

New stability-indicating ultra-fast liquid chromatographic method for the determination of eplerenone - An antimineralocorticoid

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Abstract

Introduction: A new stability indicating RP-UFLC method has been developed for the assay of Eplerenone in presence of and internal standard. Eplerenone is used for the treatment of hypertension, central serious retinopathy and especially in the treatment cardio vascular diseases. **Materials and Methods:** Shimadzu HPLC system (PDA detector) using Agilent column (150 mm × 4.6 mm i.d., 3.5 μm particle size) and (isocratic mode) was used for the chromatographic study of Eplerenone with the mixture of 10 mM tetra butyl ammonium hydrogen sulphate and acetonitrile (50:50, v/v) (UV detection at 242 nm) with flow rate 0.8 mL/min. Stress degradation studies were performed by treating Eplerenone with different reagents to study the degradation profile and the proposed method was validated as per ICH guidelines. **Results and Discussion:** Beer-Lambert's law was obeyed 0.1-40 μg/mL (Correlation coefficient 0.9998). The LOD and LOQ are found to be 0.0291 μg/mL and 0.0863 μg/mL respectively. **Conclusions:** The proposed RP-UFLC method is precise, accurate and sensitive and the method can be used for the routine analysis of Eplerenone pharmaceutical formulations (Tablets).

Key words: Eplerenone, internal standard, reverse-phase ultra-force liquid chromatographic, umifenovir, validation

INTRODUCTION

Eplerenone (ELR) chemically known as 9-11α-epoxymexrenone; 9,11α-Epoxy-7α methoxycarbonyl-3-oxo-17α-pregn-4-ene-21, 17-carbolactone is used as an antimineralocorticoid. ELR inhibits the overactivation of the mineralocorticoid receptor pathways. It belongs to spironolactone group and is used for the management of chronic heart failure.^[1-3] ELR blocks aldosterone activity which is responsible for the increase of blood pressure and metabolized by cytochrome P450 enzyme CYP3A4.^[4-6] Food and Drug Administration has given approval in 2002. ELR is available with brand name INSPRA (Pfizer) as tablets (label claim: 25 mg; 50 mg). Different analytical techniques such as thin-layer chromatography/densitometry,^[7] LC-mass spectrometry (MS),^[8] reverse-phase

high-performance liquid chromatograph (RP-HPLC),^[9,10] spectrophotometry,^[11,12] and LC-MS/MS^[13] have been reported for the determination of ELR. Umifenovir (UFV) is an antiviral drug and used as internal standard (IS). In the present study, the authors have reported a new stability-indicating RP ultra-force liquid chromatographic (RP-UFLC) method for the quantification of ELR in the presence of IS, and the method was validated as per ICH guidelines.^[14]

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Chemicals and Reagents

All other chemicals are of analytical reagent grade and all solvents are of HPLC grade (Merck). ELR was obtained from Pfizer (India). It is available with brand name INSPRA (Pfizer) as tablets with label claim: 25 mg; 50 mg.

Preparation of Standard Solutions

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

Optimized Chromatographic Conditions

Chromatographic was performed using Shimadzu model CBM-20A/20 Alite HPLC system (Shimadzu Co., Kyoto, Japan) equipped with SPD M20A prominence PDA detector. 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 tetrabutylammonium hydrogen sulfate and ACN (50:50, v/v) (isocratic mode). The same mobile phase mixture but with different composition was used as diluent (50:50, v/v). UFV (10 µg/mL) was used as an IS throughout the analysis [Figure 1].

MATERIALS AND METHODS

Method Validation

ELR solutions (0.1–40 µg/mL) were prepared from the stock solution and 10 µg/mL UFV was added to each solution and 20 µL of these solutions was injected into the UFLC system and the mean peak area of ELR with respect to UFV was noted from the respective chromatograms and a calibration curve was drawn by plotting concentration of ELR on the x-axis and the corresponding mean peak area ratio (ELR/UFV) on the y-axis. Intraday and interday precision studies were performed, and the accuracy of the method was calculated from the standard addition method. 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 ($\pm 2\%$), and detection wavelength (237 nm and 247 nm).

Assay of Commercial Formulations

A total of 20 tablets of available marketed brand - INSPRA (label claim: 25 mg; 50 mg) were procured and powdered.

