Simultaneous estimation of Atorvastatin calcium, Ramipril and Aspirin in capsule dosage form using HPTLC

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Abstract

A simple, sensitive, precise and accurate high performance thin layer chromatographic (HPTLC) method has been developed for the simultaneous estimation of atorvastatin (AT) calcium, ramipril (RA) and aspirin (AS) in combined dosage form. The method was developed using precoated silica gel 60F254 as stationary phase. The mobile phase used was a mixture of benzene: ethyl acetate: toluene: methanol: glacial acetic acid (4.0:4.5:1.0:0.5:0.1 v/v/v/v/v). Detection was carried out with ultra-violet detection at 220 nm. The Rf values were about 0.45±0.02, 0.28±0.01 and 0.72±0.02 for AT, RA and AS, respectively. The developed method was validated for linearity, accuracy, precision, limit of Detection (LOD), limit of quantification (LOQ) and robustness. The linearity ranges were 0.5-2.5 µg/spot for AT, 0.5-2.5 µg/spot for RA and 0.75-3.75 µg/spot for AS with mean recoveries of 100.29±0.94, 99.06±1.79 and 98.85±0.61 for AT, RA and AS, respectively. The proposed method can be used for the estimation of these drugs in combined dosage forms.

Key words: Atorvastatin Calcium, Ramipril, Aspirin, Capsule and HPTLC.

INTRODUCTION

Atorvastatin (AT) is [R-(R*, R*)]-2-(4-fluorophenyl)-β, δ -dihydroxy-5-(1-methyl-ethyl)-3-phenyl-4-[(phenyl amino) carbonyl]-1H-pyrrole-1-heptanoic acid, a synthetic lipid-lowering agent which is about a 100 times as potent as the other drugs in its class and at lower costs than most of the others [1]. AT is an inhibitor of 3-hydroxy-3- methylglutaryl-coenzyme A reductase [2].

Ramipril (RA) is (1S, 5S, 7S)-8[(2S-2-[(1S)-1-ethoxycarbonyl-3-phenyl propyl] amino) propanoyl]-8-azabicyclo[3.3.0]octane-7-carboxylic acid, an angiotensin converting enzyme inhibitor, used to treat hypertension and congestive heart failure [3]. Aspirin (AS) is 2-acetyloxybenzoic acid, often used as an analgesic, antipyretic, anti-inflammatory and an
antiplatelet agent [4]. It suppresses the production of prostaglandins and thromboxanes due to inactivation of the cyclo-oxygenase enzyme [5].

Literature survey revealed that several analytical methods such as spectrophotometry, spectrofluorimetry, High performance Liquid Chromatography (HPLC), Raman spectroscopy, LCMS- MS and LC-ESI-MS have been reported for the determination of AT [6-16], RA [17-26] and AS [27-38] in pharmaceutical dosage forms and biological samples. The single RP-LC method has been reported to determine AT, RA and AS in combined capsule dosage form. [39]. There is no single HPTLC method reported for the estimation of AT, RA and AS in combine formulation.

Hence, the aim was to develop a selective, sensitive and accurate method which can estimate the three components simultaneously by HPTLC. The proposed method describes a simple, accurate, sensitive and precise HPTLC method for the simultaneous estimation of AT, RA and AS in marketed pharmaceutical dosage form.

**MATERIALS AND METHODS**

A Linomate V auto sampler, scanner III, flat bottom and twin trough developing chambers and viewing cabinet with dual wavelength UV lamps (Camag, Muttenz, Switzerland), used. HPTLC plates used were silica gel with layer thickness 0.2 mm, 20x 10 cm, aluminium backing (E Merck, Germany).

AT, RA and AS pure powder were procured as gift sample from Torrent pharmaceutical, (Ahmadabad, India) used as standard. Capsule dosage form (MODLIP CAD; 10 mg AT, 5 mg RA and 75 mg AS per capsule) of torrent pharmaceutical, (Ahmadabad, India) were purchased from local market. Benzene, ethyl acetate, toluene, methanol, glacial acetic acid were procured from S.D. fine Chemicals (Mumbai, India) and were of analytical grade.

**Chromatographic Conditions**

Solutions of AT, RA and AS were applied to precoated silica gel 60F254 HPTLC plates (20 x 10 cm) by means of Linomate V automatic spotter equipped with a 100 µL Syringe and operated with settings of band length, 6 mm; distance between bands, 8 mm; distance from the plate edge, 10mm; and distance from bottom of the plate, 10 mm. The plates were developed in a twin trough chamber previously saturated for 30 min with mobile phase, benzene: ethyl acetate: toluene: methanol: glacial acetic acid (4.0:4.5:1.0:0.5:0.1 v/v/v/v/v), for a distance of 8 cm. The spots on the air dried plate were scanned with a scanner III at 220 nm using the deuterium source.

