ABSTRACT
A simple, fast, precise and accurate high performance thin layer chromatographic method has been developed for estimation of atorvastatin calcium (AST) and nicotinic acid (NA) simultaneously from a combined dosage forms. The chromatographic separation was developed using a pre coated silica gel 60 GF 254 TLC plate as stationary phase and the chromatogram was developed using benzene: ethyl acetate: toluene : methanol : glacial acetic acid (3:4:2:6:0.4:0.5v/v/v/v) as mobile phase. Densitometric evaluation was performed at 247 nm. Atorvastatin calcium and nicotinic acid resolved satisfactorily at Rf values 0.64 ± 0.002 and 0.35 ± 0.008 respectively. The developed method was validated for accuracy, linearity, precision, limit of detection (LOD) and limit of quantification (LOQ) and robustness as per the ICH guidelines. The proposed method can be used for the estimation of these drugs in combined dosage form.

Keywords: Simultaneous estimation, Atorvastatin calcium, Nicotinic acid, Tablet form and HPTLC.

INTRODUCTION
Atorvastatin calcium is chemically a calcium salt of (β R, 8 R)-2-(4 – fluro-phenyl) – α, δ di hydroxyl 5(1 methyl ethyl) 1, 3, phenyl, 4 (phenyl amino) carbonyl -1 H pyrrole- heptanoic acid tri hydrate used as antihyperlipidaemic. It is official in Indian pharmacopoeia. Nicotinic acid is chemically pyridine-3-carboxylic acid, used for pellagra and hyperlipoproteinemia. It is official both in I.P and B.P.

Detailed survey of literature for atorvastatin calcium (AST) revealed several methods based on different techniques like extractive Spectrophotometry, HPLC, HPTLC for its determination in human serum, capillary electrophoresis, HPTLC for its determination in pharmaceutical samples.

Similarly literature survey for Nicotinic acid (NA) revealed several methods based on Spectrophotometry, RP- HPLC, and tandem mass Spectrophotometry. Methods are reported for estimation of atorvastatin calcium and nicotinic acid in combination by RP- HPLC, both in tablet and capsule dosage form, however no HPTLC method has been reported. Hence the aim of this paper is to develop a sensitive, accurate and selective method which can estimate both the drugs simultaneously by HPTLC both in bulk and tablet dosage forms. The proposed method describes a simple, sensitive accurate and precise HPTLC method for the simultaneous estimation AST and NA in bulk and in marketed dosage forms.

MATERIALS AND METHODS
Chemical and reagents
AST was the generous gifts from Biocon Limited Bangalore, and nicotinic acid was procured from Qualigens Fine Chemicals (Gliax Ltd). Combination of these drugs was purchased from the local market (TONACT FORTE containing Atorvastatin calcium 10 mg and nicotinic acid 500 mg as per the label claim, marketed by Lupin Pharmaceuticals, India). Benzene, ethyl acetate, toluene, methanol, glacial acetic acid, were of HPLC grade and were procured from Merck.

HPTLC instrumentation and chromatographic conditions
Chromatographic separation of drug was performed on Merck TLC plates pre coated with silica gel 60F 254 (10cmx10 cm) with 250 μm layer thickness from E Merck, Germany. The samples were applied on to plates as a band with 6mm width with slit dimension of 5x0.45 mm micro using Camag 100 µl sample syringe (Hamilton, Switzerland) with the Linomat 5 applicator (Camag Switzerland) Linear ascending development was carried out in a twin through glass chamber (10 cm x 10 cm) previously saturated with the mobile phase, benzene: ethyl acetate: toluene: methanol: glacial acetic acid (3:4:2:6:0:4:0.5v/v/v/v/v) at room temperature, using 30 minutes of chamber saturation. The development distance was approximately 70mm. Densitometry scanning was performed using Camag TLC Scanner 3 in the range of 200-400nm and operated with Win cats software (V1.43, Camag) using deuterium lamp as source of radiation. Evaluation was by peak area with linear regression.
Preparation of standard stock solution

A standard stock solution of 500 µg/ml of AST and 100 µg/ml of NA were prepared separately using methanol as solvent.