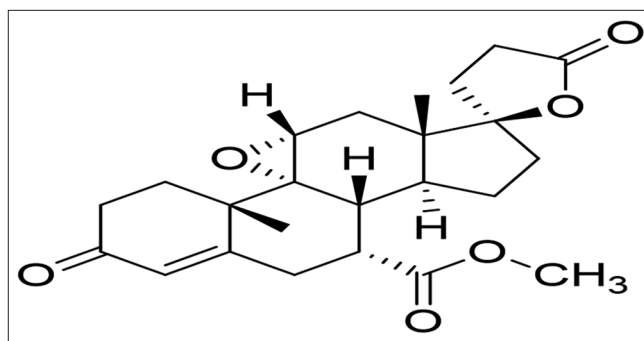


Figure 1: Structure of Eplerenone

The powder equivalent to 25 mg ELR was extracted using the mobile phase, sonicated for ½ h, and filtered through 0.45 µm membrane filter. 20 µL of this solution was mixed with 10 µg/mL UFV 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 applied stress conditions. ELR 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 H₂O₂ 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 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 ELR and UFV were noted from the corresponding chromatograms, and finally, ELR/UFV was calculated. The percentage recovery in all the degradation studies was calculated from the linear regression equation.

RESULTS AND DISCUSSION

A new stability-indicating RP-UFLC method has been developed for the determination of ELR in its tablet dosage forms in the presence of an IS, i.e., UFV. Previously

reported analytical techniques in the literature were summarized in Table 1. Trials were made with different columns, flow rates, and different mobile phases and mobile phase compositions. The optimized conditions are shown in Table 2.

Method Validation

The present UFLC method was validated by linearity, precision, accuracy, and robustness as per the ICH guidelines. ELR was eluted at 3.420 ± 0.01 min in the presence of IS UFV. UFV was eluted at 2.175 ± 0.02 min. The typical chromatogram of ELR standard in the presence of IS (UFV) is shown in Figure 2. ELR obeys Beer-Lambert's law (0.1–40 $\mu\text{g/mL}$) [Table 3] with linear regression equation, $y = 0.1401x + 0.0136$ [Figure 3]. The method was

validated as per ICH guidelines. The limit of detection and limit of quantification were found to be 0.0291 $\mu\text{g/mL}$ and 0.0863 $\mu\text{g/mL}$, respectively.

The % relative standard deviation (RSD) in intraday and interday precision studies was found to be 1.21–0.12 and 0.16–0.52, respectively ($<2.0\%$), indicating that the method is precise. The accuracy of the method was proved by the standard addition method, and the recovery values were 98.34–100.7%. The % RSD in accuracy and robustness study was found to be 0.66–1.02 ($<2.0\%$) and 0.91–1.56 which is <2.0 . The above results specify that the method is precise, accurate, and robust. In validation as well as stress degradation studies, the IS was incorporated just before injection.

Table 1: Comparison of present UFLC method with other analytical techniques

| Method/reagents/mobile phase (v/v) | Linearity ($\mu\text{g/mL}$) | Comments | Ref |
|---|--------------------------------|---------------------------|----------------|
| TLC/densitometry (ethyl acetate: toluene: TEA) | 200–1200 (ng/band) | Mixture of solvents | [7] |
| LC-MS | 0.01–2.5 | Human plasma | [8] |
| HPLC (tetraethyl Amm. Phos.):ACN (40:60) | 15–45 | pH maintenance | [9] |
| HPLC (ammonium acetate: ACN) (55:45) | 10–100 | Stability indicating | [10] |
| Spectrophotometry (HCl) | 5–15 | Low linearity range | [11] |
| Spectrophotometry (CH_3OH : H_2O) (80:20) | 5–45 | Costly | [12] |
| LC-MS/MS | 0.05–1.0 | Human urine | [13] |
| UFLC (TBAHS: ACN) (50:50) | 0.1–40 | Stability indicating (IS) | Present method |

TLC: Thin-layer chromatography, LC-MS: Liquid chromatography–mass spectrometry, ACN: Acetonitrile, HPLC: High-performance liquid chromatograph, UFLC: Ultra-force liquid chromatographic, TBAHS: Tetrabutylammonium hydrogen sulfate, IS: Internal standard

