STABILITY INDICATING HPTLC METHOD FOR DETERMINATION OF EPROSARTAN MESYLATE FROM BULK DRUG AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A simple, selective, precise and stability-indicating high-performance thin-layer chromatographic (HPTLC) method for analysis of eprosartan mesylate both as a bulk drug and in formulations was developed and validated. The method employed TLC aluminium plates precoated with silica gel 60F\(_{254}\) as the stationary phase. The solvent system consisted of ethyl acetate: acetonitrile: glacial acetic acid (5: 5: 0.7, v/v/v). This system was found to give compact spots for eprosartan mesylate (R\(_f\) value of 0.48 ± 0.02). Densitometric analysis of eprosartan mesylate was carried out in the absorbance mode at 238 nm. The linear regression analysis data for the calibration plots showed good linear relationship with r\(^2\) = 0.996 in the concentration range 100-700 ng per spot. The method was validated for specificity, precision, accuracy, limit of detection, limit of quantification and robustness. Statistical analysis proved that the method is specific, precise and accurate for the estimation of said drug. Eprosartan mesylate was subjected to acidic, alkaline, hydrolytic, oxidative and photolytic degradation. As the method could effectively separate the drug from its degradation product, it can be employed as a stability-indicating one.

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INTRODUCTION

Eprosartan mesylate [Fig. 1], mono-methane sulfonate salt of (E)-2-butyl-1-(p-carboxybenzyl)-α-2-thienylmethylimidazo-5-acrylic acid, is a non-biphenyl non-tetrazole angiotensin II receptor (AT1) antagonist [1]. Analytical methods like UV [2-7], HPLC [8-14] and HPTLC [7, 15] are reported for estimation of drug in bulk and formulation.

![Figure 1 Eprosartan mesylate](image)

An ideal stability indicating method is one that quantifies the drug per se and also resolves its degradation products. One stability indicating UPLC method is reported for estimation of eprosartan mesylate in bulk and formulation [16]. Over the past decade HPTLC has been successfully used in the analysis of pharmaceuticals, plant constituents and bio macromolecules. Several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus lowering analysis time and cost per analysis. It also facilitates automatic application and scanning in situ. A very viable alternative for stability indicating analysis of eprosartan mesylate is High-Performance Thin-Layer Chromatography (HPTLC).

Thus, the objective of the present work was to develop an accurate, specific and reproducible method for the determination of eprosartan mesylate in presence of its degradation products for the assessment of the purity of the bulk drug and stability of its pharmaceutical dosage forms.

EXPERIMENTAL

Instrumentation

TLC alumunium precoated silica gel 60F<sub>254</sub> plate (10cm×10cm) 250 µm thicknesses (E. Merck, Darmstadt, Germany) was used as stationary phase. Sample application was done by using Hamilton 100 µl syringe (Camag, Switzerland) and Camag Linomat 5 applicator (Camag, Switzerland). Linear ascending development was carried out in 10cm×10cm twin trough glass chamber (Camag, Switzerland). The densitometric scanning was performed by using Camag TLC scanner 3 (Camag, Switzerland) supported by using planar chromatography manager-wintCATS (CAMAG Ver. 1.4.1).

Chemicals

Eprosartan mesylate was procured as gift sample from Mylan Pharmaceuticals Pvt. Ltd. Analytical reagent grade ethyl acetate, acetonitrile, methanol and glacial acetic acid were purchased from SD Fine Chem. Ltd., Mumbai, India.

Calibration curves of eprosartan mesylate

A stock solution of eprosartan mesylate (100 µg/ml) was prepared in methanol. Different volumes of stock solution, 1, 2, 3, 4, 5 and 7 µl were spotted on TLC plate to obtain concentrations of 100, 200, 300, 400, 500, 600 and 700 ng per spot of eprosartan mesylate, respectively. The data of peak area versus drug concentration were treated by linear least-square regression.

METHOD VALIDATION

Specificity

The specificity of the method was ascertained by comparing the densitogram of blank (no drug) and standard drug in the mobile phase. The peak purity was assessed by comparing the spectrum of standard eprosartan mesylate with spectrum of sample.

Precision

Precision of the assay was tested at 200, 400 and 600 ng of eprosartan mesylate, in triplicate. The intra-day variation was evaluated three times a day. The inter-day variation was similarly evaluated over a period of 3 days.

Limit of detection and limit of quantification

The LOQ and LOD were based on the standard deviation of the response and the slope of the constructed calibration curve (n=3), as described in International Conference on Harmonization guidelines.
Recovery

Recovery determination for eprosartan mesylate was carried out at levels of 80%, 100% and 120%. The analyzed samples were spiked with extra 80%, 100% and 120% of the eprosartan mesylate (200 ng per spot) and the mixture was reanalyzed by the proposed method. The experiment was conducted in triplicate. This was done to check for the recovery of the drug at different levels in the formulations.

Robustness

The robustness of a method was evaluated by introduction of small change in the mobile phase composition (±0.2 ml for major component) and saturation time of development chamber (±5) min. The robustness of the method was determined at two concentration levels of 200 and 400 ng per spot.

