ORIGINAL ARTICLE

New Stability Indicating Liquid Chromatographic Method for the Determination of Eplerenone in the Presence of Internal Standard

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Abstract

Introduction: A simple stability indicating reverse-phase high-performance liquid chromatographic (RP-HPLC) method has been developed for the determination of Eplerenone (EPL) in its tablet dosage forms. EPL is an aldosterone antagonist. It is administered either alone or in combination with other drugs to treat cardiovascular diseases. **Materials and Methods:** EPL was eluted on SHIMADZU HPLC system using Agilent column (150 mm × 4.6 mm i.d., 3.5 µm particle size) (isocratic mode). A mixture of 10 mM tetra butyl ammonium hydrogen sulfate and methanol (30: 70, v/v) was used for the chromatographic separation and was monitored at 242 nm (PDA detector) with flow rate 1.0 mL/min. The total run time was 10 min. Forced degradation studies were performed, and the method was validated. **Results and Discussion:** EPL has obeyed Beer-Lambert's law 0.1–40 µg/mL with correlation coefficient 0.9996. The limit of detection and limit of quantification are found to be 0.0283 µg/mL and 0.0846 µg/ mL, respectively. EPL was found to be highly resistant toward hydrolysis and acidic conditions. **Conclusions:** It is observed that this RP-HPLC method is accurate, precise, sensitive, and reproducible for the estimation of EPL in tablets. The method was validated as per the ICH guidelines and very much specific and selective.

Key words: Eplerenone, high-performance liquid chromatographic, ICH guidelines, umifenovir, validation

INTRODUCTION

Eplerenone(EPL)isanantimineralocorticoid that inhibits the over-activation of the mineralocorticoid receptor pathways. It is chemically known as 9-11α-epoxymexrenone; 9,11α Epoxy 7α methoxycarbonyl-3-oxo-17α-pregn-4-ene-21,17-carbolactone. It is a white to off-white crystalline powder with a molecular formula $C_{24}H_{30}O_6$ and molecular weight 414.50. EPL belongs to spironolactone group and is used in the management of chronic heart failure.[1-3] It is used for the treatment of hypertension, central serous retinopathy. It acts by blocking aldosterone activity which is responsible for the increase in blood pressure. It is

metabolized by cytochrome P450 enzyme CYP3A4.[4-6] It is approved by Food and Drug Administration in 2002 for sale in the US, and it is available with brand name INSPRA (Pfizer) as tablets (Label claim: 25 mg; 50 mg). Analytical methods such as LC-mass spectrometry (MS),[7] LC-MS/MS,[8] spectrophotometry,[9,10] thin layer chromatography/densitometry,[11] and reverse-phase high-performance liquid chromatographic (RP-HPLC)[12,13] have been reported for the determination of EPL in biological fluids as well as in formulations. The authors have proposed new stability indicating RP-HPLC method for the determination of EPL in the presence of Umifenovir (UMI) as internal standard (IS). UMI is an anti-viral drug, and the method was validated in the presence of the IS to get more reliable results.

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Chemicals and reagents

EPL was obtained from Pfizer (India). It is available with brand name INSPRA (Pfizer) as tablets with label claim: 25 mg; 50 mg. All other chemicals are of AR grade, and all solvents are of HPLC grade (Merck). Chromatographic was performed using Shimadzu model CBM-20A/20 Elite HPLC system (Shimadzu Co., Kyoto, Japan) equipped with SPD M20A prominence PDA detector.

Preparation of standard solutions

Stock solution of EPL and UMI was prepared by dissolving 25 mg of EPL and UMI in two 25 mL volumetric flasks separately with HPLC grade methanol (1000 µg/mL), diluted with mobile phase and filtered through membrane filter. A 10 µg/mL UMI was used as an IS throughout the analysis.

Optimized chromatographic conditions

The chromatographic system (PDA detector) was optimized with Agilent column (150 mm × 4.6 mm i.d., 3.5 µm particle size) using a mixture of 10 mM tetra butyl ammonium hydrogen sulfate and methanol (30:70, v/v) (isocratic mode). The same mobile phase mixture but with different composition was used as a diluent (50:50, v/v). UMI (10 µg/mL) was used as an IS throughout the analysis. The system was monitored at 242 nm with flow rate 1.0 mL/min and the overall run time was 10 min and the study was observed at ambient temperature (25°C ± 2°C). All the drug solutions were periodically monitored for their stability on a daily basis [Figure 1].

