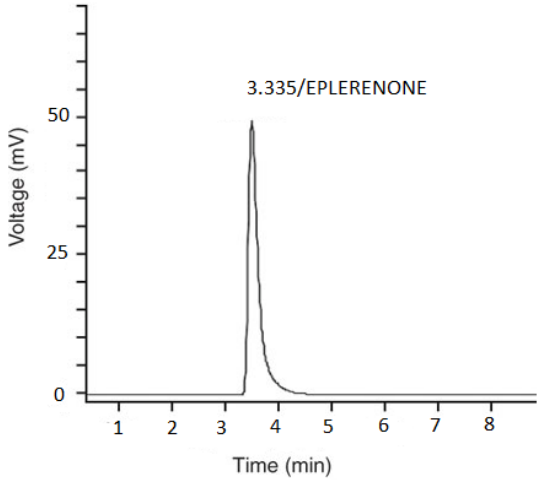

Figure 2. Isolated peak of eplerenone.

Selectivity to degradation products

This was confirmed by 'Forced degradation' of reference solution by exposing the solution to four stress conditions.

Acidic degradation

This was done through addition of the test solution 10 ml of 1N hydrochloric acid was added to the first dilution and kept at ambient temperature for 4 hours. Volume was made up with diluent and chromatograph was obtained.

Alkaline degradation

This was done through addition of the solution 10 ml of 1N solution of sodium hydroxide to the first dilution and kept at ambient temperature for 4 hours. Volume was made up with diluent and chromatograph was obtained.

Reductive degradation

This was done through addition of the solution 10% sodium sulfite was added to the first dilution and kept at ambient temperature for 4 hours. Volume was made up with diluent and chromatograph was obtained.

Oxidative degradation

This was done through addition of the solution 10 ml of hydrogen peroxide was added to the first dilution and kept at ambient temperature for 4 hours. Volume was made up with diluent and chromatograph was obtained.

Ultraviolet degradation

The solution prepared in the diluent, while preparing the first dilution 20 ml of diluent was added and exposed to short wavelength UV light for 4 hours. Volume was made up with diluent and chromatograph was obtained.

The chromatogram of the treated samples in acidic, alkaline, reductive and ultraviolet degradation the treated samples no change was observed in the peak area of the analyte. Moreover, no peak of degradation product was detected suggesting the analyte is stable under stress conditions. Except Oxidative reduction where the peak of analyte is completely degraded.

Linearity

The linear relationship response was confirmed by plotting the graph between the varying concentrations of the analyte in the solution of linearity test and their area.

Table 2. Eplerenone.

S. No.	Concentration of Analyte Solution	Area of Analyte Solution
1	0.0202 mg / ml	614426.5000
2	0.0228 mg / ml	692335.5000
3	0.0253 mg / ml	772646.5000
4	0.0278 mg / ml	852901.5000
5	0.0304 mg / ml	931659.5000

The calibration graph (Table **2**) indicates linear relationship between the concentration and area of the solutions. The R^2 of calibration for data point was calculated to be 1. This indicates that the test procedure obeys Beer's Law.

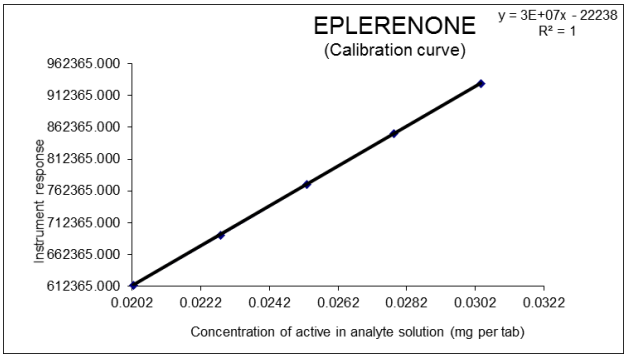

Figure 3. Calibration curve of Eplerenone.

The calibration graph (Figure **3**) shows linear relationship between the area and concentration of analyte in the solution.

Sensitivity

Limit of Quantitation*: LOQ*

This refers to the amount of analyte that can be assayed acceptable accuracy and precision. The value found to be 0.0578 milligrams per liter.

Limit of Detection*: LOD*

This is the least amount of the analyte whose presence could be detected but not quantified accurately. It is found to be equal to 0.01156 milligrams per liter.

