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Quantitative determination of eplerenone in bulk drug and tablet dosage form by TLC/densitometry

Brajesh Mahajan[1]*, Naina Keskar[2] and Shashank Shah[3]
1, Central India Institute of Pharmacy, Indore (M.P.) - India
2, Indore Institute of Science and Technology – II, Indore (M.P.) - India

Abstract

A simple, selective and precise thin-layer chromatography /densitometry method has been developed for determination of eplerenone, both as a bulk drug and in tablet formulation. The method was validated as per International Conference on Harmonization (ICH) guidelines. Aluminium foil TLC plates precoated with silica gel $60F_{254}$ were used as the stationary phase and ethyl acetate: toluene: triethylamine 6:4:0.4 (*v/v*) as mobile phase. A compact band (R_f 0.45 ± 0.02) was obtained for eplerenone. Densitometric analysis was performed in the absorbance mode at 241 nm. Linear regression analysis revealed a good linear relationship with (r^2 = 0.9991 ± 0.0004) between peak area and concentration in the range 200 - 1200 ng band $^{-1}$. The mean value ± S.D. of the slope and intercept were 4.84 ± 0.069 and 372.26 ± 7.66, respectively. The method was validated for precision, recovery and robustness. The limits of detection and quantitation were 11 and 32 ng, respectively. The % RSD for intra-day and inter-day precision of eplerenone was found to be 0.65% and 0.97 %, respectively. The mean percentage recovery for eplerenone was found to be in the range of 99 - 101%. Eplerenone was subjected to acid and alkali hydrolysis, oxidation, photochemical and thermal degradation. The drug undergoes degradation under acidic, basic and oxidation conditions. This indicates that the drug is susceptible to acid, base hydrolysis, oxidation and stable for photochemical and thermal conditions.

Key-Words: Eplerenone, TLC/densitometry, Validation

Introduction

Chemically, eplerenone is known as (Pregn-4-ene7, 21-dicarboxylic acid, 9, 11-epoxy-17- hydroxy-3-oxo, γ-lactone, methyl ester is an selective aldosterone blocker[1-2] (Fig 1). 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 liquid chromatography – tandem mass spectrometric[4], validated SPE-LC - MS[5] have been reported for the determination of eplerenone in biological fluids. RP-HPLC method has been found for determination of eplerinone in tablet formulation[6].

However, to our knowledge, no information related to the TLC/densitometric method for estimation of eplerenone in pharmaceutical dosage forms has ever been mentioned in literature.

*** Corresponding Author:**
E-mail: brajeshveemahajan@yahoo.co.in
Mob. 09039357508

Therefore the aim of this work is to develop an accurate, specific, repeatable and stability-indicating method for the determination of eplerenone in the presence of its degradation products as per ICH guidelines[7-11].

Material and Methods

Eplerenone was supplied by Aristo Pharmaceutical Bhopal, India as a gift sample. Eptus tablets containing 25 mg of eplerenone were obtained from commercial sources within their shelf life period. All the reagents used were of HPLC grade.

Chromatography

Chromatography was performed on 20 cm x 10 cm on aluminium foil plates precoated with 250 µm layers of silica gel $60F_{254}$ (E. Merck, Germany). Before use the plates were developed with methanol then dried in the current of dry air and activated at 60^0C for 5 min. Samples were applied as band, 6 mm wide, 15 mm apart, by use of a Camag (Switzerland) Linomat 5 equipped with 100 µL syringe. A constant application rate of 150 nL sec^{-1} was used. Ethyl acetate: toluene: triethylamine 6:4:0.4 (v/v) was used as mobile phase. Linear ascending development was performed in a twin

trough glass chamber previously saturated with mobile phase vapour for 25 min at room temp (RT, $25^0C \pm 2$) and relative humidity 60 ± 5%. The development distance was approximately 80 mm. After development the plates were dried in current of air. Densitometric scanning, at 241 nm, was performed with Camag TLC Scanner 3 in the absorbance mode. The source of radiation was a deuterium lamp emitting a continuous UV spectrum in the range of 190 - 400 nm.

Calibration curve of eplerenone

A stock standard solution containing 0.2 mg /ml of eplerenone was prepared in methanol. Different volumes of this solution (1 - 6 µL) were applied on TLC plate to obtain concentration in the range of 200 to 1200 ng band $^{-1}$. Each concentration was applied six times on to the TLC plate, developed and scanned as described above. Peak areas were plotted against corresponding concentrations to furnish the calibration plot.

Analysis of the marketed formulation

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 0.45 µm filter (Millifilter, Milford, MA, USA). An appropriate volume of 4 ml was diluted to 10 ml with methanol. This solution (4 µL, 400 ng band $^{-1}$) was applied to a plate for assay of eplerenone.

