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 60F254 were used as the stationary phase and ethyl acetate: toluene: triethylamine 6:4:0.4 (v/v) as mobile phase. A compact band (Rf 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 (r² = 0.999 ± 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

**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 60F254 (E. Merck, Germany). Before use the plates were developed with methanol then dried in the current of dry air and activated at 60°C 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...
tough glass chamber previously saturated with mobile phase vapour for 25 min at room temp (RT, 25°C ± 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 (3.3xA.S.D.)/b and quantification limit was calculated as (10xA.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 Rf 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.

**Ruggness**

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 (1 mL) 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°C. 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 1.

**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 \(\text{H}_2\text{O}_2\)-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 furnishing 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>
<thead>
<tr>
<th>Table I: Summary of validation parameter</th>
<th>Method characteristic</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>Linear range (ng (\text{band}^{-1}))</td>
<td>200 - 1200</td>
<td></td>
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<tr>
<td>Correlation coefficient</td>
<td>0.9991</td>
<td></td>
</tr>
<tr>
<td>Limit of detection (ng )</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Limit of quantitation (ng )</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>% Recovery ((n = 9)); % RSD</td>
<td>99.73; 0.86</td>
<td></td>
</tr>
<tr>
<td>Ruggedness (%RSD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Analyst I ((n = 3))</td>
<td>1.74</td>
<td></td>
</tr>
<tr>
<td>Analyst II ((n = 3))</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>Precision (% R.S.D.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repeatability of application ((n = 6))</td>
<td>1.19</td>
<td></td>
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<tr>
<td>Inter-day ((n = 3))</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>Intra-day ((n = 3))</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>Robustness</td>
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<td></td>
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<tr>
<td>Specificity</td>
<td>Specific</td>
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Table II: Forced degradation of eplerenone

<table>
<thead>
<tr>
<th>Sample exposure condition</th>
<th>Number of degradation products (R_f values)</th>
<th>Recovery (%)</th>
</tr>
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<tbody>
<tr>
<td>0.1 M HCl, 8h, RT</td>
<td>3 (0.26, 0.28, 0.58)</td>
<td>94.20</td>
</tr>
<tr>
<td>0.1 M NaOH, 8h, RT</td>
<td>2 (0.04, 0.16)</td>
<td>88.03</td>
</tr>
<tr>
<td>10 % H_2O_2, 8h, RT</td>
<td>2 (0.13, 0.32)</td>
<td>87.27</td>
</tr>
<tr>
<td>Photo, 8h</td>
<td>No degradation</td>
<td>100.13</td>
</tr>
<tr>
<td>Heat, 3h, 55°C</td>
<td>No degradation</td>
<td>100.02</td>
</tr>
</tbody>
</table>

RT - Room temperature

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The authors are grateful to R.C. Patel College of Pharmacy, Shirpur (M.S.) India for providing necessary facilities and Aristo Pharmaceuticals Ltd. Bhopal (MP) India for providing eplerenone as a gift sample.

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)

References