Validated HPTLC Method for Simultaneous Estimation of Losartan potassium and Metolazone in Bulk Drug and Formulation

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ABSTRACT

This paper describes a new, simple, precise, and accurate HPTLC method for simultaneous estimation of Losartan potassium and Metolazone as the bulk drug and in tablet dosage forms. Chromatographic separation of the drugs was performed on aluminum plates precoated with silica gel 60 F254 as the stationary phase and the solvent system consisted of toluene : ethyl acetate : methanol : glacial acetic acid (6 : 4 : 1 : 0.1 v/v/v/v). Densitometric evaluation of the separated zones was performed at 237 nm. The two drugs were satisfactorily resolved with Rf values 0.33 ± 0.02 and 0.46 ±0.02 for Losartan potassium and Metolazone respectively. The accuracy and reliability of the method was assessed by evaluation of linearity (60-210 ng/spot for Losartan potassium and 120-420 ng/spot for Metolazone, precision (intra-day % RSD was 1.28 – 1.58 and inter-day % RSD was 1.14 – 1.83 for Losartan potassium and intra-day % RSD was 0.67 – 1.03 and inter-day % RSD was 0.49 – 1.18 for Metolazone), accuracy 99.58 ± 0.15 for Losartan potassium and 99.40 ± 0.39 for Metolazone), and specificity in accordance with ICH guidelines.

Keywords: Thin layer chromatography, Densitometry, Validation and Quantification, Losartan potassium and Metolazone.

INTRODUCTION

Losartan potassium is chemically 2-n-butyl-4-chloro-5-hydroxymethyl-1-[2‘-(1H-te-trazol-5-yl)(biphenyl-4-yl)methyl]imidazole, potassium salt (1) (Figure 1). It is a strong non-peptide antihypertensive agent, which exerts its action by specific blocking of angiotensin II receptors. It has a gradual, long-lasting effect as an antihypertensive.
Metolazone is chemically 7-chloro-2-methyl-3-(2-methylphenyl)-4-oxo-1,2-dihydroquinazoline-6-sulfonamide (2,3) (Figure 2). Metolazone is an oral diuretic drug, commonly classified with the thiazide diuretics. It is primarily used to treat congestive heart failure and high blood pressure. Metolazone indirectly decreases the amount of water reabsorbed into the bloodstream by the kidney, so that blood volume decreases and urine volume increases. This lowers blood pressure and prevents excess fluid accumulation in heart failure.

Hence, the combination of Losartan potassium and Metolazone (extended release) complement each other and provides an additive effect on blood pressure control, which is sustained for at least 24 hours.

Today TLC is rapidly becoming a routine analytical technique due to its advantages of low operating costs, high sample throughput and the need for minimum sample preparation. The major advantage of TLC is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC thus reducing the analysis time and cost per analysis.

Literature review reveals that methods have been reported for analysis of Losartan potassium by HPTLC in combination with other drugs (4, 5). Simultaneous estimation of Losartan potassium and Metolazone by LC in combination with other drugs in tablet dosage form have been reported (6, 7, 8, 9, 10, 11, 12). LC-MS-MS development and validation for simultaneous quantitation of Losartan potassium and Metolazone in human plasma have been reported (13, 14, 15).

To date there has been no published reports on simultaneous quantitation of Losartan potassium and Metolazone by HPTLC in bulk drug and in tablet dosage form. This present study reports for the first time the simultaneous quantitation of Losartan potassium and Metolazone by HPTLC in bulk drug and in tablet dosage form. The proposed method is validated as per ICH Guidelines (16).

MATERIALS AND METHODS

Materials
Working standards of pharmaceutical grade Losartan potassium (Batch No. LTP/1001002) and Metolazone (Batch No. 20103601P) were obtained as generous gifts from Centaur Pharmaceuticals Pvt. Ltd., Ambarnath (Maharashtra, India). They were used without further purification and certified to contain 99.70 % (w/w) and 99.80 % (w/w) on dry weight basis for Losartan potassium and Metolazone respectively. Fixed dose combination tablets (Brand Name: METOZ L-25) containing 25 mg of Losartan potassium and 2.5 mg of Metolazone were procured from Centaur Pharmaceuticals Pvt. Ltd, India. All chemicals and reagents of analytical grade were purchased from Merck Chemicals, Mumbai, India.