MATERIALS AND METHODS

Method validation

The method was validated by evaluating linearity, recovery, precision, accuracy, system suitability, solution stability, limit of detection (LOD), limit of quantification (LOQ), and robustness as per the ICH guidelines[14] for the determination of EPL.

Linearity, precision, accuracy, and robustness studies

A series of solutions (0.1–40 µg/mL) were made from the EPL stock solution along with 10 µg/mL UMI. 20 µL of each solution was injected into the HPLC system, and the peak areas of EPL and UMI were noted from the chromatograms. A graph was drawn by plotting concentration of EPL on the X-axis and the corresponding mean peak area ratio (EPL/UMI) on the Y-axis. Intraday and inter-day precision were also studied at three different concentrations of Eplerenone on the same day and on three consecutive days in presence of internal standard.

The accuracy of the method was proved by the standard addition method and the recovery values were determined.. The accuracy of the method was proved by the standard addition method and the recovery values IS were determined. The robustness of an analytical procedure indicates its ability to remain unaffected by small and deliberate changes in method parameters and provides an assurance of its reliability for routine analysis. The proposed method was checked for the robustness by slightly changing the optimized conditions such as flow rate (±0.1 mL), mobile phase composition (±2%) and detection wavelength (247 nm and 237 nm).

Assay of commercial formulations

A total of 20 tablets of the available marketed brand - INSPRA (Label claim: 25 mg; 50 mg) was procured and powdered. The powder equivalent to 25 mg EPL was extracted using the mobile phase sonicated for half an hour and filtered through 0.45 mm membrane filter. 20 µL of this solution was mixed with 10 µg/mL UMI and injected into the HPLC system, and the peak area ratio was determined from the respective chromatogram, and the percentage recovery was calculated.

Stress degradation studies

Forced degradation studies were performed[15] to determine the ability of the drug to withstand its properties in the

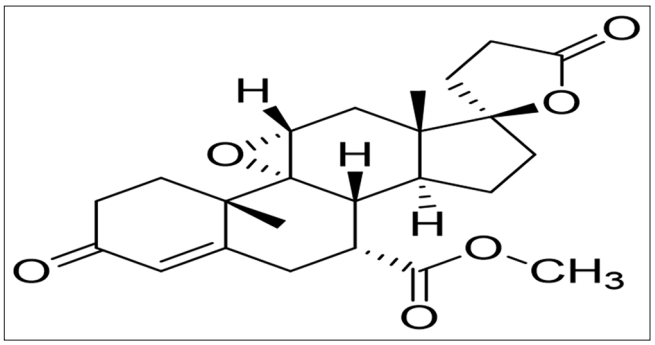

Figure 1: Chemical structure of eplerenone

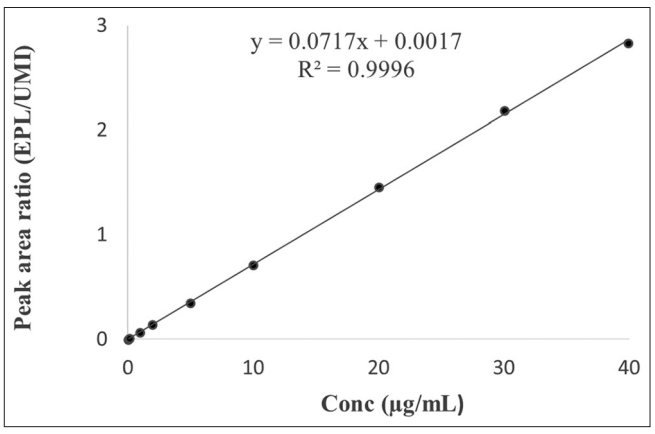

Figure 2: Calibration of eplerenone in the presence of Umifenovir (internal standard)

applied stress conditions. EPL was exposed to different stress conditions such as acidic hydrolysis, basic hydrolysis, oxidation, and hydrolysis. Acidic degradation was performed by treating the drug solution with 0.1 N HCl for 30 min at 80°C in a thermostat and later the solution was cooled, neutralized using sodium hydroxide solution and the solution was made up to volume to the required concentration with the mobile phase. Similarly, the alkaline degradation was performed by treating the drug solution at room temperature with 0.1 N NaOH just for 30 min at 80°, neutralized with hydrochloric acid and diluted with mobile phase. Oxidative degradation was performed by treating the drug solution with 30% v/v H_2O_2 at 80° in the thermostat for 30 min. Hydrolysis was performed by heating the drug solution with 1 ml of HPLC grade water at 80°C for 30 min and then the drug solutions were diluted according to the requirement. All the solutions were filtered through Whatman membrane filter No. 45. 10 µg/mL IS (UMI) was added to all the solutions just before injection so that the IS will not be affected by the reagents used for the stress degradation studies. The peak area values of EPL and UMI were noted from the corresponding chromatograms, and finally, EPL/UMI was calculated. The percentage recovery in all the degradation studies was calculated from the linear regression equation.