**Preparation of standard stock solution**

Accurately weighed AT (25 mg), RA (25 mg) and AS (37.5 mg) were transferred to 50 mL volumetric flasks, dissolved in and diluted to the mark with methanol to obtain a working standard solution of AT (500 µg/ mL),RA (500 µg /mL) and AS (750 µg /mL) for this proposed method.

**Method Validation**

**Calibration curve:**

Calibration curves were plotted over a concentration range of 0.5-2.5, 0.5-2.5 and 0.75-3.75 µg/spot for AT, RA and AS, respectively. Accurately prepared working standard solution of AT, RA and AS (1.0, 1.5, 2.0, 3.5, 4.0, 4.5 and 5.0 µL) were applied to plates. The calibration curves
were constructed by plotting peak areas versus concentrations with the help of win CATS software. Each reading was the average of three determinations.

**Accuracy (% recovery):**
The accuracy of the methods was determined by calculating recoveries of AT, RA and AS by standard addition method. Known amount of standard solutions of AT (0.5, 1.0 and 1.5 µg/spot) RA (0.25, 0.5 and 0.75 µg/spot) and AS (3.75, 7.5 and 11.25 µg/spot) were added to prequantified sample solutions of AT (1 µg/spot), RA (0.5 µg/spot) and AS (7.5 µg/spot) for proposed method, respectively. The amounts of AT, RA and AS were estimated by applying these values to the regression equation of the calibration curve.

**System Suitability test:**
System suitability tests are used to verify that the resolution and repeatability of the system were adequate for the analysis intended. The precision of the instruments was checked by repeatedly spotting (n = 6) solution of AT (1.0 µg/spot), RA (1.0 µg/spot) and AS (1.5 µg/spot) without changing the position of plate for HPTLC method.

**Intermediate Precision (Reproducibility):**
The intraday and interday precisions of the proposed method was determined by estimating the corresponding responses three times on the same day and on three different days over a period of one week for three different concentration of AT (1.0, 1.5 and 2.0 µg/spot), RA (1.0, 1.5 and 2.0 µg/spot) and AS (1.5, 2.25 and 3.0 µg/spot). The results are reported in terms of relative standard deviation.

**Limit of detection (LOD) and Limit of quantification (LOQ):**
The LOD and LOQ were calculated using following equations as per International conference on Harmonization (ICH) guideline.

\[
\text{LOD} = 3.3 \times \sigma / S \\
\text{LOQ} = 10 \times \sigma / S
\]

Where \( \sigma \) = standard deviation of the response and \( S \) = the standard deviation of y intercept of regression lines.

**Robustness:**
Here, small deliberate changes in the chromatographic conditions like mobile phase composition, detection wavelength and saturation time for mobile phase were done. Obtained results were compared with original chromatographic conditions.

**Analysis of Pharmaceutical Dosage form (Capsules):**
All the capsules were purchased from local market. The response for capsule dosage form was measured at 220 nm for the quantification of AT, RA and AS by using HPTLC as described above. The amounts of the above mentioned drugs present in the sample solutions were determined by fitting the responses into the regression equation for AT, RA and AS.

**RESULTS AND DISCUSSION**

To optimize the HPTLC parameters, several mobile phase compositions were tried. Satisfactory separations for AT, RA and AS were obtained with mobile phase consisting of benzene: ethyl acetate: toluene: methanol: glacial acetic acid (4.0:4.5:1.0:0.5:0.1 v/v/v/v/v). Quantification was
achieved with UV detection at 220 nm based on peak area. Better resolution of the peaks with clear baseline separation was found. (Fig.1).

Fig 1: Chromatogram of RA, AT and AS

Specificity (Selectivity):
The selectivity of HPTLC method was checked by comparison of chromatograms obtained from samples (capsules) and corresponding placebo. Additives are practically insoluble in methanol or mobile phase whereas the active constituents are freely soluble. No interference from additives of the capsules was obtained.

