ORIGINAL ARTICLE

A Sensitive Reversed-phase High-performance Liquid Chromatography Method for the Analysis of Eplerenone

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Abstract

Introduction: Eplerenone is an anti-hypertensive drug. A sensitive reversed-phase high-performance liquid chromatography method was developed for the assay of Eplerenone (Isocratic mode) in the presence of an internal standard, teneligliptin (an antidiabetic drug). **Materials and Methods:** Mobile comprising formic acid and methanol was selected (flow rate: 0.4 ml/min), and Eplerenone as well as the internal standard were eluted a detection wavelength of 244 nm. Phenomenex C8 column was used during the study, and the HPLC system was from Shimadzu with photodiode array detector. **Results and Discussion:** Eplerenone follows linearity according to Beer–Lambert's law 0.5–60 µg/mL with regression equation, y = 0.2054x + 0.0351 correlation coefficient 0.9996. The LOD and LOQ are found to be 0.0141 µg/mL and 0.3947 µg/mL, respectively. **Conclusions:** The liquid chromatographic method is simple and sensitive, and the method was validated for the determination of Eplerenone in pharmaceutical dosage forms.

Key words: Eplerenone, internal standard, isocratic mode, reversed-phase high-performance liquid chromatography, validation

INTRODUCTION

Eplerenone (EPLR) is an anti-mineralo corticoid used to block the aldosterone activity and thereby reduces the blood pressure.[1-6] Analytical techniques are established for the quantification of Eplerenone such as liquid chromatography-mass spectrometry (LC-MS),[7] LC-MS/MS,[8] thin-layer chromatography (TLC)/densitometry,[9] spectrophotometry,[10,11] and reversed-phase high-performance liquid chromatography (RP-HPLC).[12-15] Teneligliptin was introduced into the study as internal standard (IS) in the present study. The authors describe a simple and sensitive liquid chromatographic method for the assay of Eplerenone and the method was validated [Figure 1].[16]

MATERIALS AND METHODS

Chemicals and reagents

Eplerenone was obtained from Pfizer (India) and Zydus Cadila (India) as gift samples and was used as supplied and all other chemicals were of HPLC grade (Merck). Eplerenone is available as tablets (label claim: 25 mg; 50 mg) with brand name INSPRA (Pfizer).

Preparation of Eplerenone and Internal standard stock solutions

Stock solutions of Eplerenone and internal standard (teneligliptin) were prepared by dissolving 25 mg of teneligliptin and Eplerenone separately in two separate 25 mL volumetric flasks with methanol (HPLC grade) (1000 µg/mL) and diluted with mobile phase on a daily basis for the

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study. A 10 µg/mL teneligliptin internal standard was used during the study.

Method validation

Chromatographic separation was performed on isocratic mode with methanol and formic acid mixture (75:25 v/v) at a flow rate of 0.4 mL/min and with detector signal at 244 nm. 0.5–60 µg/mL of Eplerenone solutions were prepared along with the internal standard (teneligliptin) (10 µg/mL) and injected into the HPLC system. The mean peak area of Eplerenone and teneligliptin was calculated from the chromatograms, and calibration curve was drawn using concentration of Eplerenone on the X-axis and the mean peak area ratio of Eplerenone to that of the internal standard on theY-axis (Eplerenone/internal standard). Precision and accuracy (spiked 50%, 100%, and 150%) studies were performed, and percentage RSD was calculated.

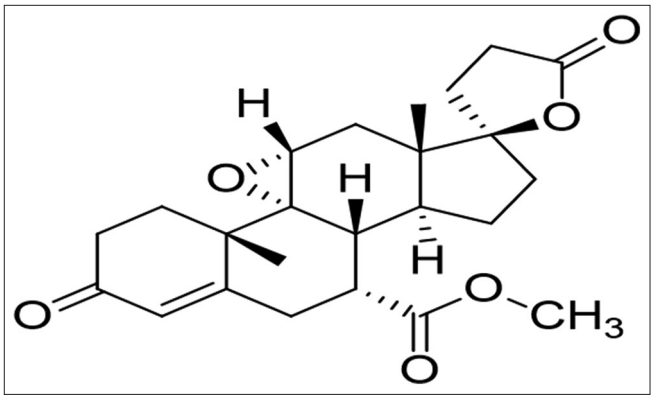

Figure 1: Structure of eplerenone

Assay of Eplerenone tablets

Tablets of different pharmaceutical companies were bought, powdered and powder equivalent to 25 mg Eplerenone was extracted with methanol and filtered after sonication for 30 min. The resulting solutions were mixed with internal standard solution and then injected into the HPLC system. The peak area of the chromatograms of Eplerenone and internal standard was noted from the calibration curve.