RESULTS AND DISCUSSION

A simple, precise, robust, and accurate stability indicating RP-HPLC method have been developed for the determination of EPL in active pharmaceutical ingredient (API) and its tablet dosage forms in the presence of UMI, an IS. The proposed RP-HPLC method was compared with the previously reported analytical techniques in Table 1. Trials were made with different mobile phases, flow rates and different columns and the optimized conditions were shown in Table 2.

Method validation

The proposed method was validated by linearity, precision, accuracy, robustness as per the ICH guidelines for the EPL determination. EPL obeys Beer-Lamberts law (0.1–40 µg/ mL) [Table 3] with linear regression equation, y = 0.0717 x + 0.0017 [Figure 2] and the LOD and LOQ

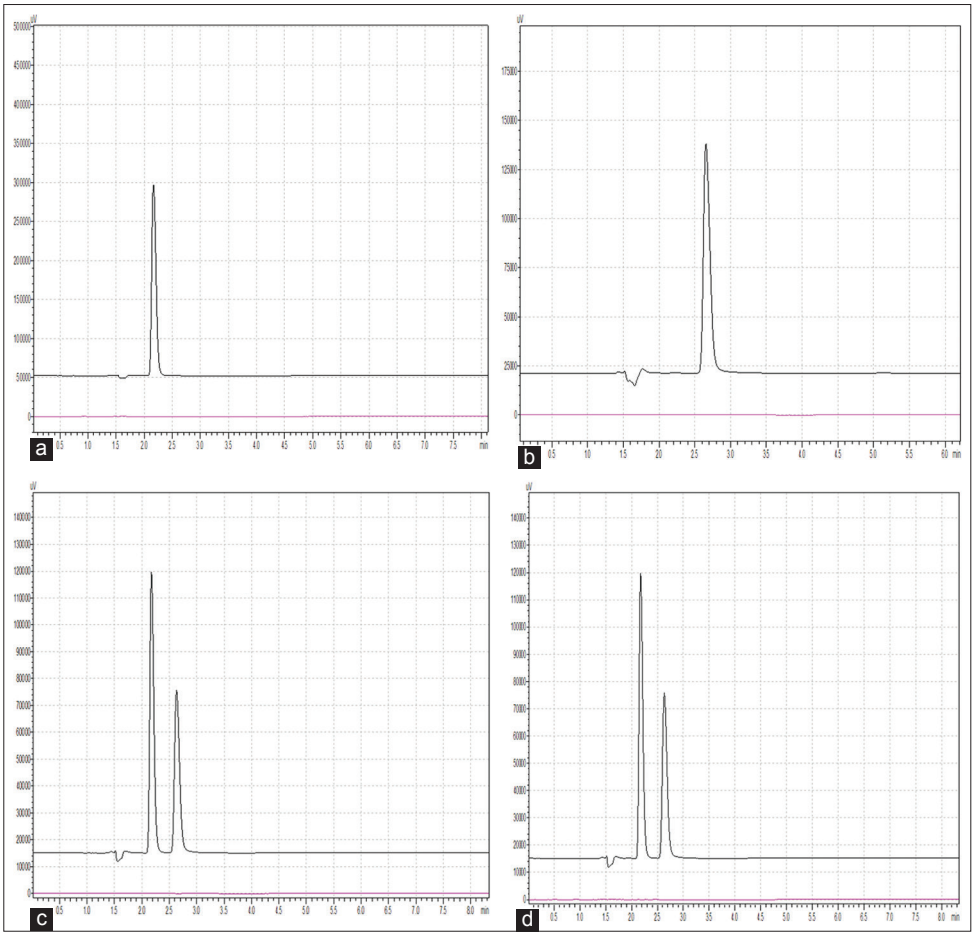

Figure 3: 3D Chromatograms of eplerenone in presence of internal standard during (a) acidic hydrolysis (b) hydrolysis (c) oxidation (d) alkaline hydrolysis