Instrumentation
The samples were spotted in the form of bands of width 6 mm with a Camag 100 microlitre sample (Hamilton, Bonaduz, Switzerland) syringe on silica gel precoated aluminum plate 60 F 254 plates, [20 cm × 10 cm with 250 µm thickness; E. Merck, Darmstadt, Germany] using a Camag Linomat V (Switzerland) sample applicator. The plates were prewashed with methanol and activated at 110 °C for 5 min prior to chromatography. A constant application rate of 0.1
µL/s was used and the space between two bands was 5 mm. The slit dimension was kept at 5 mm × 0.45 mm and the scanning speed was 10 mm/s. The monochromator bandwidth was set at 20 nm, each track was scanned three times and baseline correction was used. The mobile phase consisted of toluene : ethyl acetate : methanol : glacial acetic acid (6 : 4 : 1 : 0.1 v/v/v/v) and 11.1 mL of mobile phase was used per chromatographic run. Linear ascending development was carried out in a 20 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 40 min at room temperature (25 °C ± 2) at relative humidity of 60 % ± 5. The saturation time was kept 30 min for chromatographic run. Each chromatogram was developed over a distance of 8 cm. Following the development, the TLC plates were dried in a stream of air with the help of an air dryer in a wooden chamber with adequate ventilation. The flow in laboratory was maintained unidirectional (laminar flow, towards the exhaust). Densitometric scanning was performed using a Camag TLC scanner III in the reflectance absorbance mode at 237 nm and operated by CATS software (V 3.15, Camag). The source of radiation used was deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm. Concentrations of the compound chromatographed were determined from the intensity of the light. Evaluation was performed by linear regression of peak areas determined by UV absorption as a function of sample amounts.

Preparation of Standard Stock Solutions
Standard stock solutions with a concentration of 1000 µg/mL were prepared in methanol for Losartan potassium and Metolazone respectively. From the standard stock solutions, diluted mixed standard solutions were prepared containing 500 µg/mL for Losartan Potassium and 50 µg/mL for Metolazon respectively. The stock solution was stored at 2-8 °C protected from light.

Optimization of the HPTLC method
The TLC procedure was optimized with a view to develop a simultaneous assay method for Losartan potassium and Metolazone respectively. The mixed standard stock solution (500 µg/mL of Losartan potassium and 50 µg/mL of Metolazone) was taken and 2 µL sample was spotted on to TLC plates and run in different solvent systems. Optimization of HPTLC method was very difficult in this case as Losartan potassium was not moving at all in toluene : ethyl acetate : methanol (6 : 4 : 1 v/v/v). After many trials it was found that glacial acetic acid is necessary for movement of Losartan potassium. Finally the mobile phase consisting of toluene : ethyl acetate : methanol : glacial acetic acid (6 : 4 : 1 : 0.1 v/v/v/v) was found optimum (Figure 3).

In order to reduce the neckless effect TLC chamber was saturated for 30 min using saturation pads. The mobile phase was run up to a distance of 8 cm; which takes approximately 20 min for complete development of the TLC plate.

Validation of the method
Validation of the optimized TLC method was carried out with respect to the following parameters.

Linearity and range
From the mixed standard stock solution, 30 µg/mL Losartan potassium and 60 µg/mL of Metolazone, 2 to 7 µL solution were spotted on TLC plate to obtain final concentration 60-210 ng/spot for Losartan potassium and 120-420 ng/spot for Metolazone. Linearity of the method...
was studied by applying six concentrations of the drug, each concentration was applied three times to the TLC plates. The plate was then developed using the previously described mobile phase and the peak areas were plotted against the corresponding concentrations to obtain the calibration curves.

**Precision**

The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations (60 ng/spot, 120 ng/spot and 180 ng/spot for Losartan potassium and 120 ng/spot, 240 ng/spot and 360 ng/spot for Metolazone respectively) six times on the same day. The intermediate precision of the method was checked by repeating studies on three different days.

**Limit of detection and limit of quantitation**

Limits of detection (LOD) and quantification (LOQ) represent the concentration of the analyte that would yield signal-to-noise ratios of 3 for LOD and 10 for LOQ, respectively. LOD and LOQ were determined by measuring the magnitude of analytical background by spotting a blank and calculating the signal-to-noise ratio for Losartan potassium and Metolazone by spotting a series of solutions until the S/N ratio 3 for LOD and 10 for LOQ. To determine the LOD and LOQ, serial dilutions of mixed standard solution of Losartan potassium and Metolazone were prepared from the standard stock solution in the range of 10–700 ng/spot. The samples were applied to TLC plate and the chromatograms were run and measured signal from the samples was compared with those of blank.