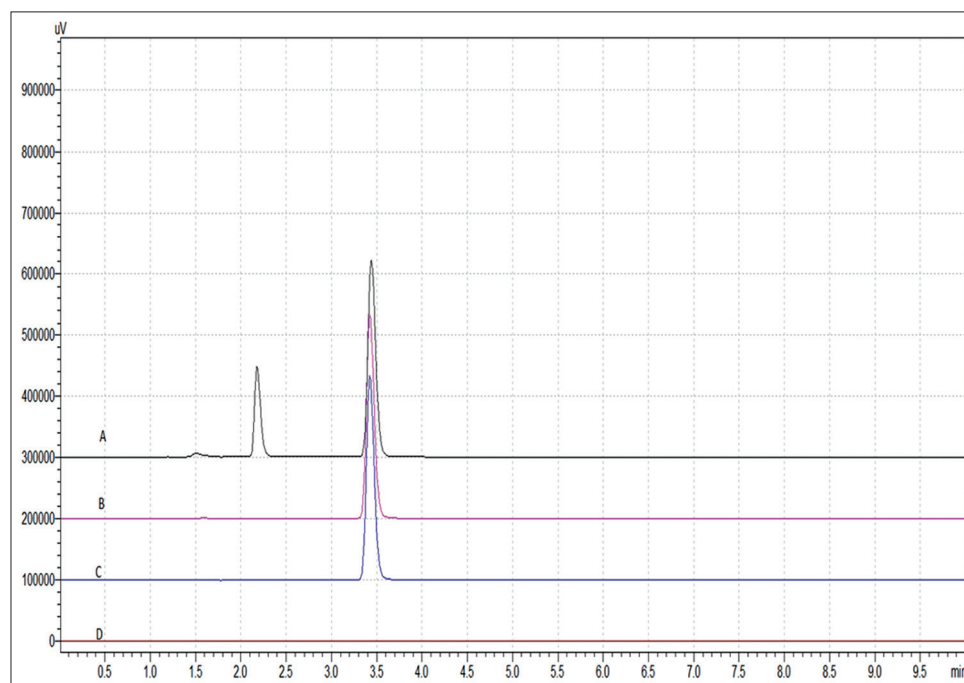


Figure 2: Overlay chromatogram of a) Eplerenone tablet (INSPIRA) in presence of Umifenovir (IS) b) Eplerenone tablet c) Eplerenone standard (20 $\mu\text{g/mL}$) d) Blank

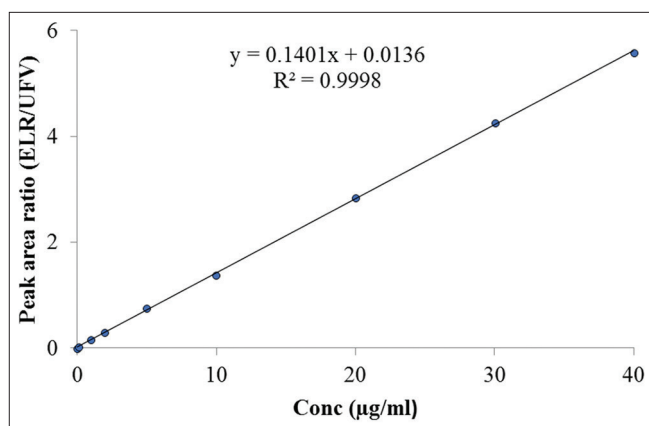


Figure 3: Calibration of eplerenone in presence of umifenovir (IS)

Table 2: Optimized conditions for the determination of ELR

| Parameter | Optimized chromatographic conditions |
|---------------------|--|
| Mobile phase | Tetra butyl ammonium hydrogen sulphate: Acetonitrile (50: 50, v/v) |
| Column particulars | Agilent column (150 mm×4.6 mm i.d., 3.5 µm particle size) |
| Detector | SPD M20A prominence PDA |
| Flow rate | 0.8 mL/min |
| UV detection | 242 nm |
| Column temp. | (25±2°C) |
| Injection volume | 20 µL |
| Elution | Isocratic mode |
| Retention time (Rt) | 3.420±0.02 min (ELR) |
| Internal standard | Umifenovir (Rt 2.175 ± 0.02 mins) |
| Total runtime | 10 mins |

PDA: Photodiode array, UV: Ultraviolet, TBAHS: Tetrabutylammonium hydrogen sulfate, IS: Internal standard, ACN: Acetonitrile, ELR: Eplerenone

Assay of Commercial Formulations

ELR has shown 99.87% recovery in the marketed formulation in the presence of IS. The recovery was calculated from the linear regression equation. The chromatogram obtained during the assay is shown in Figure 2 and no interference of the excipients was observed.

Stress Degradation Studies

ELR was forced to undergo degradation using HCl, NaOH, hydrogen peroxide, and water at 80°C for 30 min. In acidic hydrolysis, about 5.4% of ELR has undergone degradation and in hydrolysis about 38.5% of ELR has undergone degradation. In alkaline hydrolysis, about 9.3% degraded and oxidation the degradation was reported to be 3.5%. The ELR drug peak was separated well in all the degradation studies along with the IS UFV without any interference indicating that the method is selective and specific. The system suitability parameters were well in the acceptance criteria [Table 4]. The typical chromatograms obtained during the stress degradation studies are shown in Figure 4, whereas the three-dimensional chromatograms are shown in Figure 5.

CONCLUSION

The proposed RP-UFLC method is simple, precise, accurate, robust and economical and was validated as per ICH guidelines. The reported method can be used for the routine analysis of Eplerenone tablets. Eplerenone is found to be more sensitive towards hydrolysis and the method is selective and specific.

