Estimation of Losartan Potassium and Ramipril in Their Combined Dosage Form by Validated HPTLC Method

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ABSTRACT
A sensitive, specific and precise high performance thin layer chromatographic method for estimation of Losartan potassium (LOS) and Ramipril (RAM) has been developed and validated. The method employed TLC aluminium plates pre-coated with silica gel 60 F$_{254}$ as the stationary phase. The solvent system consisted of toluene: methanol: ethyl acetate (6: 3: 2, v/v/v). This system was found to give compact and dense spots for LOS and RAM (R$_f$ value 0.35 ± 0.02 and 0.57 ± 0.02, respectively). Densitometric analysis of drug was carried out in the absorbance mode at 210 nm. The method was validated with respect to linearity, specificity, precision, limit of detection, limit of quantification and recovery. The linear regression analysis data for the calibration plots showed a good linear relationship with R$_2$ = 0.9943 and 0.9963 for LOS and RAM respectively, in the concentration range of 200-1000 ng/spot for both the drugs. The LOD and LOQ were found to be 26.03 ng/spot and 78.88 ng/spot, respectively for LOS, 14.37 ng/spot and 43.56 ng/spot, respectively for RAM. Recovery of LOS and RAM were achieved in the range of 100.42-101.44 % and 100.80-102.41 %, respectively by developed method. Statistical analysis proves that the method is repeatable and specific for the estimation of LOS and RAM. Developed method was successfully applied for estimation of LOS and RAM in their combined dosage form.

Keywords: HPTLC, losartan potassium, ramipril, simultaneous estimation

INTRODUCTION
Losartan potassium (LOS), chemically known as monopotassium salt of 2-butyl-4-chloro-1-[[2'-(1H-tetrazol-5-yl)] [1,1'-biphenyl]-4-yl]methyl]-1H-imidazole-5-methanol, is an
angiotensin II receptor antagonist [1-3]. Drug is official in IP 2010, BP 2010, and USP32-NF27 [4-6]. Ramipril (RAM), chemically known as (2S,3aS,6aS)-1-[(S)-2-[[S]-1-(ethoxycarbonyl)-3-phenyl propyl]amino]propanoyl] octahydrocyclopenta[b]pyrrole-2-carboxylic acid, is an angiotensin-converting enzyme (ACE) inhibitor [1-3]. Drug is official in IP 2010 and USP32-NF27 [4-6]. Chemical structures of LOS and RAM are depicted in Figure 1.

![Chemical structure of Losartan potassium (a) and Ramipril (b)](image)

**Figure 1.** Chemical structure of (a) Losartan potassium (b) Ramipril

The literature review described HPLC, RP-HPLC and HPTLC method for determination of LOS individually and in combination with other drugs in their pharmaceutical dosage forms [7-13]. A literature survey reveals that RP-HPLC, stability indicating HPLC, HPTLC, liquid chromatography (LC) coupled with tandem mass spectrometric methods have been available for determination of RAM individually and in combination with other drugs in their pharmaceutical dosage form [13-17]. No HPTLC method was reported for simultaneous estimation of these two drugs in combined dosage form (tablet). Therefore, it was thought of interest to develop and validate HPTLC method for simultaneous estimation of Losartan potassium and Ramipril in their combined dosage form. The present work deals with estimation of these two drugs in combined dosage form by HPTLC method.

**EXPERIMENTAL**

**Instrumentation**

The HPTLC system (Camag, Switzerland) consisting of Linomat V semiautomatic spotting device, TLC Scanner IV (Camag, Muttenz, Switzerland), twin-trough developing chamber (20 x 10 cm), UV cabinet with dual wavelength UV lamps, winCATS software,
syringe (100 µl capacity, Hamilton) were used for chromatographic study. Electronic analytical balance (Shimadzu AUX-220) was used for all the weighing purpose.

**Chemicals and Reagents**

LOS and RAM were kindly supplied as gift samples by Themis Medicare Pvt. Ltd., Mumbai, India and Cadila Pharmaceutical, Ahmedabad, Gujarat, India, respectively. All chemicals and reagents used were of LR grade and purchased from s.d. Fine-Chem Limited, Mumbai, India. Loram tablets (Unisearch / Unichem laboratories), containing LOS (50 mg) and RAM (5 mg) were procured from local pharmacy.