Table 1. Regression analysis of the calibration curves for atorvastatin calcium, ramipril and aspirin by the proposed HPTLC method

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AT</th>
<th>RA</th>
<th>AS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range</td>
<td>0.5-2.5 µg/spot</td>
<td>0.5-2.5 µg/spot</td>
<td>0.75-3.75 µg/spot</td>
</tr>
<tr>
<td>Slope</td>
<td>2.9165</td>
<td>1.0318</td>
<td>2.0539</td>
</tr>
<tr>
<td>Intercept</td>
<td>-257.36</td>
<td>235.95</td>
<td>3148.7</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.998</td>
<td>0.9978</td>
<td>0.9946</td>
</tr>
</tbody>
</table>

Linearity:
The linear correlation between the peak area and compound was checked for each component. Data for five solutions of different concentration of AT (500-2500 ng/spot), RA (500-2500
ng/spot) and AS (750-3750 ng/spot) were collected and analyzed. The least squares method was used for calculation of slope, intercept and correlation coefficient(r). For all the compounds the correlation between the peak area and substance concentration were described by linear regression equation with high values of correlation coefficient(r). (Tab 1)

**Limit of detection (LOD) and Limit of quantification (LOQ):**
LOD for AT, RA and AS were found to be 65.50, 87.82 and 98.23ng/spot, respectively by this method. LOD for AT, RA and AS were found to be 198.49, 266.13 and 297.67ng/spot, respectively by this method. (Tab 2).

**Table 2. Summary of validation parameters for atorvastatin calcium, ramipril and aspirin by the proposed HPTLC method**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AT</th>
<th>RA</th>
<th>AS</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOD (ng/spot)</td>
<td>65.50</td>
<td>87.82</td>
<td>98.23</td>
</tr>
<tr>
<td>LOQ (ng/spot)</td>
<td>198.49</td>
<td>266.13</td>
<td>297.67</td>
</tr>
<tr>
<td>Accuracy (%)±RSD</td>
<td>100.29±0.94</td>
<td>99.06±1.79</td>
<td>98.85±0.61</td>
</tr>
<tr>
<td>Precision (% RSD)</td>
<td>0.79-1.18</td>
<td>0.90-1.62</td>
<td>0.56-0.94</td>
</tr>
<tr>
<td>Intra-day (n = 3)</td>
<td>0.84-1.25</td>
<td>1.09-1.73</td>
<td>0.63-1.12</td>
</tr>
<tr>
<td>Inter-day (n =3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repeatability(% RSD)</td>
<td>1.06</td>
<td>1.23</td>
<td>1.28</td>
</tr>
</tbody>
</table>

**Accuracy:**
The recovery experiment carried out by standard addition method. The percentage of the recoveries obtained were 100.29 ± 0.94, 99.06 ± 1.79, 98.85 ± 0.61 for AT, RA and AS, respectively. (Tab. 2). The recovery of the method was good.

**Precision:**
The low % RSD values of inter-day (0.63-1.73%) and intra-day (0.56-0.94 %) implies that the reproducibility of the proposed method was good. (Tab 2)

**Robustness:**
The method was found to be robust, although small deliberate changes in method conditions did have a negligible effect on the chromatographic behavior of the solutes. The results indicate that changing in mobile phase composition had no large effect on the chromatographic behavior of the drugs. Even a small change in saturation time of mobile phase did not cause a notable change in retention factor (Rf), tailing of the peaks of the used drugs for this method. Alteration of the detection wavelength (± 5 nm) caused no variation of the peak areas and did not affect the chromatographic behavior of AT, RA and AS.

**System Suitability Test:**
The percentages of relative standard deviation (%RSD) for AT, RA and AS were found to be 1.06, 1.23 and 1.28, respectively using this method (Tab. 2). All the results were within acceptable range.

**Assay of Pharmaceutical dosage form:**
The proposed validate method was successfully applied to determine AT, RA and AS in pharmaceutical product. The results obtained were comparable with the corresponding labeled amounts. (Tab.3)
Table 3: Assay results for AT, RT and AS

<table>
<thead>
<tr>
<th>Brand</th>
<th>Modlip-CAD</th>
<th>Content Label</th>
<th>Amount</th>
<th>Mean±SD</th>
<th>% ± RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT</td>
<td>10 mg</td>
<td>9.9525±0.10649</td>
<td>99.53±1.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RA</td>
<td>5 mg</td>
<td>5.03793±0.04634</td>
<td>100.75±0.092</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AS</td>
<td>75 mg</td>
<td>74.3796 ±0.51770</td>
<td>99.17±0.69</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSION

The validated HPTLC method employed here proved to be simple, specific, accurate, precise, sensitive and robust. It can be successfully used for routine analysis of AT, RA and AS in combined dosage form without any interference from common excipients and impurity. With the developed method, only single mobile phase is sufficient for quantification of AT, RA and AS either in combination or in single dosage form as per availability of formulation for many pharmaceutical industries. Hence, this proposed method can save labor, cost and analysis time for changing mobile phases.

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