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Table 3: Linearity of ELR in the presence of UFV (IS)

| Concentration (µg/mL) | | *Mean peak area | | Peak area ratio (ELR/UFV) | % RSD |
|-----------------------|-----|-----------------|--------|---------------------------|-------|
| ELR | UFV | ELR | UFV | | |
| 0.1 | 10 | 10807 | 719053 | 0.015 | 0.05 |
| 1 | 10 | 108090 | 719632 | 0.150 | 0.06 |
| 2 | 10 | 216380 | 719435 | 0.301 | 0.49 |
| 5 | 10 | 540442 | 719574 | 0.752 | 1.8 |
| 10 | 10 | 990585 | 719826 | 1.376 | 0.11 |
| 20 | 10 | 2038603 | 718542 | 2.837 | 1.26 |
| 30 | 10 | 3064701 | 718597 | 4.260 | 0.76 |
| 40 | 10 | 4016618 | 719021 | 5.581 | 0.10 |

*Mean of three replicates. ELR: Eplerenone, UFV: Umifenovir, RSD: Relative standard deviation, IS: Internal standard

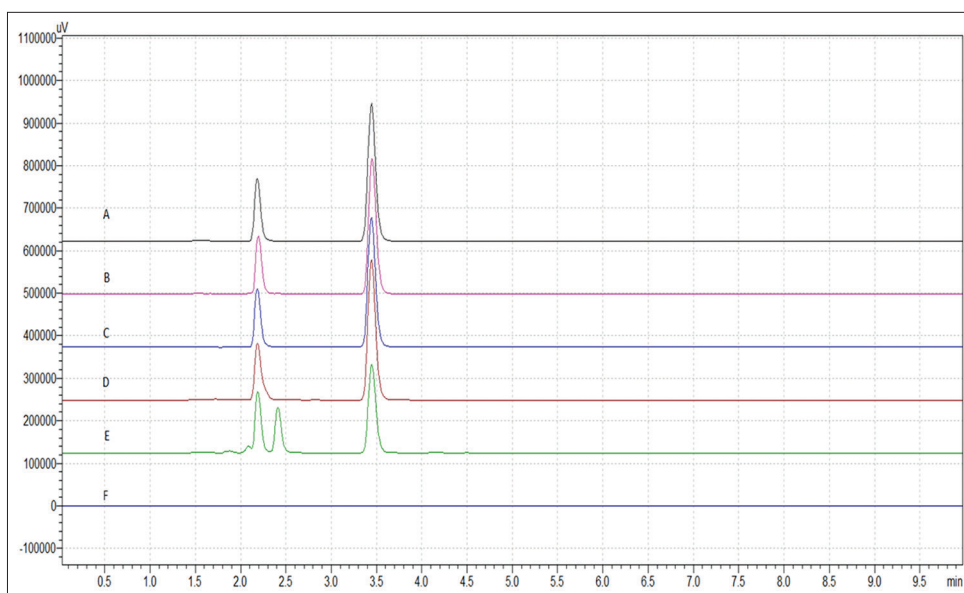


Figure 4: Overlay Chromatogram of (a) Eplerenone (20 µg/mL) in presence of Umifenovir (b) Acidic hydrolysis (c) Hydrolysis (d) Oxidation (e) Alkaline hydrolysis (f) Baseline

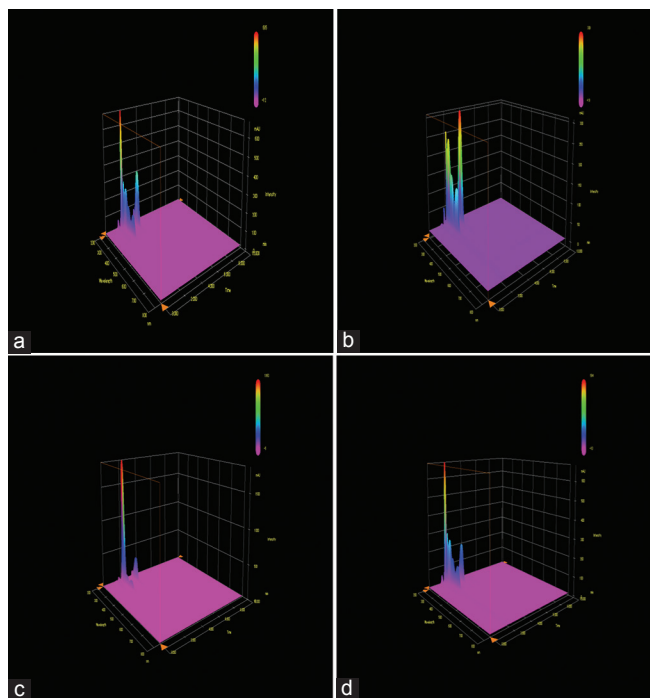


Figure 5: Three-dimensional chromatograms of eplerenone in the presence of internal standard during (a) acidic hydrolysis (b) hydrolysis (c) oxidation (d) alkaline hydrolysis

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