**Chromatographic Conditions**

Chromatographic separation was performed on 10 × 10 cm aluminium plates pre-coated with 250 µm layer of silica gel 60 F254 (E. Merk, Darmstadt, Germany). The TLC plate was pre-washed with methanol and activated at 70 °C for 15 min prior to spotting. The samples were spotted on TLC plate 15 mm from the bottom edge by Linomat V semi-automatic spotter using following parameters: band width, 6 mm; track distance, 11.6 mm; application rate, 0.1 µl/s. TLC plate was developed in twin trough chamber (20 cm x 10 cm) using toluene: methanol: ethyl acetate (6 : 3 : 2, v/v/v) as mobile phase at temperature, 27 ± 2 °C; relative humidity, 35-40%; chamber saturation time, 30 min; migration distance, 75 mm. The TLC plate was dried, scanned and analysed by TLC Scanner IV and winCATS software using following parameters: slit dimension, 4 mm × 0.30 mm; scanning speed, 20 mm/sec; detection wavelength, 210.

**Preparation of Solutions**

*Preparation of stock solutions of LOS and RAM*

Accurately weighed 25 mg of standard LOS and 25 mg of standard RAM were transferred in to two separate 25 ml volumetric flasks, dissolved in methanol and diluted up to mark with methanol separately to get standard stock solutions having strength of 1000 µg/ml of each drug.

*Preparation of combined working standard solution*

The combined working standard solution was prepared by mixing of 0.5 ml of LOS standard stock solution and 0.5 ml of RAM standard stock solution in to 10 ml volumetric flask and diluted up to mark with methanol to get a solution having strength of 50 µg/ml of each, LOS and RAM.

**Calibration Curve Preparation**

From combined working standard solution, 4, 8, 12, 16 and 20 µl were spotted on a TLC plate. The TLC plate was developed, dried and analysed as described under chromatographic conditions [Section 2.3]. Calibration curves were obtained by plotting peak area against respective concentration of each drug.
Method Validation

Specificity

The specificity of the method was ascertained by analysing drugs from standard and sample. The bands for LOS and RAM from the sample were confirmed by comparing the $R_f$ and UV spectra of the respective band with those obtained from standard. The peak purity was assessed by comparing UV spectra acquired at three different positions on the band, i.e. peak start (s), peak apex (m), and peak end (e).

Linearity

Linearity response was determined by analysing five independent levels of calibration curve in the concentration range of 200-1000 ng/spot for LOS and RAM, respectively (n=5). The calibration curves of peak area versus respective concentration of each drug were plotted and correlation coefficients and regression line equations were computed.

Precision

Repeatability of measurement of peak area: From combined working standard solution, 12 µl was spotted on a TLC plate. The plate was developed, dried and analysed as described under chromatographic conditions [Section 2.3]. The spots were scanned for seven times without changing plate position and RSD for measurement of peak area was calculated for both drugs.

Repeatability of sample application: From combined working standard solution, 12 µl was spotted seven times on a same TLC plate. The TLC plate was developed, dried and analysed as described under chromatographic conditions [Section 2.3]. The peak area of seven spots for each drug was measured and RSD of peak area was calculated for both drugs.

Intra-day and inter-day precision: Intra-day precision (RSD) was determined by analysing combined working standard solution over the entire calibration range for three times on same day, the inter-day precision (RSD) was determined by analysing combined working standard solution over the entire calibration range for three days.

Accuracy

The accuracy of the method was determined by calculating recovery of LOS and RAM using the standard addition method. Accurately weighed known quantity of standard LOS and RAM was spiked at 80, 100 and 120 % level to preanalysed sample of LOS and RAM. After appropriate dilutions, samples were analysed with optimized chromatographic conditions. The recovery of LOS and RAM was calculated at each level (n = 3) from respective linear regression equations.
Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ of the method were estimated from the set of five calibration curves using following equations.

\[
\text{LOD} = 3.3 \frac{N}{S} \quad \text{and} \quad \text{LOQ} = 10 \frac{N}{S}
\]

where \(N\) is the standard deviation of intercepts of five calibration curves, and \(S\) is the mean slope of five calibration curves.