**Robustness of the method**

Following the introduction of small changes in the mobile phase composition (± 0.1 mL for each component), the effects on the results was examined. Mobile phases having different compositions, e.g. toluene : ethyl acetate : methanol : glacial acetic acid (6.1 : 4 : 1 : 0.1 v/v/v), (6 : 4.1 : 1 : 0.1 v/v/v), (6 : 4 : 1.1 : 0.1 v/v/v), (6 : 4 : 1 : 0.2 v/v/v) were tried and chromatograms were run. The amount of mobile phase was varied over the range of ± 5 %. The plates were prewashed with methanol and activated at 110 °C for 2, 5, and 7 min respectively prior to chromatography. The time from spotting to chromatography and from chromatography to scanning was varied from + 10 min. The robustness of the method was determined at three different concentration levels for 60 ng/spot, 120 ng/spot and 180 ng/spot for Losartan potassium and 120 ng/spot, 240 ng/spot and 360 ng/spot for Metolazone.

**Specificity**

The specificity of the method was determined by analyzing standard drug and test samples. The spot for Losartan potassium and Metolazone in the samples was confirmed by comparing the R<sub>f</sub> and spectrum of the spot with that of standard. The peak purity of Losartan potassium and Metolazone was determined by comparing the spectrum at three different regions of the spot i.e. peak start (S), peak apex (M) and peak end (E).

**Recovery studies**

Accuracy of the method was carried out by applying the method to drug sample (Losartan potassium and Metolazone combination tablet) to which known amount of Losartan potassium and Metolazone standard powder corresponding to 80, 100 and 120% of label claim had been
added (standard addition method), mixed and the powder was extracted and analyzed by running chromatogram in optimized mobile phase.

**Analysis of a marketed formulation**

To determine the content of Losartan potassium and Metolazone in conventional tablet (Brand Name: METOZ L-25, Batch No. 107, Manufacturer – Centaur Pharmaceutical Pvt. Ltd., Label claim: Losartan potassium 25 mg and Metolazone 2.5 mg per tablet), twenty tablets were weighed, their mean weight determined and finely powdered. The weight of the tablet triturate equivalent to 25 mg Losartan potassium and Metolazone 2.5 mg was transferred into a 50 mL volumetric flask containing 35 mL methanol, sonicated for 30 min with occasional shaking and diluted to 50 mL with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min and the drug content of the supernatant was determined (500 µg/mL for Losartan potassium and 50 µg/mL for Metolazone). (Then 2 µL of the spot was applied which gave final concentration of 1000 ng/spot for Losartan potassium and 100 ng/spot for Metolazone. The analysis was repeated in triplicate. The possibility of excipient interference with the analysis was examined.

**Results and discussion**

The results of validation studies on simultaneous estimation of the method developed for Losartan potassium and Metolazone in the current study using as the mobile phase toluene : ethyl acetate : methanol : glacial acetic acid (6 : 4 : 1 : 0.1 v/v/v/v) for TLC are given below.

**Linearity**
The drug response was linear (r² 0.9998 for Losartan potassium and 0.9997 for Metolazone) over the concentration range between 60 - 210 ng/spot for Losartan potassium and 120 - 420 ng/spot Metolazone respectively.

**Precision**
The results of the repeatability and intermediate precision experiments are shown in **Table 1**. The developed method was found to be precise as the RSD values for repeatability and intermediate precision studies were < 2%, as recommended by ICH guidelines.

**LOD and LOQ**
Signal-to-noise ratios of 3 : 1 and 10 : 1 were obtained for LOD and LOQ respectively. The LOD and LOQ were found to be, 100 ng/spot and 120 ng/spot for Losartan potassium, 50 ng/spot and 60 ng/spot for Metolazone respectively.

**Robustness of the method**
The standard deviation of peak areas was calculated for each parameter and the % RSD was found to be less than 2. The low values of the % RSD, as shown in **Table 2** indicated the robustness of the method.

**Specificity**
The peak purity of Losartan potassium and Metolazone was assessed by comparing their respective spectra at the peak start, apex and peak end positions of the spot i.e., r (S, M) = 0.9973 and r (M, E) = 0.9981. A good correlation (r = 0.9994) was also obtained between the standard and sample spectra of Losartan potassium and Metolazone respectively.
Recovery studies
As shown from the data in Table 3 good recoveries of the Losartan potassium and Metolazone in the range from 98.40 % w/w to 100.18 % w/w were obtained at various added concentrations.

