

STABILITY-INDICATING HPTLC METHOD FOR DETERMINATION OF
EPLERENONE

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ABSTRACT

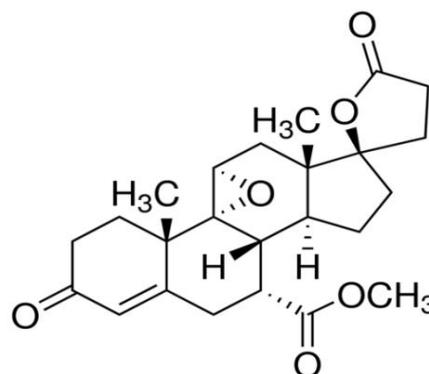
A simple and economical thin-layer chromatography /densitometry method has been developed for determination of eplerenone. The method was validated as per International Conference on Harmonization (ICH) guidelines. Aluminium TLC plates precoated with silica gel 60F₂₅₄ were used as the stationary phase and ethyl acetate: toluene 7:3 (v/v) as mobile phase. A compact band (R_f 0.52 \pm 0.03) was obtained for Eplerenone. Densitometric analysis was performed in the absorbance mode at 238 nm. Linear regression analysis revealed a good linear relationship with ($R^2 = 0.990 \pm 0.0004$) between peak area and concentration in the range 1500 - 9000 ng/band. The mean value of the slope and intercept were 2.29 and 7126.9 respectively. The limits of detection and quantitation were 114.06 ng/band and 345.64 ng/band, respectively. Eplerenone was subjected to acid, alkali hydrolysis, oxidation, photochemical and thermal degradation.

KEYWORDS: Eplerenone, HPTLC, Forced degradation, Validation.

INTRODUCTION

Eplerenone is chemically Pregn-4-ene-7,21-dicarboxylic acid, 9,11-epoxy-17-hydroxy-3-oxo-, γ -lactone, methyl ester, (7 α ,11 α ,17 α), an antihypertensive drug that is highly selective aldosterone receptor antagonist (SARA).^[1-2] Eplerenone binds to the mineralocorticoid receptor and blocks the binding of aldosterone, a component of rennin-angiotensin aldosterone system.^[3] In literature few analytical methods such as LC/MS/MS^[4] UP-LC,^[5] UV^[6] method have been reported for the determination of Eplerenone in biological fluids. RP-HPLC method has been reported for determination of Eplerenone in tablet formulation,^[7-8] SIM-HPLC.^[9] There was information related to the TLC/densitometric method for estimation of Eplerenone in pharmaceutical dosage forms reported in literature.^[10] Hence, considering inherent advantage of HPTLC over HPLC, the objective of current work was to develop Stability Indicating HPTLC Method (SIM) as per ICH Q1A (R2) guidelines.^[11] It was aimed to establish inherent stability of the Eplerenone through stress studies under a variety of stress conditions and to validate the Stability-Indicating Assay method.^[12]

Chemical structure of Eplerenone



MATERIALS AND METHODS

Reagents and Chemicals

Working standard of Eplerenone were obtained from Alkem laboratories Mumbai (India). Acetonitrile (AR grade), Toluene (AR grade), Ethylacetate (AR grade), Hydrochloric acid (HCl), sodium hydroxide (NaOH), hydrogen peroxide (H₂O₂, 30% v/v), and methanol (AR grade) were purchased from Merck specialties Pvt. Ltd. (Mumbai, India).

Instrumentation and chromatographic conditions

Precise analytical weighing balance (Shimadzu AY120) was used for weighing. Chromatographic separation of drug was performed using aluminium plate precoated

with silica gel 60 F₂₅₄ (10 × 10) with 250 µm thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). Samples were applied on the plate as a band with 6 mm width using CAMAG 100 µL sample syringe (Hamilton, Switzerland). Thermal degradation study was carried out in hot air oven (Make-Kumar lab). Linear ascending development was carried out in 10 x 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) by using ethyl acetate: toluene 7:3 (v/v) as mobile. The optimized chamber saturation time for mobile phase was 15 min. The length of chromatogram run was 9 cm and development time was approximately 15 min. TLC plates were dried in a current of air. Densitometric scanning was performed on CAMAG thin layer chromatography scanner at 238 nm operated by WINCATS software version 1.4.2. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm.