Method validation

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 (0.1 mg /ml) was prepared and different volume of stock solution in the range 200 to 400 ng were applied in triplicate. Amounts of eplerenone per band were plotted against average response (peak area) and the regression equation was determined. The standard deviations (S.D.) of responses and the average standard deviations (A.S.D.) were calculated. Detection limit was calculated as (3.3×A.S.D.)/b and quantification limit was calculated as (10×A.S.D.)/b, where "b" denotes to the slope obtained in the linearity study.

Precision

Intra-day and inter-day variation for determination of eplerenone was measured at three different concentrations (400, 600 and 800 ng band $^{-1}$). Repeatability of sample application and measurement of peak area were assessed by chromatography of six replicates of same concentration (400 ng band $^{-1}$ eplerenone).

Robustness

Robustness of the method was studied by making variations in different parameters such as mobile phase composition; mobile phase volume; development distance; duration of saturation; activation of prewashed TLC plates; time from spotting to chromatography and time from chromatography to scanning. The robustness of the method was studied by applying 400 ng band^{-1} of eplerenone and effects on results were examined.

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 **.**

Recovery studies

To check the recovery of the drug at different levels in formulations, analysed samples were spiked with an extra 80, 100 and 120% of eplerenone standard and the mixtures were reanalyzed by the proposed method. The experiment was conducted in triplicate.

Ruggedness

The ruggedness of the method was assessed by analysis of 400 ng eplerenone by two different analysts under the same experimental and environmental conditions.

Forced degradation of standard eplerenone

A stock solution containing 10 mg eplerenone in 10 mL methanol was prepared. This solution was used for forced degradation to provide an indication of the stability- indicating property and specificity of the proposed method. In all degradation studies the average peak area of eplerenone after application (400 ng spot $^{-1}$) of six replicates was obtained after development and scanning of the plate as described above.

Acid and base induced degradation

Eplerenone (10 mg) of was separately dissolved in 10 mL of methanolic solution of 0.1M HCl and 0.1 M NaOH. These solutions were kept for 8 h at room temperature in the dark in order to exclude the possible degradative effect of light. The solutions (1ml) were taken and neutralized, then diluted up to 10 mL with methanol. The resultant solutions were applied on TLC plate in triplicate (4 µL each, i.e. 400 ng band $^{-1}$). The plate was chromatographed as described above.

Hydrogen peroxide-induced degradation

Eplerenone (10 mg) was dissolved in 10 mL of methanolic solution of hydrogen peroxide (10%, v/v)

and the mixture was kept for 8 h at room temperature in the dark, to exclude the possible degradative effect of light. The solution (1 mL) was taken diluted to 10 mL with methanol and treated as described for acid and base-induced degradation.

Dry heat degradation product

The powdered drug was stored for 3 h under dry heat condition at 55^0C. A solution of the treated powder was then prepared and 400 ng band $^{-1}$ was applied to a plate in triplicate. The plate was then chromatographed and treated as described above.

Photochemical degradation product

Eplerenone (10 mg) was dissolved in 10 mL of methanol. The solution was left in sun light for 8 h. The resultant solution was treated as described for hydrogen peroxide induced degradation.

Results and Conclusion

Method Development

Ethyl acetate: toluene: triethylamine (6:4:0.2 v/v) was selected as mobile phase because it resulted in acceptable resolution of the bands with R_f values of 0.45 ± 0.02 for eplerenone. The densitogram obtained from standard eplerenone is shown in Fig 2.

Validation

The calibration plots were found to be linear over the range 200 - 1200 ng band $^{-1}$ for eplerenone with mean value (± SD) of correlation coefficient, slope and intercept were 0.9991 (± 0.0001), 4.84 (± 0.069) and 372.26 (± 7.66), respectively. The LOD and LOQ was 11 and 32 ng band $^{-1,}$ respectively. Intra-day and Inter-day variations, as RSD (%), were found to be 0.65 and 0.97 respectively. Study of the robustness of the method revealed that the peak areas were unaffected (RSD < 2%) by small changes of the operating conditions. The low R.S.D. values indicated the method is robust. When the method was performed by two different analysts under the same experimental and environmental conditions, the mean % RSD were found to be 1.74 (analysts I) and 0.84 (analysts II), showed the robustness of the method. The method was also evaluated for assay of commercially available tablets containing eplerenone. The drug content was found to be 100.57 % (% R.S.D. = 0.95%). To study the accuracy and precision of the method, recovery was determined. For eplerenone, recovery was found to in the range of 99 – 101 %, with RSD ranging from 0.59 to 0.87 %. The results obtained from validation of the method are summarized in Table I

