HPTLC METHOD DEVELOPMENT AND VALIDATION OF SIMVASTATIN AND ASPIRIN IN COMBINE DOSAGE FORM


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ABSTRACT
There is a simple, precise and accurate methods are described for the direct determination of Simvastatin and Aspirin in combined dosage form. The method is based on HPTLC separation of the two drugs followed by the densitometry measurements of their spots at 238 nm for both drugs. The separation was carried out on silica gel 60GF254 using mobile phase Hexane: ethyl acetate: chloroform: glacial acetic acid (6:3:0.5:0.5). The linearity for Simvastatin and Aspirin lies between 200-1200 ng/spot and 1000-6000 ng/spot with co-relation co-efficient > 0.999. The Rf values are 0.52 and 0.29 for Simvastatin and Aspirin respectively. All the developed methods are validated according to ICH guidelines. Accuracy was performed and result of accuracy was lies between the limit 98% to 102%. Percentage recovery for SIM was 98.38-102.89%, while for ASP, it was found to be in range of 100.89-.95%.Recovery between 98 % - 102% justifies the accuracy of the method. In HPTLC method the regression line equation for Simvastatin and Aspirin are, Y = 8.4698x + 1212.8 for Simvastatin. And y = 0.4598x + 877.1 for Aspirin. The proposed methods were applied successfully for the determination of the two drugs in bulk powder and pharmaceutical dosage form. The LOD and LOQ value are 26.12 and 111.5 for Simvastatin and 79.16 and 339.23 for aspirin in HPTLC Method.

KEYWORDS: Simvastatin, Aspirin, Simultaneous method and HPTLC method validation.

INTRODUCTION
Simvastatin is a lipid-lowering agent and potent competitive inhibitor of hydroxy methyl glutaryl CoA reductases.[1,2] Aspirin is a Nonsteroidal Antinflammatory drug (NSAID) effective in treating fever, pain, and inflammation in the body. It also prevents blood clots.[1-3] Simvastatin and aspirin in combine dosage form is used for treatment of CVS disease. In recent years HPTLC method was reported for the quantification of Simvastatin. But referring to the literature survey, there is no any published HPTLC method for Simvastatin and Aspirin combined capsule form. The present paper describes a simple, accurate and precise method for simultaneous estimation of Simvastatin and Aspirin in combined capsule dosage form. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guideline.[5] IUPAC Name of Simvastatin is S,3R,7S,8S,8aR)-8-{2-[2R,4R)-4-hydroxy-6-oxooxan-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydonaphthalen-1-yl 2,2-dimethylbutanoate
Aspirin chemically known as acetyl salicylic acid and is used as non-steroidal anti-inflammatory
and analgesic drug.\textsuperscript{[3,6,7]} A variety of chromatographic methods were introduced for the determination of Simvastatin and Aspirin, including conventional RP-HPLC\textsuperscript{[8-12]}, RP-UPLC\textsuperscript{[13]}, HPTLC\textsuperscript{[14]} method.

**MATERIAL AND METHODS**

- Simvastatin (Gift sample from Macloed’s laboratory Ltd.)
- Aspirin (Gift sample from Sidmac Laboratories Ltd.)
- Methanol AR Grade
- Distilled water

**HPTLC METHOD**

**APPARATUS AND INSTRUMENT:**
Applicator Linomat V (Camag) - Semiautomatic application, band application by spray on technique, twin trough glass chamber (Camag) - (20x10cm), TLC scanner, III (Camag), Spectral range 190–800 nm, U.V cabinet with dual wavelength U.V lamp (Camag) - Dual wavelength 254/366 nm, Stationary Phase- Pre-coated Silica gel on aluminum sheet 60GF254, Applicator syringe (Hamilton, Bonaduz, Schweiz) - 100 μL, Data resolution-100 μm/step.

**REAGENTS AND MATERIALS:**
Simvastatin API, Aspirin API, hexane, ethyl acetate, chloroform, G.A.A (ARGrade)

**CHROMATOGRAPHIC CONDITIONS:**
Stationary phase: Pre-coated Silica gel on aluminium sheet 60GF254, Applicator syringe (Hamilton, Bonaduz, Schweiz) - 100 μL, Data resolution-100 μm/step, Mobile phase- Hexane: ethyl acetate: chloroform: G.A.A.(6:3:0.5:0.5), Chamber saturation time: 20 min, Distance run: 70 mm, Ambient temperature: 25-26°C, Source lamp: Deuterium, Measurement Mode: Absorbance, Wavelength of detection: 238 nm, Slit dimension: 6 x 0.03 mm, Band width: 6 mm, Syringe capacity: 100 μL, Space between band: 10mm, Scanning rate: 20 mm/sec

**METHODOLOGY:**

**Preparation of mobile phase:**
A mixture of Hexane: ethyl acetate: chloroform: G.A.A.(6:3:0.5:0.5)ml were mixed properly and it was used as a mobile phase.

**Preparation of Standard Solution:** 10 mg of simvastatin and 50 mg Aspirin (ASP) were dissolved and diluted with methanol upto 100mL (100 μg/mL of SIM and 500 μg/mL of ASP). Prepration of calibration curve
Preparation of Sample Solution: 20 tablets were weighed. Powder equivalent to 10 mg of SIM and 50 mg of ASP transferred into 100mL volumetric flask. Methanol was added to adjust level up to mark and sonicated for 30 min. The solution was filtered through whatman filter paper no. 42. First few mL of filtrate was discarded. The sample solution was used for estimation of SIM and ASP. 1 & 5 μL of sample solution was used for estimation purpose.