Preparation of Stock Solution

Standard stock solution of Eplerenone was prepared by dissolving 10 mg of drug in 10 ml of acetonitrile to get concentration of 1000 µg/ml from which 1 ml was further diluted to 10 ml with acetonitrile to get concentration of solution 100 µg/ml.

Selection of Detection Wavelength

The UV spectrum of Eplerenone (10 µg/ml) solution was obtained over the range of 200-400 nm. It was observed that drug showed considerable absorbance at 238 nm. So, wavelength 238 nm was selected as the wavelength for detection.

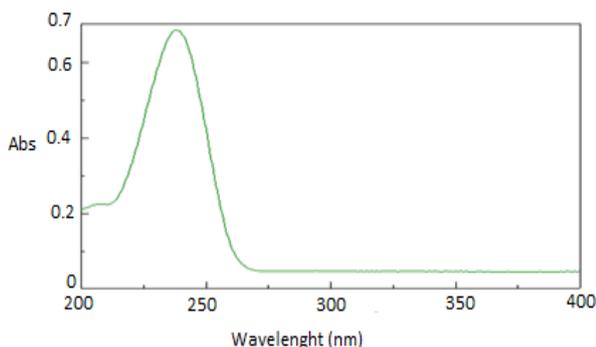


Fig. 1: Absorbance Spectrum of Eplerenone.

Stress degradation studies of bulk drug

The forced degradation studies were carried out on bulk drug substance in order to prove the stability-indicating property and selectivity of the developed method. The degradation was carried out under acid, base, oxidative, thermolytic and photolytic conditions. Stress conditions were optimized to achieve 10 to 30 % degradation.

Acid treatment

To 1 ml of stock solution of Eplerenone (10,000 ng/band), 1 ml of 0.1N HCL was added. The volume was made up to 10 ml by Acetonitrile reflux for 1 hour. The 4

µl of resultant solution were applied on the TLC plate and densitogram was developed. 86.78% of Eplerenone was recovered with no peak of degradant.

Alkali treatment

To 1 ml of stock solution of Eplerenone (10,000 ng/band), 1 ml of 0.1 N NaOH was added. The volume was made up to 10 ml by Acetonitrile kept for 10 min. The 4 µl of resultant solution were applied on the TLC plate and densitogram was developed. 88.79 % of Eplerenone was recovered with no peak of degradant.

Oxidative treatment

To 1 ml of stock solution of Eplerenone (10,000 ng/band), 1 ml of 30% v/v H₂O₂ of was added. The volume was made up to 10 ml by Acetonitrile kept for 10 min. The 4 µl of resultant solution were applied on the TLC plate and densitogram was developed. 82.50 % of Eplerenone was recovered with no peak of degradant.

Dry heat treatment

The powdered drug was stored for 2 hours under dry heat condition at 60° C. A solution of the treated powder was then prepared and 4 µL was applied to a plate. The plate was then chromatographed and treated as described above.

Photochemical treatment

The solution was kept in UV till illumination achieved was 200 watt hrs/square meter and was applied to a plate. The plate was then chromatographed and treated as described above. Weighed sample was exposed to Florescence upto 1.2 million Lux. Hrs and 4 µL was applied to a plate. The plate was then chromatographed and treated as described above.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

Ethyl acetate: toluene (7:3v/v) was selected as mobile phase because it resulted in acceptable resolution of the bands with R_f values of 0.52 ± 0.03 for Eplerenone. The densitogram obtained from standard Eplerenone is shown in Fig.2.