Precision

Validation of tests for assay includes an investigation of precision. This was tested by intermediate precision. It was estimated by six replicate assays of the sample containing analyte at 100% of label claim. The results are as under in Table **3**.

Intermediate precision

Five test samples equivalent to 100% of label claim were prepared and assayed individually by two analysts in order to compensate. The results obtained were under acceptable limits which are presented in Table **4**.

Accuracy

The accuracy of test method is determined by spiking the placebo with predetermined amount of active equivalent to 80%, 100% and 120% of the label claim. Three sample from each were prepared and tested. Results are presented in Table **5**.

The results of assay represent contents of the analyte equivalent to the theoretical contents in the placebo. The standard deviations were within the usual acceptable limits.

Table 3. Percentage assay of Eplerenone.

S. No.	Weight of Sample (mg)	Assay (mg per Tablet)	Assay (% of Label Claim)	Mean	STD %	RSD %
1	230.2	51.36	102.72	50.24 mg/tablet (100.48% of label claim)	0.6948	1.3829%
2	230.6	50.39	100.79			
3	230.8	50.2	100.41			
4	228.6	49.27	98.54			
5	226.3	50.39	100.78			
6	227.9	49.82	99.64			

Table 4. Intermediate precision.

	Weight of Sample (mg)	Assay (mg per Tablet)	Assay (% of Label Claim)	Mean	STD %	RSD %
Analyst 1:	230.2	49.07	98.14	49.53 mg/tablet (99.05% of label claim)	0.7631	1.5408%
	230.6	49.23	98.47			
	230.2	49.25	98.49			
	238	50.88	101.77			
	230.5	49.19	98.38			
Analyst 2:	222.6	49.68	99.35	49.82 mg/tablet (99.63% of label claim)	0.5899	1.1841%
	230.8	50.62	101.24			
	230.9	49.34	98.69			
	228.5	49.23	98.46			
	222.7	50.21	100.43			

Table 5. Accuracy.

Theoretical Contents of Active Added in Placebo		Assay (mg / Tablet)	Assay (% of Label Claim)	Difference (%)	Mean Difference (%)	Standard Deviation	% Recovery
Placebo with 80% of active							
	1.	40.85	81.71	1.708			**102.13**
	2.	40.79	81.58	1.576	1.56	0.15	**101.97**
	3.	40.70	81.41	1.410			**101.76**
Placebo with 100% of active							
	1.	50.48	100.97	0.966			**100.97**
	2.	49.98	99.96	-0.043	-0.11	1.12	**99.96**
	3.	49.37	98.74	-1.264			**98.74**
Placebo with 120% of active							

	1.	60.04	120.09	0.087			**100.07**
	2.	59.79	119.59	-0.413	0.13	0.56	**99.66**
	3.	60.36	120.71	0.710			**100.59**

Table 6. Robustness.

Storage Condition	Assay (mg/ Tablet)	Assay (% of Label Claim)
Stored at 4°C	49.68	99.35
Stored at ambient temperature (25°C)	50.45	100.89
Stored at 35°C	50.22	100.43

Robustness

It is the measure how stable the test procedure is under slight variation in test procedure. The following changes were made deliberately in testing procedure. The test solution prepared according to the test procedure and kept at 4°C, 25°C and 35°C for 4 hours and assayed according to the test procedure. The results were compared with initial results and tabulated in Table **6**.

The reference and test samples are stable for up to four hours kept under the temperature as low as 4°C and as high as 35°C, the variations were within the acceptable limits of ± 2%.

DISCUSSION

Eplerenone is a widely used selective aldosterone blocker for hypertension due to its marked protective effect in renal and cardiac patients. The drug requires selective, precise and accurate quantitative determination for its analysis. This method validated as per ICH guidelines would help achieve quality control parameters of routine laboratory investigation of bulk and pharmaceutical dosage forms available for the drug. The prepared solution of standard and reference were also found to be stable for up to four hours under low temperature with minor variations.

CONCLUSION

A simple, selective and precise method has been developed for determination of Eplerenone suitable for both bulk drug and in tablet formulation analysis. Moreover, the method was validated as per recommended criteria in guidelines of International Conference on Harmonization (ICH).

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