Analysis of Marketed Formulation

Twenty tablets were accurately weighed and finely powdered. Tablet powder equivalent to 100 mg of LOS and 10 mg of RAM was accurately weighed, transferred to 25 ml volumetric flask, mixed with 20 ml of methanol and sonicated for 25 minutes. Resulting solution was filtered through Whatman filter paper no. 41 and diluted up to 25 ml with methanol. Aliquot of 2.5 ml of filtrate was transferred into 10 mL volumetric flask and diluted up to mark with methanol. From resulting solution, 1 ml was transferred into 10 ml volumetric flask and diluted up to mark with methanol. Two separate bands were spotted by applying 6 \(\mu\)l and 40 \(\mu\)l of resulting solution for estimation of LOS and RAM, respectively. Plate was developed and analysed as described in section 2.3. Amount of LOS and RAM from marketed formulation was calculated using calibration curve of respective drug.

RESULTS AND DISCUSSION

Selection of Wavelength

After chromatographic development, both spots were scanned from 200 nm to 700 nm and the spectra were overlain. It was observed that both drugs showed considerable absorbance at 210 nm (Figure 2). Therefore, 210 nm was selected as wavelength for detection.

Mobile Phase Optimization

Different solvent systems have been tried for separation of LOS and RAM, but good separation was found to be in toluene: methanol: ethyl acetate (6: 3: 2, v/v/v). TLC plates were observed in UV chamber using short UV (254 nm), long UV (366 nm) and white light. The \(R_f\) values were found to be 0.35 ± 0.02 and 0.57 ± 0.02 for LOS and RAM, respectively. It was observed that drying of TLC plate under IR lamp after spotting and pre-saturation of TLC chamber with mobile phase for 30 min ensured good reproducibility of value.

Calibration Curve Preparation

Calibration curves were prepared by plotting peak area against respective concentration of each drug. For both LOS and RAM, measured peak area were found to be linearly increasing with concentration over the range of 200-1000 ng/spot. Thus, same range was selected for validation of method with respect to linearity and range.
Method Validation

Linearity and range

Representative calibration curves of LOS and RAM were obtained by plotting mean peak area of LOS and RAM against concentration over range of 200-1000 ng/spot (n=5). They were found to be linear in above mentioned range with correlation coefficient of 0.9943 and 0.9963 for LOS and RAM, respectively (Table 1). RSD for LOS and RAM were found to be in range of 1.00-2.50 % and 1.17-2.73 % respectively. Average linear regressed equation for curves were $y = 6.4459x + 841.76$ and $y = 3.0317x + 159.70$ for LOS and RAM respectively.

### Table 1. Calibration curve data for LOS and RAM

<table>
<thead>
<tr>
<th>LOS</th>
<th>Peak Area (mean ± SD)</th>
<th>RSD (%)</th>
<th>RAM</th>
<th>Peak Area (mean ± SD)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>1962.12 ± 19.71</td>
<td>1.00 %</td>
<td>200</td>
<td>717.9 ± 9.03</td>
<td>1.17 %</td>
</tr>
<tr>
<td>400</td>
<td>3735.04 ± 88.44</td>
<td>2.36 %</td>
<td>400</td>
<td>1373.46 ± 37.22</td>
<td>2.61 %</td>
</tr>
<tr>
<td>600</td>
<td>4778.2 ± 124.72</td>
<td>2.50 %</td>
<td>600</td>
<td>1919.40 ± 46.82</td>
<td>2.73 %</td>
</tr>
<tr>
<td>800</td>
<td>6091.08 ± 81.02</td>
<td>1.33 %</td>
<td>800</td>
<td>2676.88 ± 69.61</td>
<td>2.60 %</td>
</tr>
<tr>
<td>1000</td>
<td>7179.94 ± 145.89</td>
<td>2.03 %</td>
<td>100</td>
<td>3151.86 ± 55.55</td>
<td>1.76 %</td>
</tr>
</tbody>
</table>
**Figure 3.** Densitogram of LOS and RAM in pure form

**Figure 4.** Densitogram of LOS in tablet under optimized chromatographic conditions (600 ng/spot)

**Figure 5.** Densitogram of LOS and RAM in tablet under optimized chromatographic conditions (4000 ng.band and 400 ng/spot respectively)
Specificity