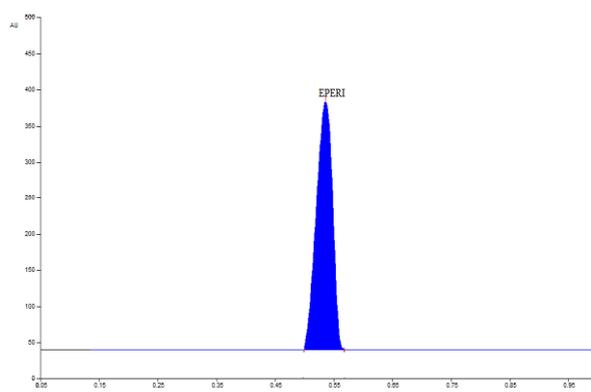


Fig. 2: Densitogram obtained for Eplerenone (R_f - 0.52 ± 0.03) 1500 ng/bond.

Results of forced degradation studies

Forced degradation study showed that the method is highly specific and there was no interference of degradation products observed at retention factor of drug.

Acid degradation

In acid hydrolysis condition, 86.78 % of was recovered with no peak of degradation.

Alkali treatment

In alkali hydrolysis condition, 88.79 % was recovered with no peak of degradation.

Oxidative degradation

In oxidative hydrolysis condition, 82.50 % was recovered with no peak of degradation.

Dry heat degradation

When the drug substance was exposed to dry heat at 60° C for 2 hrs 94.49% of Eplerenone was recovered with no peak of degradant.

Photo degradation

Eplerenone exhibited 84.36% was recovered, when exposed to ultraviolet light (200 Watthours/Square meter) and 85.52 % was recovered when exposed to fluorescence light (1.2 million lux hours).

Table 1: Data of forced degradation studies of Eplerenone.

Stress conditions/ duration	% Recovery	% Degradation	Peak Purity	
			r(s,m)	r(s,m)
Base (0.1 N NaOH, Kept for 10 min)	88.79	13.21	0.9995	0.9998
Acid (0.1 N HCl reflux for 1 Hour)	86.78	11.20	0.9992	0.9993
H ₂ O ₂ 30% v/v (kept for 10 min)	82.50	17.49	0.9994	0.9997
Thermal (60° C, 2 hrs)	94.49	5.50	0.9996	0.9997
UV light (200 watt hrs/square meter)	84.36	15.63	0.9994	0.9992
Florescence light (1.2 million Lux. Hrs)	85.52	14.47	0.9996	0.9993

Validation

1. Specificity

The specificity of the method was determined by analysis of drug standard and sample. The band for Eplerenone in sample was identified by comparing the R_f values and spectrum of the band with those of the band from a Eplerenone standard. The peak purity of Eplerenone was accessed by comparing the spectra at three different positions on the peak, i.e., peak start (S), peak apex (M) and peak end (E) positions of the band.

2. Assay

To determine the Eplerenone content of conventional tablets Eptus; twenty tablets were weighed and powdered in a glass mortar. An amount of powder equivalent to 25 mg Eplerenone was transferred to 100 ml volumetric flask, extracted with methanol, sonicated for 20 min and diluted to mark with same solvent. The resulting solution was filtered through whatman filter paper. An appropriate volume of 4 ml was diluted to 10 ml with acetonitrile .This solution (3 μL, 3000 ng/band) was applied to a plate for assay of Eplerenone.

3. Linearity and range

The standard stock solutions of Eplerenone (100μg/ml) were applied by spotting on TLC plate in range of, 15, 30, 45, 60, 75 and 90 μl (Fig. 3). Straight-line calibration graphs were obtained $y = 2.2977x + 7126.9$ in concentration range 1500-9000 ng/band with high correlation coefficient > 0.99. The results obtained are shown in, the peak areas were plotted against the corresponding concentrations to obtain the calibration curve as shown in Fig.3 for Eplerenone.