Validation of the method

The method was validated as per ICH guide line. The parameters checked were linearity, accuracy, precision, limit of detection, limit of quantification, robustness and specificity.

Calibration curve

From the working standard solution of AST (500 µg/ml) and NA (100 µg/ml), 1-3µl solutions and 2-10µl solutions were spotted for AST and NA respectively on a HPTLC plate to obtain a final concentration of 500 ng/spot to 1500 ng/spot for AST and 200 ng/spot to 1000ng/spot for NA. The plates were then developed as per procedure described above and the peak areas were plotted against corresponding concentration to obtain the calibration curves.

Specificity

The specificity of the method was determined by analysis of standard drug and samples. The band for AST and NA in the sample was identified by comparing the Rf value and the spectrum of the band with those of the band obtained from a standard drug solution.

Accuracy: (% Recovery)

For accuracy of method, recovery studies were carried out by applying a known amount of standard AT and NA at a level of 80,100,120 % to the sample solution (standard addition method). Three determinations were performed at each level, using same chromatographic condition as describe above.

Precision: (Reproducibility)

The precision of the method was verified by performing the intraday and interday precision. The intraday and interday precision of the proposed method was determined by estimating the corresponding response three times on the same day and on three different days over a period of one week for five concentration of AST (500, 750, 1000, 1250, 1500 ng/spot) and NA (200, 400, 600, 800, 1000 ng/spot). The results are expressed 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 guide line

LOD= 3.3 x σ/S

LOQ= 10 x σ/S

Where σ is standard deviation of the response and S is the standard deviation of y intercept of regression lines.

Robustness

Robustness was checked by making a slight deliberate change in the experimental procedure like slight change in the mobile phase, saturation time and the values were compared with the original chromatographic conditions.

Analysis of the marketed products

To find the content of the marketed formulation, (TONACT FORTE, Label Claim, 10 mg of AST and 500 mg of NA), twenty tablets were weighed and average weight was determined, powered, from this equivalent weight of 25 mg for AST and 50 mg of NA was transferred into a 50 ml volumetric flask, containing 15 ml of methanol and sonicated for 30 minutes, filtered through Whatmann filter paper No.41and then volume was made up to 50 ml with methanol. From this stock solution 100 ng/spot was spotted for AST and 200 ng/spot was spotted for NA on a HPTLC plate and chromatogram was developed as described earlier. The analysis was repeated for three times and interference for excipients was analyzed.

RESULTS AND DISCUSSION

Validation

To optimize the HPTLC parameters, several mobile phase were tried and satisfactory results were obtained by using the mobile phase benzene: ethyl acetate: toluene: methanol: glacial acetic acid (3:4:2.6:0.4:0.5v/v/v/v). Quantification was achieved under UV detection at 247nm. A sharp and symmetrical peak was resolved with an Rf of 0.64 ± 0.002 for AST and 0.35 ± 0.008 for NA (Figure 1).

![Figure 1: Chromatogram of AST and NA](image)

Linearity

The linear regression data revealed a good linear relationship over the concentration range of 500 ng/spot to 1500 ng/spot for AST and 200 ng/spot to 1000ng/spot for NA with correlation coefficient \( r^2 = 0.9997 \) for AST and correlation coefficient \( r^2 =0.9991 \) for NA respectively. The results are showed in Table 1, figure 2 and figure 3.
LINEARITY OF ATORVASTATIN CALCIUM

\[ y = 3.9881x + 1979.1 \]
\[ R^2 = 0.9997 \]

Figure 2: Representative of linearity of atorvastatin calcium average of six trials

LINEARITY OF NICOTINIC ACID

\[ y = 5.4655x + 330.43 \]
\[ R^2 = 0.9991 \]

Figure 3: Representative of linearity of nicotinic acid Average of six trials

Figure 4: UV absorption spectra of Standard AST and Sample AST

Figure 5: UV absorption spectra of Standard NA and Sample NA

Figure 6: Overlay spectra of Standard AST and Standard NA

Specificity

The method was found to be specific since no interferences spots were seen when carried out in presence of additives, as active constituent was soluble in methanol where as additives were insoluble in methanol. Figure 1 and UV absorption spectra's in figure 4 - 6.