Comparison of chromatograms for LOS and RAM from standard and that from formulation showed identical Rf values for both drugs, i.e. 0.34 ± 0.02 for LOS and 0.57 ± 0.02 for RAM (Figure 3, 4 & 5). Excipients and other components present in tablet formulation did not interfere in separation and resolution of LOS and RAM. Apart from Rf values, UV spectra of individual bands from sample were also correlated with spectrum of standard drugs. Comparison of spectra scanned at peak start (s), peak apex (m) and peak end (e) positions of individual bands of sample LOS and RAM showed high degree of correlation [i.e., $r(s, m) = 0.9989$ and $r(m, e) = 0.9979$, $r(s, m) = 0.9993$ and $r(m, e) = 0.9992$] respectively confirmed purity of corresponding band (Figure 6 & 7).
Precision

Precision of instrument was checked by repeated scan of same band of respective drugs seven times without changing plate position. RSD for measurement of peak area was found to be 0.58 % and 1.11 % for LOS and RAM, respectively. Repeatability of sample application (RSD) for area of LOS and RAM was found to be 1.39 % and 1.28 %, respectively which ensures the precision of spotting device. The intraday precision (RSD) was found to be in range of 0.93-1.48 % for LOS and 0.87-1.56 % for RAM. RSD for inter-day precision was found to be in range of 1.12-2.36 % for LOS and 1.29-2.52 % for RAM.

Accuracy

Accuracy was determined by standard addition method. The proposed method was applied for estimation of LOS and RAM in their combined pharmaceutical dosage forms after spiking with known quantity of standard drugs. The percentage recovery was found to be 100.42 % - 101.44 % for LOS and 100.80 % - 102.41 % for RAM (Table 2).

**LOD and LOQ**

The LOD was found to be 26.03 ng/spot for LOS and 14.37 ng/spot for RAM. The LOQ was found to be 78.88 ng/spot for LOS and 43.56 ng/spot for RAM.

Summary of validation parameters has been presented in Table 3.

**Assay of Marketed Formulation**

The spots at Rf 0.34 for LOS and 0.57 for RAM were observed in the chromatogram of the drug sample from marketed formulation. The drug content was found to be 99.46 % ± 0.25 and 98.37% ± 0.15 for LOS and RAM of label claim of their combined dosage form respectively (Table 4). There was no additional peak observed in the chromatogram of combined marketed formulations except LOS and RAM that indicate no interference of excipients in estimation of LOS and RAM in their pharmaceutical dosage form.

**Table 2.** Recovery data for LOS and RAM from tablet formulation

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount of drug in preanalysed sample (mg)</th>
<th>Amount of standard drug spiked (mg)</th>
<th>Total amount (mg)</th>
<th>Amount recovered (mg) (n = 3)</th>
<th>Mean Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOS</td>
<td>100</td>
<td>80</td>
<td>180</td>
<td>80.3 ± 0.56</td>
<td>100.4</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>100</td>
<td>200</td>
<td>101.4 ± 1.02</td>
<td>101.4</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>120</td>
<td>220</td>
<td>120.6 ± 1.36</td>
<td>100.5</td>
</tr>
<tr>
<td>RAM</td>
<td>10</td>
<td>8</td>
<td>18</td>
<td>8.06 ± 0.08</td>
<td>100.8</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10</td>
<td>20</td>
<td>10.15 ± 0.16</td>
<td>101.6</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>12</td>
<td>22</td>
<td>12.27 ± 0.11</td>
<td>102.4</td>
</tr>
</tbody>
</table>
CONCLUSION

HPTLC method was developed for estimation of LOS and RAM in tablet formulation. The method was validated as per ICH (Q2 R1) guidelines. The proposed method was found to be specific, accurate, precise and sensitive. The developed method was successfully applied for quantitative analysis of LOS and RAM in their combined tablet formulation. Results were found to be in good agreement with label claim of their combined tablet formulation. The proposed method can be applied for routine analysis of LOS and RAM in their combined dosage form.

ACKNOWLEDGEMENTS

We would like to thank, Themis Medicare Pvt. Ltd., Mumbai, India for providing the gift sample of LOS and Cadila Pharmaceutical, Ahmedabad, Gujarat, India for providing gift sample of RAM.

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