Stability-indicating property

Chromatograms obtained from acid-degraded samples of eplerenone contained additional peaks at R_f 0.26, 0.28 and 0.58. Chromatograms obtained from base-degraded samples of eplerenone contained additional peaks at 0.04 and 0.16. The concentration of the drug was found to change from the initial concentration, indicating eplerenone undergoes degradation under acidic and basic conditions. Chromatograms obtained from H_2O_2-degraded samples of eplerenone contained additional peaks at 0.13 and 0.32. The bands of the degradation products were well resolved from that of the pure drug Fig 3. Samples degraded under dry heat conditions furnished no additional peak, so eplerenone is stable under these conditions. No additional peak was observed when drug solution was left in sun light for 8 h. These results indicate the drug is susceptible to acid/base hydrolysis, oxidation and stable in sun light and dry heat-induced degradation. The results of accelerated degradation studies are listed in Table II. This TLC-densitometric method is simple precise, specific and accurate. Statistical analysis proved the method is reproducible and selective for the analysis of eplerenone as the bulk drug and in tablet formulations. The method can be used to determine the purity of the commercially available drug by detecting the related impurities. Because the method could effectively separate the drugs from their degradation products, it can be regarded as stability indicating.

Table I: Summary of validation parameter

Method characteristic	Value
Linearity range (ng band $^{-1}$)	200 - 1200
Correlation coefficient	0.9991
Limit of detection (ng)	11
Limit of quantitation (ng)	32
% Recovery (n = 9); % RSD	99.73 ; 0.86
Ruggedness (%RSD)	
Analyst I (n = 3)	1.74
Analyst II (n = 3)	0.84
Precision (% R.S.D.)	
Repeatability of application (n = 6)	1.19
Inter-day (n = 3)	0.97
Intra-day (n = 3)	0.65
Robustness	Robust
Specificity	Specific

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Table II: Forced degradation of eplerenone

Sample exposure condition	Number of degradation products (R_f values)	Recovery (%)
0.1 M HCl, 8h, RT	3 (0.26, 0.28, 0.58)	94.20
0.1 M NaOH, 8h, RT	2 (0.04, 0.16)	88.03
10 % H_2O_2, 8h, RT	2 (0.13, 0.32)	87.27
Photo, 8 h	No degradation	100.13
Heat, 3H, 55^0C	No degradation	100.02

RT- Room temperature

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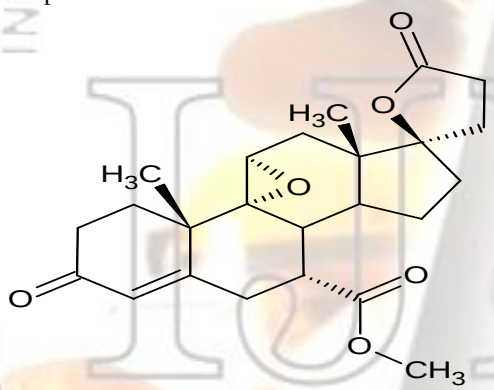

Fig 1. Chemical structure of eplerenone

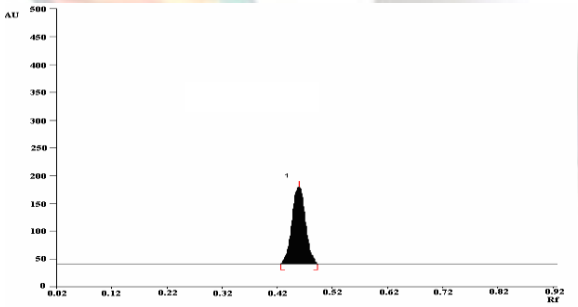

Fig 2. Densitogram obtained from eplerenone (R_f 0.45) in Ethyl acetate: toluene: triethylamine (6:4:0.4)

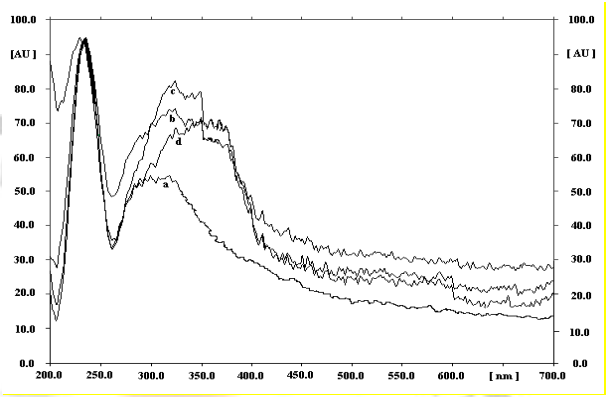

Fig 3.Typical overlain spectra of eplerenone standard (a), and eplerenone recovered after treatment with 0.1 N HCl (b), 0.1 N NaOH (c), and 10% H_2O_2 (d)

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