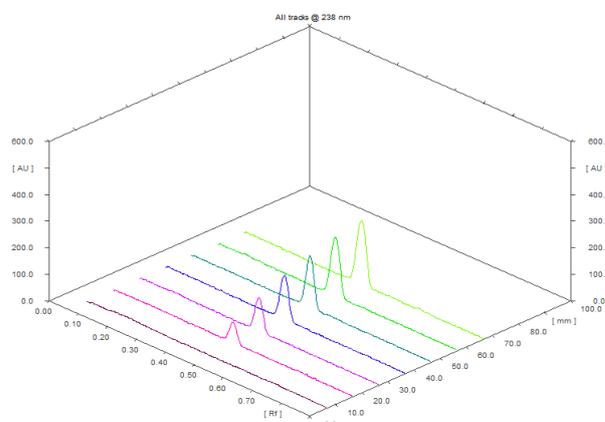


Fig. 3: Densitogram of linearity of Eplerenone (1500-9000 ng/band).

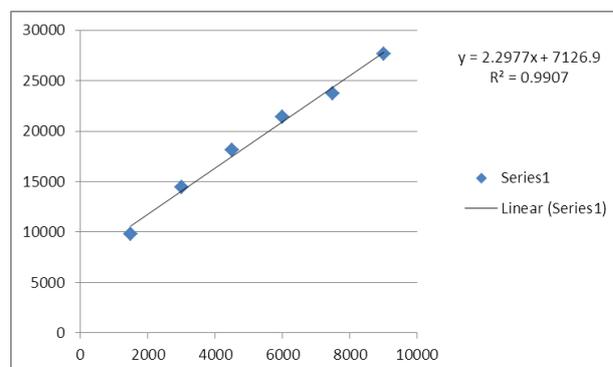


Fig. 4: Calibration curve for Eplerenone.

4. Accuracy

To check accuracy of the method, recovery studies were carried out by adding standard drug to sample at three different levels 80, 100 and 120 %. Basic concentration

of sample was 3000 ng/band from SB. The drug concentrations were calculated from respective linearity equation. The results of the recovery studies indicated

that the method is accurate. The results obtained are shown in Table 2.

Table 2: Recovery studies of Eplerenone.

Drug	Amount taken (ng/ band)	Amount added (ng/ band)	Total amount found (ng/ band)	Mean peak Area	% Recovery	% RSD
Eplerenone	3000	2400	5400	19461.456	100.17	1.37
	3000	3000	6000	20863.114	99.63	1.41
	3000	3600	6600	22512.973	01.45	0.04

5. Precision

The Precision of the method was demonstrated by Intra-day and Inter-day variation studies. In the Intra-day studies, 3 replicates of 3 different concentration were analyzed in a day and percent RSD was calculated. For the Inter-day variation studies, 3 different concentration were analyzed on 3 consecutive days and percent RSD was calculated. The results obtained for Intra-day and Inter-day variations are shown in table 3.

Table 3: System precision.

Inter-day precision of Eplerenone				
Concentration (ng/band)	Area	Mean Area	SD	RSD %
1500	9573.9	9571.46	0.81	1.15
	9542.2			
	9598.3			
3000	14216.6	14226.67	0.77	0.75
	14284.3			
	14179.1			
4500	17433	17386.5	1.64	1.65
	17198.1			
	17528.4			
Intra-day precision of Eplerenone				
Concentration (ng/band)	Area	Mean Area	SD	RSD %
1500	9593.6	99551.53	1.07	1.53
	9537.5			
	9523.5			
3000	14313.3	14384.27	1.23	1.16
	14478.3			
	14361.2			
4500	17195.1	17198.5	1.26	1.29
	17069.7			
	1330.7			

Table 4: Method precision.