Table 1: Comparison of the present study with the reported methods in literature

Method/reagents	Linearity (μg/mL)	Comments	Ref
LC-MS	0.01–2.5	Human plasma	7
LC-MS/MS	0.05–1.0	Human urine	8
Spectrophotometry (HCl)	5–15	Low linearity range	9
Spectrophotometry (CH_3OH:H_2O) (80:20)	5–45	Costly	10
TLC/densitometry (ethylacetate: toluene: TEA)	200–1200 (ng/band)	Mixture of solvents	11
HPLC (40:60 v/v) tetra ethyl ammonium phosphate (pH 2.3):acetonitrile	15–45	pH maintenance	12
HPLC (55:45 v/v) ammonium acetate: acetonitrile	10–100	Stability indicating method	13
HPLC (30:70 v/v) tetra butyl ammonium hydrogen sulfate: Methanol	0.1–40	Stability indicating method in the presence of IS	Present method

LC-MS: Liquid chromatographic-mass spectrometry

Table 2: Optimized conditions for determination of EPL

Parameter	Optimized chromatographic conditions
Mobile phase	Tetra butyl ammonium hydrogen sulfate: methanol (30:70 v/v)
Column particulars	Agilent column (150 mm×4.6 mm i.d., 3.5 μm particle size)
Detector	SPD M20A prominence photodiode array detector
Flow rate	1.0 mL/min
UV detection	242 nm
Column temperature	(25°±2°C)
Injection volume	20 μL
Elution	Isocratic mode
Retention time	2.159±0.02 min (EPL) and 2.651±0.02 min (UMI)
Total runtime	10 min

EPL: Eplerenone, UMI: Umifenovir

were found to be 0.0283 μg/mL and 0.0846 μg/mL, respectively. EPL was eluted at 2.158 ± 0.01 min in the presence of IS (UMI) [Figure 3a and b]. The combination of EPL standard (API) along with the IS UMI was shown in Figure 3c.

Intraday and interday precisions were studied using three different concentrations of EPL on the same day and on 3 consecutive days, respectively. The percentage relative standard deviation (RSD) was found to be 0.16–0.47 and 0.67–0.92, respectively (<2.0%), demonstrating that the method is precise. The accuracy of the method was proved by the

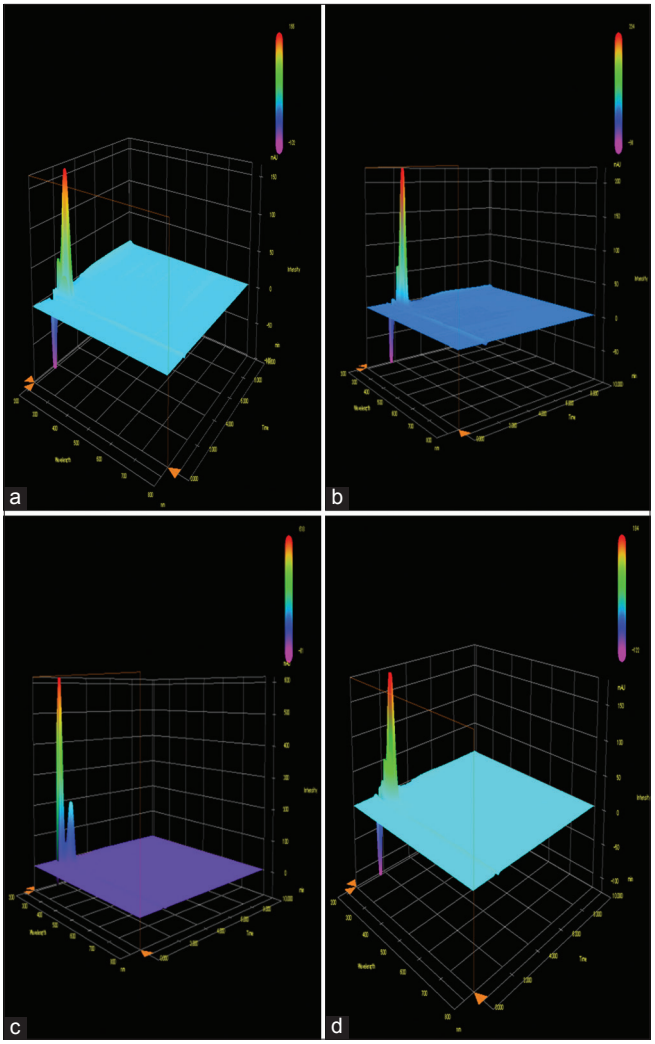

Figure 4: Overlay chromatogram of eplerenone in presence of internal standard (a) acidic hydrolysis (b) hydrolysis (c) oxidation (d) alkaline hydrolysis (e) standard (f) blank