Accuracy

The proposed method when used for the estimation of AST and NA from pharmaceutical dosage form after spiking with the standard, afforded recovery of 99.51 ± 1.553 (80%), 101.65 ±0.4895 (100%), 99.67±0.1097 (120%) at different levels were found for AST and 99.97±0.4262 (80%), 98.21±0.4253 (100%), 100.61±1.362 (120%) for NA respectively.

Precision

The proposed method was found to be precise as indicated by percent RSD not more than 2% as per ICH guidelines for interday and intraday determination. The results are shown in the Table 2.

Limit of Detection (LOD) and Limit of quantification (LOQ)

The limit of detection was found to be 7.24 ng/spot and 0.66 ng/spot and limit of quantification were found to be 21.95 ng/spot and 2.008 ng/spot for AST and NA respectively. The results are shown in Table 2.

Robustness

Robustness was checked by making a slight deliberate change in the experimental procedure by slight change in the mobile phase composition, saturation time. The method was found to be robust since the peak area values were not significantly affected for AST and NA respectively.

Analysis of the marketed products

The proposed method was applied successfully to determine the content of AST and NA in pharmaceutical product. The results are expressed in terms of percentage in Table 2.
Table 1: Regression analysis of the calibration curve of atorvastatin calcium and nicotinic acid by the proposed HPTLC method

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AST</th>
<th>NA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range</td>
<td>500-1500ng/spot</td>
<td>200-1000ng/spot</td>
</tr>
<tr>
<td>Slope</td>
<td>3.9881</td>
<td>5.4655</td>
</tr>
<tr>
<td>Intercept</td>
<td>1979.1</td>
<td>330.41</td>
</tr>
<tr>
<td>Correlation coefficient *</td>
<td>0.9997</td>
<td>0.9991</td>
</tr>
</tbody>
</table>

* Indicates mean of six observation

Table 2: Summary of validation parameters for atorvastatin calcium and nicotinic acid by the proposed HPTLC method

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AST</th>
<th>NA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy (% ± RSD) #</td>
<td>99.13 ±0.1528</td>
<td>99.46±0.5962</td>
</tr>
<tr>
<td>Recovery studies (% ± RSD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80%</td>
<td>99.51 ± 1.553</td>
<td>99.97±0.4262</td>
</tr>
<tr>
<td>100%</td>
<td>101.65 ± 0.4895</td>
<td>98.21±0.4253</td>
</tr>
<tr>
<td>120%</td>
<td>99.67 ± 0.1097</td>
<td>100.61±1.362</td>
</tr>
<tr>
<td>LOD</td>
<td>7.24 ng/spot</td>
<td>0.66 ng/spot</td>
</tr>
<tr>
<td>LOQ</td>
<td>21.95 ng/spot</td>
<td>2.008 ng/spot</td>
</tr>
<tr>
<td>Precision (± RSD) #</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inter day</td>
<td>0.4478-1.657</td>
<td>0.256-1.123</td>
</tr>
<tr>
<td>Intra day</td>
<td>0.7793-1.148</td>
<td>0.091-1.745</td>
</tr>
</tbody>
</table>

± indicates standard deviation; # Average of three trials

CONCLUSION

The proposed HPTLC method is a simple, precise, specific and accurate for the determination of atorvastatin calcium and nicotinic acid as bulk drug and in pharmaceutical formulation without any interference in the excipients. With the developed method only one single mobile phase is sufficient for quantification of AST and NA. The chromatographic method is validated as per ICH guide lines. Statistical data’s indicates that the proposed method is suitable for routine analysis in quality control laboratories for estimating both the drugs simultaneously in a dosage form.

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