System suitability tests

The chromatographic systems used for analysis must pass the system suitability limits before sample analysis can commence. The injection repeatability, tailing factor (T) and resolution (Rs) for the principle peak and its degradation product were evaluated using eprosartan mesylate solution of 800 ng per spot.

Analysis of the prepared formulation

To determine the content of eprosartan mesylate from marketed tablet (label claim: eprosartan mesylate 400 mg/tablet), the tablets were powdered and weight equivalent to 10 mg was extracted in methanol. To ensure complete extraction of the drug, solution was sonicated for 15 min and the volume was made up to 100 ml. The resulting solution was filtered and the filtrate was analyzed for the drug content wherein 4 µl of the solution was spotted onto the plate followed by development and scanning. The analysis was done in triplicate.

Forced degradation of eprosartan mesylate

Acid degradation

From eprosartan mesylate, drug equivalent to 100 mg of eprosartan was dissolved in 100 ml of methanolic solution of 0.1M HCl. The acidic mixture was kept for 1 hr and in the dark in order to exclude the possible degradative effect of light. The resultant solutions were diluted 10 times, and applied on the TLC plate in triplicate (4 µl each, i.e. 400 ng per spot).

Base induced degradation

From eprosartan mesylate, drug equivalent to 100 mg of eprosartan was dissolved in 100 ml of methanolic solution of 0.1M NaOH. The acidic basic mixture for 3 hr in the dark in order to exclude the possible degradative effect of light. The resultant solutions were diluted 10 times, and applied on the TLC plate in triplicate (4 µl each, i.e. 400 ng per spot).

Oxidation

From eprosartan mesylate, drug equivalent to 100 mg of eprosartan was dissolved in 100 ml of methanol. To 50 ml of methanolic solution of eprosartan mesylate, 50 ml of hydrogen peroxide (30.0%, v/v) was added separately. The solution was kept for 3 hr in dark. The resultant solutions were diluted 5 times and applied on TLC plate in triplicate (4 µl each, i.e. 400 ng per spot).

Hydrolysis

From eprosartan mesylate, drug equivalent to 100 mg of eprosartan was dissolved in 100 ml of distilled water. The aqueous solution is kept in dark for 6 hr. The resultant solutions were diluted 10 times, and applied on the TLC plate in triplicate (4 µl each, i.e. 400 ng per spot).

Photochemical degradation

From eprosartan mesylate, drug equivalent to 100 mg of eprosartan was kept in UV chamber at 254 nm for 24 hr. It was later dissolved in 100 ml methanol and diluted 10 times, and applied on the TLC plate in triplicate (4 µl each, i.e. 400 ng per spot).

Thermal Degradation

From eprosartan mesylate, drug equivalent to 100 mg of eprosartan was kept in oven at 120 °C for 24 hr. It was later dissolved in 100 ml methanol and diluted 10 times, and applied on the TLC plate in triplicate (4 µl each, i.e. 400 ng per spot).

RESULTS AND DISCUSSION

Development of the optimum mobile phase

TLC procedure was optimized with a view to develop a stability-indicating assay method. A solvent system that would give dense and compact spots with appropriate and significantly different $R_f$ values for eprosartan mesylate and its degraded products was desired for quantification of eprosartan mesylate in the pharmaceutical formulations. Ultimately, mobile phase used consisted of ethyl acetate: acetonitrile: glacial acetic acid (5.0:5.0:0.7, v/v/v) which gave good resolution and sharp peaks for eprosartan mesylate at 238 nm. The sample was sprayed in the form of narrow bands of 6 mm length at a constant rate of 0.15 µl/s. Time for chamber saturation was optimized to 15 min. The length of chromatographic development was 80 mm. System suitability parameters was calculated and compared with the standard limit as per ICH.
Calibration curves

The linear regression analysis of the data for the calibration curves (n=3) with equation $y = 12.78x + 478.9$ ($r^2 = 0.996$) showed a good linear relationship over the concentration range 100-700 ng per spot with respect to peak area. No significant difference was observed in the slopes of standard curves (ANOVA, $p > 0.0001$).

Specificity

The chromatogram of commercial formulation showed only one peak at $R_f$ value of 0.48 and the spectrum of sample and standard spot matched with each other, indicating that there is no interference of the excipients in the formulation.

Precision

The repeatability of sample application and measurement of peak area were expressed in terms of % RSD and results are depicted in Table 2, which revealed intra- and inter-day variation of eprosartan mesylate at three concentration levels of 200, 400 and 600 ng per spot.

<table>
<thead>
<tr>
<th>Amount (ng per spot)</th>
<th>Intra-day precision</th>
<th>Inter-day precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean area</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>200</td>
<td>2967.77</td>
<td>82.23</td>
</tr>
<tr>
<td>400</td>
<td>5719.17</td>
<td>74.34</td>
</tr>
<tr>
<td>600</td>
<td>7959.21</td>
<td>65.47</td>
</tr>
</tbody>
</table>

LOD and LOQ

LOD for eprosartan mesylate was found to be 29.49 ng per spot and LOQ for eprosartan mesylate was found to be 89.37 ng per spot.