Inter-day precision of Eplerenone				
Concentration (ng/band)	Area	Mean Area	SD	RSD %
1500	9839.1	9816.3	0.59	0.76
	9810.2			
	9799.6			
3000	14110.9	14128.34	0.29	0.29
	14150.1			
	14121.03			
4500	18191.7	18088.97	1.28	1.21
	18136.77			
	17938.46			
Intra-day precision of Eplerenone				
Concentration (ng/band)	Area	Mean Area	SD	RSD %
1500	9739.9	9758.83	1.35	1.78
	9812.2			
	9724.4			
3000	14209.9	14137.4	1.19	1.17
	14154.1			
	14048.2			
4500	17191.7	17062.53	1.35	1.40
	17021.4			
	16974.5			

6. Limit of detection (LOD) and limit of quantification (LOQ)

To determine the limit of detection and quantification, concentrations in the lower part of the linear range of the calibration curve were used. Stock solution of Eplerenone (100 mg/ml) was prepared and different volume of stock solution in the range 1500 to 9000 ng/band were applied six times. Amounts of Eplerenone per band were plotted against average response (peak area) and the regression equation was determined. Detection limit was calculated as $(3.3 \times S.D \text{ of response at lowest concentration})/b$ and quantification limit was calculated as $(10 \times S.D \text{ of response at lowest concentration})/b$, where "b" denotes to the slope obtained in the linearity study.

7. Robustness

Robustness of the method was studied by making variations in different parameters such as mobile phase composition; mobile phase volume; development time;

duration of saturation; activation of prewashed TLC plates; time from spotting to chromatography and time from chromatography to scanning; chamber size. The robustness of the method was studied by applying 4500 ng/band of Eplerenone and effects on results were examined.

Table 5: Robustness Study.

Sr. No.	Parameters	Robust condition	% RSD
1	Saturation time	13min	1.16
		17min	0.50
2	Mobile phase composition Toluene: Ethyl Acetate (3:7v/v) ±0.2	Toluene: Ethyl Acetate (7: 2v/v)	0.988
		Toluene: Ethyl Acetate (3.1: 6.9 v/v)	0.681
		Toluene: Ethyl Acetate (2.9: 7.1v/v)	0.89
3	Chamber size	Large chamber	0.86
		Small chamber	0.85

8. Specificity

The specificity of the method was determined by analysis of drug standard and sample. The band for Eplerenone in sample was identified by comparing the R_f values and spectrum of the band with those of the band from a Eplerenone standard. The peak purity of Eplerenone was accessed by comparing the spectra at three different positions on the peak, i.e., peak start (S), peak apex (M) and peak end (E) positions of the band.

Table 6: Summary of validation parameters.

Sr. No	Validation parameters	Eplerenone
1	Specificity	Specific
2	Precision	% Recovery
	System precision - Interday (n=3) Intraday (n=3)	0.75-1.65 1.16-1.53
	Method precision - Interday (n=3) Intraday (n=3)	0.29-1.21 1.17-1.78
3	Assay	101.49
4	Accuracy	% Recovery
	80%	100.17%
	100%	99.63%
	120%	101.45 %
5	Linearity	$y = 2.2977x + 7126.9$
6	Range	1500-9000 ng/band
7	LOD	114.06 ng/band
8	LOQ	345.64 ng/band
9	Robustness	Robust

DISCUSSION

There are two stability indicating methods reported in the literature so far. The result of forced degradation by hydrolysis and oxidation obtained by us, fairly matches the result reported by Rane V. P. It may be noted that the work by Rane V.P, involved exposure of drug to UV light at long and short wavelengths, where as Mahajan B, have used drug exposure to sun light. We have followed ICH guideline and the drug exposure to UV radiation (200 watt hrs/square meter) and Florescent light (1.2 million Lux. Hrs) was done in a photostability chamber as per ICH guideline.

CONCLUSION

The developed method is stability indicating, since the drug peak was found to be pure as confirmed by peak purity profiling study, under all stress degradation conditions. This proves that there is no interference of degradation product in analytical peak. The method is specific, accurate, precise, and robust and can be used for routine quality control as well as assessing the stability of Eplerenone.

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