Linearity and Range: Linearity was found in the range of 200-1200 ng/spot for SIM and 1000-6000 ng/spot for ASP. The drug peak area was calculated for each concentration level and a graph was plotted of drug concentration against the peak area. Calibration parameters are given in table 1.

Table 1: linearity data for Simvastatin and Aspirin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Simvastatin</th>
<th>Aspirin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity</td>
<td>200-1200</td>
<td>1000-6000</td>
</tr>
<tr>
<td>Linearity eqn</td>
<td>$Y = 8.4698x + 1212.8$</td>
<td>$Y = 0.4598x + 877.1$</td>
</tr>
<tr>
<td>Correlation coeff.</td>
<td>0.9997</td>
<td>0.9991</td>
</tr>
<tr>
<td>Slope</td>
<td>1212.8</td>
<td>877.1</td>
</tr>
<tr>
<td>Intercept</td>
<td>8.4698</td>
<td>0.4598</td>
</tr>
</tbody>
</table>

Fig: 1: 3D spectra of Simvastatin and Aspirin in HPTLC method.

Intraday

Pure API of Simvastatin and Aspirin were taken in a ratio of 400+2000, 600+3000, 800+4000 (ng/spot) was analysed at three levels of concentration for three times in a day. Peak areas of
solutions were measured. The %RSD of Simvastatin and Aspirin were found to be 0.6699 for Simvastatin and 0.8589 for Aspirin.

**Interday**

Pure API of Simvastatin and Aspirin were taken in a ratio of 400+2000, 600+3000, 800+4000 (ng/spot) was analysed at three levels of concentration for three consecutive day. Peak areas of solutions were measured. The %RSD of Simvastatin and Aspirin were found to be 0.6797 for Simvastatin and 0.8887 for Aspirin.

**Specificity**

The purity of the chromatographic peaks were analyzed by scanning standard and sample peaks in spectral scanning mode of the wincats software. The peak purity for Simvastatin and Aspirin were tested by correlation of spectra acquired at the peak start (s), peak maximum (m), and peak end (e) positions. The correlation spectra of sample were compared with that of standard.

**Table: 2: Specificity Data For Simvastatin And Aspirin**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Correlation r (s, m)</th>
<th>Correlation r (m, e)</th>
<th>Peak purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simvastatin</td>
<td>0.9998914</td>
<td>0.9991214</td>
<td>Pass</td>
</tr>
<tr>
<td>Aspirin</td>
<td>0.9990532</td>
<td>0.9995859</td>
<td>Pass</td>
</tr>
</tbody>
</table>

**Accuracy**

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. Percentage recovery for Simvastatin and for Aspirin was found out. Recovery between 98% - 102 % justifies the accuracy of the method. The preparation of solution for the recovery study is given in the table. To above flask mgs of both drug in 80%, 100% and 120% were added and then continued assay procedure. Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. Percentage recovery for sim was 98.38-102.89%, while for asp, it was found to be in range of 99.49-102.87%. The results are shown in table. Recovery between 98 % - 102% justifies the accuracy of the method.

**Table: 3: % Recovery Data Of Simvastatin And Aspirin For HPTLC Method**

<table>
<thead>
<tr>
<th>Assay level</th>
<th>Tablet content equivalent to (ng/spot)</th>
<th>Standard added (ng/spot)</th>
<th>Total drug total recovered (ng/spot)</th>
<th>% recovery Sim</th>
<th>Asp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sim 400  Asp 2000</td>
<td>Sim - 320 Asp 1600</td>
<td>Sim 399.22 Asp 1999.55</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>80%</td>
<td>400 2000</td>
<td>320 1600</td>
<td>718.9 3599.40</td>
<td>98.38</td>
<td>101.67</td>
</tr>
<tr>
<td>100%</td>
<td>400 2000</td>
<td>400 2000</td>
<td>799.66 3999.51</td>
<td>101.53</td>
<td>99.49</td>
</tr>
<tr>
<td>120%</td>
<td>400 2000</td>
<td>480 2400</td>
<td>879.59 4400.74</td>
<td>102.89</td>
<td>102.87</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

Literature survey revealed that there was no any reported HPTLC method for simultaneous estimation of Simvastatin and Aspirin. The present study was aimed at development of speedy and cost effective HPTLC technique for determination of SIM and ASP. Various blends of solvent systems in varying proportions were tried as mobile phase. However, mobile phase consisting n-Hexane: Ethyl acetate: chloroform: G.A.A. in the ratio of (6: 3: 0.5: 0.5 v/v/v) was found to be more suitable with Rf values of 0.24±0.02 and 0.44±0.02 for SIM and ASP, respectively with saturation time of 20 minutes. The selection of wave length was based on maximum absorbance for optimum sensitivity. The drugs showed good linearity in the range of 200-1200 ng/spot for SIM and 1000-6000 ng/spot for ASP with coefficient of correlation value 0.9997 and 0.9991, respectively. From the recovery studies, the accuracy results were 99.88% for SIM and 100.04% for ASP and were found to be highly accurate.

CONCLUSION

Simvastatin and Aspirin in combined dosage form is used for the treatment of diabetes mellitus and other CVS disease. Simple, accurate, rapid and precise HPTLC method were developed and validated for simultaneous estimation of both these drugs.

HPTLC method

The linearity range lies between 200-1200 ng/spot for Simvastatin and 1000-6000 ng/spot for Aspirin with co-relation co-efficient of 0.9997 and 0.9991 respectively. The quantitation performed at 238 nm. The Rf value for Simvastatin is 0.52 and for Aspirin is 0.29. The percentage recovery for Simvastatin and Aspirin is in the range of 98.38-102.66 % and 99.60-102.89%. The excipients usually present in the pharmaceutical formulation did not interfere with determination of Simvastatin and Aspirin.

REFERENCES

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