Table 1 Precision Studies

<table>
<thead>
<tr>
<th>Concentration (ng/spot)</th>
<th>Measured conc. ±SD</th>
<th>Recovery (%)</th>
<th>Measured conc. ±SD</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Repeatability (n=6)</td>
<td>RSD</td>
<td>Intermediate precision (n=6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(%)</td>
<td>Recovery (%)</td>
<td>(%)</td>
<td>Recovery (%)</td>
</tr>
<tr>
<td>Losartan potassium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>59.43 ± 3.5</td>
<td>1.28</td>
<td>99.28</td>
<td>58.69 ± 3.2</td>
</tr>
<tr>
<td>120</td>
<td>118.12 ± 3.6</td>
<td>0.94</td>
<td>98.82</td>
<td>119.11 ± 4.7</td>
</tr>
<tr>
<td>180</td>
<td>177.37 ± 8.8</td>
<td>1.58</td>
<td>98.90</td>
<td>177.51 ± 10.4</td>
</tr>
<tr>
<td>Metolazone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>119.74 ± 10.7</td>
<td>1.03</td>
<td>98.37</td>
<td>119.79 ± 11.0</td>
</tr>
<tr>
<td>240</td>
<td>239.96 ± 16.0</td>
<td>0.67</td>
<td>99.87</td>
<td>239.38 ± 11.7</td>
</tr>
<tr>
<td>360</td>
<td>359.19 ± 37.3</td>
<td>0.91</td>
<td>98.31</td>
<td>359.62 ± 1.5</td>
</tr>
</tbody>
</table>

Table 2 Robustness testing

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SD of Peak Area for Losartan potassium (±0.1 ml)</th>
<th>% RSD</th>
<th>SD of Peak Area for Metolazone</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase composition (±0.1 ml)</td>
<td>4.07</td>
<td>0.34</td>
<td>3.74</td>
<td>0.02</td>
</tr>
<tr>
<td>Amount of mobile phase (±5%)</td>
<td>21.09</td>
<td>0.83</td>
<td>8.72</td>
<td>0.15</td>
</tr>
<tr>
<td>Time from spotting to chromatography (+ 10 min.)</td>
<td>5.31</td>
<td>0.16</td>
<td>3.31</td>
<td>0.06</td>
</tr>
<tr>
<td>Time from chromatography to scanning (+ 10 min.)</td>
<td>6.12</td>
<td>0.25</td>
<td>2.63</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Table 3 Recovery studies

<table>
<thead>
<tr>
<th>Drug</th>
<th>Label claim (mg per tablet)</th>
<th>Amount Added (%)</th>
<th>Total amount (mg)</th>
<th>Amount recovered (mg) ± %RSD</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Losartan potassium</td>
<td>25</td>
<td>80 (20 mg)</td>
<td>45</td>
<td>44.28 ± 1.13</td>
<td>98.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 (25 mg)</td>
<td>50</td>
<td>49.82 ± 1.43</td>
<td>99.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120 (30 mg)</td>
<td>55</td>
<td>54.95 ± 1.12</td>
<td>99.90</td>
</tr>
<tr>
<td>Metolazone</td>
<td>2.5</td>
<td>80 (2 mg)</td>
<td>4.5</td>
<td>4.47 ± 1.21</td>
<td>99.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 (2.5 mg)</td>
<td>5</td>
<td>4.99 ± 0.78</td>
<td>99.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120 (3 mg)</td>
<td>5.5</td>
<td>5.51 ± 1.56</td>
<td>100.18</td>
</tr>
</tbody>
</table>

Analysis of a formulation
Experimental results of the amount of Losartan potassium and Metolazone in tablets, expressed as a percentage of label claim were in good agreement with the label claims thereby suggesting
that there is no interference from any of the excipients that are normally present in tablets. Two different lots of Losartan potassium and Metolazone combination tablets were analyzed using the proposed procedures (Table 4).

**Figure 3 Densitogram of standard drugs**


Losartan potassium: $R_f 0.33 \pm 0.02$

Metolazone: $R_f 0.46 \pm 0.02$

*Concentration of drugs:* 100 µg/mL for Losartan potassium and 10 µg/mL for Metolazone

*Application volume:* 10 µL

*Wavelength:* 237 nm
CONCLUSION

Introducing TLC into pharmaceutical analysis represents a major step in terms of quality assurance. The developed TLC technique is precise, specific and accurate. Statistical analysis proves that the method is suitable for the analysis of Losartan potassium and Metolazone as bulk drug and in pharmaceutical formulation without any interference from the excipients. It may be extended to study the degradation kinetics of Losartan potassium and Metolazone also for its estimation in plasma and other biological fluids. The proposed TLC method is less expensive, simpler, rapid, and more flexible than HPLC.

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