Table 3: Linearity of eplerenone in presence of internal standard

Concentration (μg/mL)		*Mean peak area		Peak area ratio (EPL/UMI)	% RSD
EPL	UMI	EPL	UMI		
0.1	10	6595	920831	0.007	0.21
1	10	65951	922219	0.071	0.27
2	10	134902	922814	0.146	0.32
5	10	317256	922618	0.343	0.21
10	10	659513	922342	0.715	0.23
20	10	1349026	921937	1.46	0.28
30	10	2023539	922132	2.19	0.25
40	10	2608052	921654	2.829	0.30

*Mean of three replicates. EPL: Eplerenone, UMI: Internal standard, RSD: Relative standard deviation

Table 4: Stress degradation studies of eplerenone in presence of internal standard

Stress condition Medium/temperature/ duration	Rt (min)	% recovery	% drug degradation	Theoretical plates	Tailing factor
Standard drug	2.159	100	-	3088	1.45
Acidic hydrolysis (0.1N HCl/80°C/30 min)	2.190	69.25	30.75	3105	1.514
Alkaline hydrolysis (0.1N NaOH/80°C/30 min)	2.174	80.76	19.24	3133	0.011
Oxidative degradation (30% H_2O_2/80°C/30 min)	2.160	83.03	16.96	3026	1.520
Hydrolysis (80°C/30 min)	2.176	45.21	54.79	3029	1.522

EPL: Eplerenone, UMI: Umifenovir, IS: Internal standard

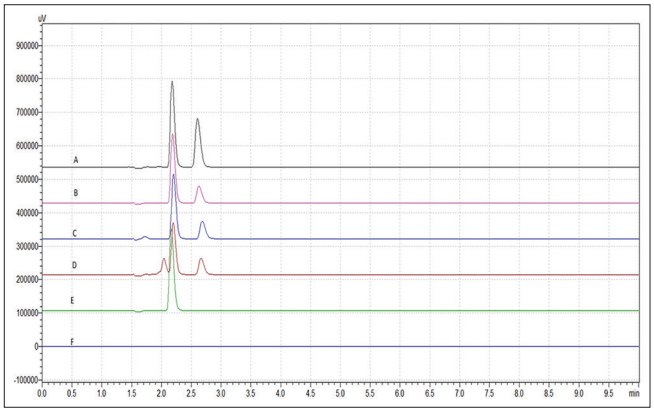

Figure 5: Overlay chromatogram of eplerenone in the presence of Umifenovir (a) acidic hydrolysis (b) hydrolysis (c) oxidation (d) alkaline hydrolysis (e) standard (f) blank

standard addition method, and the recovery values were found to be 98.34–98.97% with percentage RSD 0.56–0.71 (<2.0%) and in robustness study the percentage RSD was found to be 0.91–1.56 which is <2.0. The above results indicate that the method is precise, accurate, and robust. In all the validation studies, the IS was incorporated before injecting the solution into HPLC system.

Assay of commercial formulations

EPL has shown 99.12% recovery in the marketed formulations in the presence of IS. The recovery was calculated from the linear regression equation. The chromatogram obtained during the assay was shown in Figure 3d and no interference of the excipients.

Stress degradation studies

EPL was forced to undergo degradation using HCl, NaOH, hydrogen peroxide and water at 80°C for 30 min. In acidic hydrolysis, about 30.75% of EPL has undergone degradation, and in hydrolysis, about 54.79% has undergone degradation. In alkaline and oxidation, the degradation was reported to be <20%. EPL peak was well separated in all the degradation studies along with the IS UMI without any interference indicating that the method is selective and specific. The system suitability parameters were well in the acceptance criteria [Table 4]. The representative three-dimensional, as well as the overlay chromatograms obtained during the degradation studies, was shown in Figures 3 and 4, respectively.

CONCLUSION

The present RP-HPLC method is simple, economical and was validated as per the ICH guidelines and therefore this method can be used for the determination of EPL in pharmaceutical industries. Furthermore, EPL has shown its sensitivity toward hydrolysis, and acidic degradation and the system suitability parameters are satisfied.

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