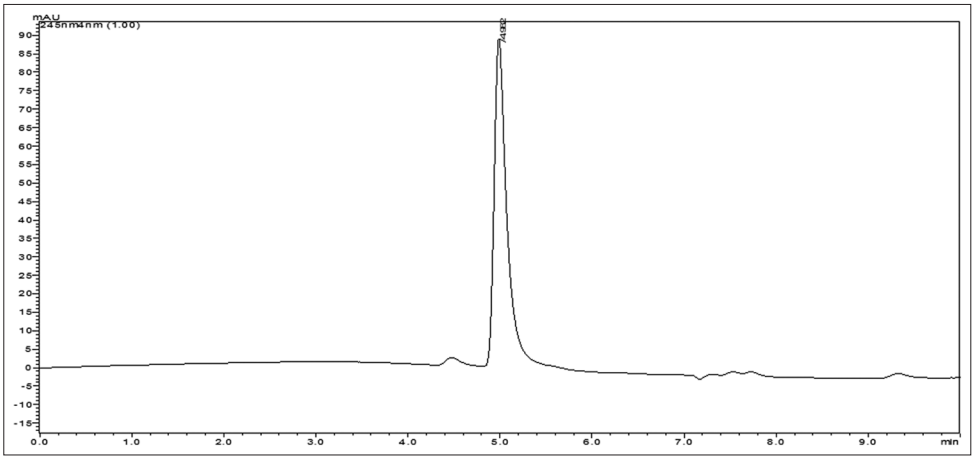

Figure 2: Chromatogram of internal standard (Teneligliptin) (10 µg/mL) at Rt: 4.982 min

Table 1: Comparison of proposed method with the previously published methods

Method/reagents/mobile phase (v/v)	Linearity (µg/mL)	Comments	Ref
LC-MS	0.01–2.5	Human plasma	[7]
LC-MS/MS	0.05–1.0	Human urine	[8]
TLC/densitometry (Ethylacetate:Toluene:TEA)	200–1200 (ng/band)	Mixture of solvents	[9]
Spectrophotometry (HCl)	5–15	Low linearity range	[10]
Spectrophotometry (CH_3OH:H_2O) (80:20)	5–45	Costly	[11]
HPLC (tetra ethyl Amm. Phos.): ACN (40:60)	15–45	pH maintenance	[12]
HPLC (ammonium acetate: ACN) (55:45)	10–100	Stability indicating	[13]
UFLC (tetra butyl ammonium hydrogen sulfate: ACN) (50:50)	0.1–40	Stability indicating (IS)	[14]
UFLC (tetra butyl ammonium hydrogen sulfate: methanol) (30:70)	0.1–40	Stability indicating (IS)	[15]
HPLC (0.1% Formic acid: methanol) (25:75)	0.5-60	IS	Present method

LC-MS: Liquid chromatography-mass spectrometry, TLC: Thin-layer chromatography, IS: Internal standard

RESULTS AND DISCUSSION

A sensitive liquid chromatographic method has been developed for the determination of Eplerenone in the presence of internal standard (teneligliptin: 10 µg/mL) using formic acid and methanol mixture as the mobile phase. Shimadzu Model CBM-20A/20Alite HPLC system (Shimadzu Co., Kyoto, Japan) equipped with SPD M20A prominence photodiode array detector was the instrument employed for the study with Phenomenex C8 column using (0.1%) formic acid:methanol mixture (25:75, % v/v) as the mobile phase. The present proposed method was compared with the previously published methods in Table 1.

Table 2: Linearity of Eplerenone

Concentration (µg/mL)	*Mean peak area		*Mean peak area ratio
	EPLR	IS	(EPLR/IS)
0	0	0	0
0.5	84375	824589	0.1023
5	857561	822158	1.0431
10	1718374	822563	2.0891
20	3500548	831547	4.2097
30	5126548	830287	6.1744
40	7023986	829625	8.4665
50	8526512	834587	10.2165
60	10198956	830154	12.2856

*Mean of three replicates

Method optimization

During the optimization, the above mobile phase was tried in different ratio – 40: 60, 35: 65, and 30: 70 with the same flow rate 0.4 ml/min where tailing was observed continuously more than 2.0. However, enhancement of organic phase overcome the tailing, i.e., (0.1%), formic acid:methanol mixture with 25:75 ratio results in a sharp peak at 8.529 min for Eplerenone and that of the internal standard at 5.096 min. The chromatogram of internal standard (teneligliptin) and Eplerenone was shown in Figures 2 and 3. Instead of quantifying Eplerenone alone, the presence of internal standard prunes the data better using HPLC and therefore all the calculations were performed using the internal standard. The system suitability parameters are within

Table 3: Intraday precision study of Eplerenone

Concentration (µg/mL)	*Mean peak area		*Mean peak area ratio	Statistical parameters
	EPLR	IS	(EPLR/IS)	Average±SD (% RSD)
5	857695	821563	1.044	1.042±0.0025 (0.24)
5	857861	825482	1.039	
5	859521	823625	1.044	
10	1729655	823654	2.099	2.095±0.004 (0.19)
10	1726594	825461	2.092	
10	1724852	826985	2.086	
20	3412569	830014	4.112	4.141±0.0295 (0.71)
20	3421456	820314	4.171	
20	3482568	826954	4.211	