Recovery

The proposed method when used for extraction and subsequent estimation of eprosartan mesylate from pharmaceutical dosage forms after spiking with 80, 100 and 120% of additional in 200 ng per spot drug gave recovery of 98–100%.

<table>
<thead>
<tr>
<th>Amount taken (ng)</th>
<th>Excess amount added (ng)</th>
<th>Theoretical Content (ng)</th>
<th>Recovery (%)</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>160 (80%)</td>
<td>360</td>
<td>99.50</td>
<td>1.05</td>
</tr>
<tr>
<td>200</td>
<td>200 (100%)</td>
<td>400</td>
<td>100.76</td>
<td>2.00</td>
</tr>
<tr>
<td>200</td>
<td>240 (120%)</td>
<td>440</td>
<td>98.75</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Robustness

The low values of %RSD obtained after introducing small changes in mobile phase composition and saturation time indicated robustness of the method as indicated in Table 3.
Table 3 Results of Robustness studies (n=3).

<table>
<thead>
<tr>
<th>Amount (ng per spot)</th>
<th>Mobile phase composition</th>
<th>Saturation time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EA: ACN: GAA (4.8:5.2:0.7)</td>
<td>%RSD</td>
</tr>
<tr>
<td>200</td>
<td>1.874</td>
<td>0.307</td>
</tr>
<tr>
<td>400</td>
<td>1.586</td>
<td>1.224</td>
</tr>
</tbody>
</table>

System suitability tests
The % RSD value was found to be 2% and tailing factor of 1.1 was obtained. The separation of drug and degradant peaks was found to be optimum. The low %RSD value of injection repeatability and resolution of peaks indicated the suitability of this method for routine analysis of eprosartan mesylate in pharmaceutical dosage forms.

Analysis of pharmaceutical formulation
A single spot at Rf 0.48 was observed in the chromatogram of eprosartan mesylate extracted from tablet. The eprosartan mesylate content was found to be 99.55 % with a %RSD of 0.98. It may therefore be inferred that degradation of eprosartan mesylate had not occurred in the formulations that were analyzed by this method.

Stability-indicating property
The chromatograms of the samples degraded with acid, water and hydrogen peroxide showing well-separated spots of eprosartan mesylate as well as some additional peaks at different Rf values. The spots of degraded products were well resolved from the drug spot. The peaks of eprosartan mesylate were not significantly shifted in the presence of the degradation peaks, which indicated the stability-indicating nature of the method. The percentage recovery was calculated and listed in Table 4.

Table 4 Forced degradation studies of eprosartan mesylate.

<table>
<thead>
<tr>
<th>Stress condition/ duration</th>
<th>Figure</th>
<th>Percent recovery</th>
<th>Rf of degradant</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidic, 0.1 N HCl/ 1 hr</td>
<td>Fig. 3</td>
<td>28.58</td>
<td>0.02</td>
<td>Labile</td>
</tr>
<tr>
<td>Basic, 0.1N NaOH/ 8 hr, 80 °C reflux</td>
<td>Fig. 4</td>
<td>81.70</td>
<td>0.72</td>
<td>Labile</td>
</tr>
<tr>
<td>Oxidation, 15 % v/v H2O2/ 3 hr</td>
<td>Fig. 5</td>
<td>92.24</td>
<td>0.2</td>
<td>Labile</td>
</tr>
<tr>
<td>Hydrolysis/ 6 hr</td>
<td>Fig. 6</td>
<td>90.55</td>
<td>0.61</td>
<td>Labile</td>
</tr>
<tr>
<td>Photolytic, UV 254 nm/ 24 hr</td>
<td>Fig. 7</td>
<td>97.81</td>
<td>-</td>
<td>Stable</td>
</tr>
<tr>
<td>Thermal, 120 °C/ 24 hr</td>
<td>Fig. 8</td>
<td>99.69</td>
<td>-</td>
<td>Stable</td>
</tr>
</tbody>
</table>

Figure 3  HPTLC densitogram of acid degradation products of eprosartan mesylate.
Figure 4 HPTLC densitogram of alkali degradation products of eprosartan mesylate

Figure 5 HPTLC densitogram of oxidative degradation products of eprosartan mesylate

Figure 6 HPTLC densitogram of hydrolytic degradation products of eprosartan mesylate
Figure 7 HPTLC densitogram of photolytic degradation products of eprosartan mesylate

Figure 8 HPTLC densitogram of thermal degradation products of eprosartan mesylate

CONCLUSION

The proposed HPTLC method is cheap, simple, rapid, and flexible. The developed HPTLC technique was found to be specific, precise, accurate, robust and stability indicating. The validated method was successfully applied for quantification of eprosartan mesylate from commercial tablets. The method may be extended to study the degradation kinetics of eprosartan mesylate and its estimation in plasma and other biological fluids.

REFERENCES