*Mean of three replicates. EPLR: Eplerenone

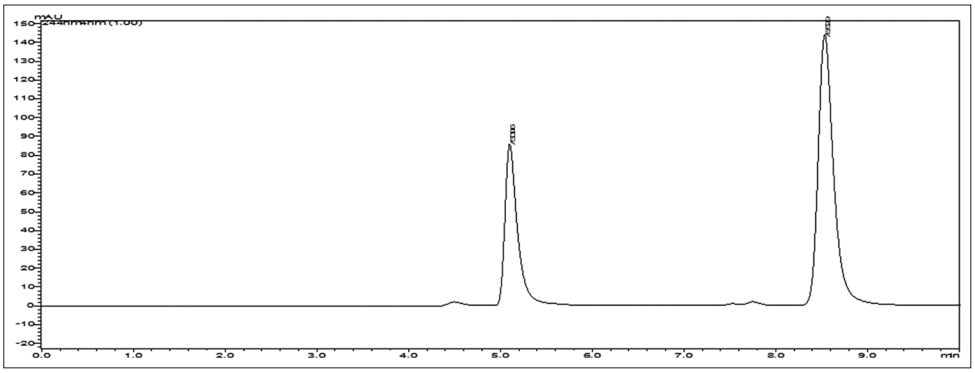

Figure 3: Chromatogram of eplerenone in the presence of IS (10 µg/mL) Eplerenone (Rt: 8.529 min; theoretical plates: 12348); internal standard (Rt: 5.096 min; theoretical plates: 6669

Table 4: Interday precision study of Eplerenone

Concentration (µg/mL)	*Mean peak area		*Mean peak area ratio	Statistical parameters
	EPLR	IS	(EPLR/IS)	Average±SD (% RSD)
5	857695	821563	1.043	1.0405±0.0025 (0.24)
5	852584	821653	1.038	
5	859521	821589	1.046	
10	1729655	823654	2.099	2.092±0.005 (0.238)
10	1726581	826598	2.089	
10	1725468	822657	2.097	
20	3412569	830014	4.112	4.113±0.001 (0.02)
20	3412569	829584	4.114	
20	3412569	828456	4.119	

*Mean of three replicates. EPLR: Eplerenone

Table 5: Accuracy study of Eplerenone

*Concentration (µg/mL)				% Recovery
Dosage form	API	Total	Obtained (% RSD)	
10	5	15	14.86 (0.36)	99.67
10	5	15		
10	5	15		
10	10	20	19.89 (0.59)	99.45
10	10	20		
10	10	20		
10	15	25	24.93 (1.03)	99.72
10	15	25		
10	15	25		

*Mean of three replicates. EPLR: Eplerenone

Table 6: Assay of Eplerenone tablets

Tablet brands	Label claim (mg)	*Amount found (mg)	*Recovery (%)
I	25	24.98	99.92
II	25	24.86	99.44
III	25	24.91	99.64

*Mean of three replicates. EPLR: Eplerenone

the acceptable criteria, i.e., theoretical plates 12348 (above 2000) and tailing factor 1.427 (<1.5) with resolution 2.569 (>2.0) for Eplerenone.

Method validation

Eplerenone follows linearity 0.5–60 µg/mL in the presence of internal standard [Table 2] and the calibration curve [Figure 4] linear regression equation, y = 0.2054x + 0.0351 (R^2 = 0.9996) [Figure 4]. Precision [Tables 3-5] and accuracy [Table 5] results were tabulated. The method is precise and accurate as percentage RSD is <2.0.

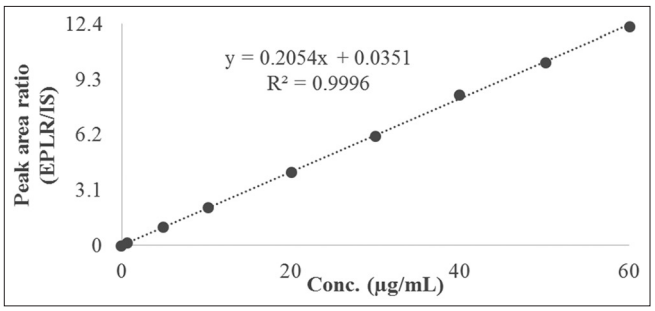

Figure 4: Calibration of eplerenone in the presence of IS

Assay of Eplerenone tablets

Eplerenone tablets of three different brands were analyzed and 99.44–99.92 recovery was observed [Table 6]. No interference of excipients was reported.

CONCLUSIONS

The proposed liquid chromatographic method for the assay of Eplerenone is simple and validated as per the ICH guidelines. It is suitable for the analysis of Eplerenone in pharmaceutical formulations.

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