**SIMULTANEOUS ESTIMATION OF AMLODIPINE AND ROSUVASTATIN IN COMBINED BULK FORMS BY RP-HPLC USING ULTRAVIOLET DETECTION**

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The objective of the study was to develop simple RP-HPLC method for the simultaneous determination of amlodipine and rosuvastatin. In this method, kromasol C18 (100 mm, 4.6 mm, 5µm) column was used. The mobile phase and flow rate used were {[(acetonitrile 40, 55, 70, 40, 40) : (phosphate buffer 60, 45, 30, 60, 60)]: (Time 0.5, 2.0, 3.0, 3.0, 2.0), UV detection was monitored at 239 nm. Calibration graphs were established for amlodipine and rosuvastatin respectively. The average retention time for amlodipine and rosuvastatin was found to be 2.40±0.16 min and 4.28±0.04 min, respectively. The intraday and Interday precision expressed as percent relative standard deviation was below 2%. The validated HPLC method was found to be rapid, precise and accurate and can be readily utilized for analysis of amlodipine and rosuvastatin in bulk forms.

**Key words:** Amlodipine, Rosuvastatin, RP-HPLC, Method development, Validation.

**INTRODUCTION**

Amlodipine besylate, 2-[(2-aminoethoxy)-methyl]-4-(2-chlorophenyl) 1,4-dihydro-6-methyl-3,5-pyridine dicarboxylic acid-3 ethyl-5 methyl ester (Figure 1), is a long-acting calcium channel blocker which is used as an anti-hypertensive and in the treatment of angina (EP, 2005; USP, 2007).

![Fig. 1. Structure of amlodipine](image)

Owing to widespread use of amlodipine in different kinds of pharmaceutical preparations, rapid and sensitive methods for the determination of amlodipine individual and in combination are being investigated (Rahman and Azmi, 2001; Zarghi et al 2005; Dongre et al 2008). The most recent methods for the determination of amlodipine besylate include chromatographic, spectrophotometric and titrimetric techniques.

Rosuvastatin, (3R, 5S, 6E)-7-[4-(4-fluorophenyl)-2-(n-methylmethanesulfonamido)-6-(propan-2-yl) pyrimidin-5-yl]-3, 5-dihydroxyhept-6-enoic acid (Figure 2), is a member of the drug class of statins which is used to treat high cholesterol and related conditions, and to prevent cardiovascular disease (O’Neil, 2006; Srinivasa Rao et al 2011). In the literature, a capillary zone electrophoretic, UV spectrophotometric, LC/MS, and high performance liquid chromatography (HPLC) methods are reported for the analysis of rosuvastatin (Sane et al 2005; Gupta et al 2009; Kaila et al 2010). More accurate, simple and widely used HPLC method has been not reported for the simultaneous estimation of amlodipine and rosuvastatin in combination formulation.
volume of 20
amlodipine
centration in the range of 1
stock solutions, dilutions were made to get the
each of
Stock solution was prepared by dissolving 10 mg
Lineari
method was applied to pharmaceutical bulk
concentration. After validation, the developed
interday variation in the expected drug
expressed with respect to the intraday and
(%) relative
linearity, precision
Validation of the method
The developed method was validated as per ICH
Validation and simultaneous estimation
of amlodipine and
More accurate, simple and widely used HPLC
method has been not reported for the
simultaneous estimation of amlodipine and
rosuvastatin in combination formulation.
So, in continuation of work done on method
validation and simultaneous estimation of drugs
by our research group (Shah et al 2011; Patil et
al 2011; Chhabra et al 2012) and other scientists
(Singh et al 2011; Basaveswara Rao et al 2012;
Jain et al 2012), the present investigation was
directed toward simultaneous estimation of
amlodipine and rosuvastatin in combined bulk
forms by RP-HPLC using ultraviolet detection.

MATERIALS AND METHODS

Chromatographic conditions
Analytical conditions were standardized through
the LC system using kromasil C 18 column (100
mm, 4.6 mm, 5 µm). The mobile phase used was
(Time 0.5, 2.0, 3.0, 3.0, 2.0) { (acetonitrile 40, 55,
70, 40, 40) : (phosphate buffer 60, 45, 30, 60,
60)}. UV detection was made at 239 nm. The
volume of injection was fixed at 20 µl. All
analyses were done at 30°C. The mobile phase
was prepared fresh each day, vacuum-filtered
through 0.50 µm millipore nylon filters.

Validation of the method
The developed method was validated as per ICH
guidelines in terms of accuracy, specificity,
linearity, precision (ICH, 1994). The precision
( % relative standard deviation, %RSD) was
expressed with respect to the intraday and
interday variation in the expected drug
concentration. After validation, the developed
method was applied to pharmaceutical bulk
forms containing amlodipine and rosuvastatin.

Linearity
Stock solution was prepared by dissolving 10 mg
each of amlodipine and rosuvastatin in 10 ml
volumetric flask with methanol. From the above
stock solutions, dilutions were made to get the
concentration in the range of 1-150 ppm of
amlodipine and 0.5-100 ppm of rosuvastatin. A
volume of 20 µl of each sample was injected into
column. All measurements were repeated three
times for each concentration and calibration
curve was constructed by plotting the peak areas
of analyte versus corresponding drug
centration.

Precision
The precision of the proposed method was
assessed as intermediate precision and
repeatability by preparing three different
sample solutions at low, medium and high
concentrations, which were prepared freshly
and daily analysed. These experiments were
repeated over a 2-day period.

RESULTS AND DISCUSSION
HPLC method was found to be accurate, simple,
economic and rapid for routine simultaneous
estimation of amlodipine and rosuvastatin in
bulk forms.

Optimization of the chromatographic
conditions
Initially, the mobile phase used was
acetonitrile:phosphate buffer (70:30%) then,
ratio of the solvents were varied. At 70:30%,
there was no good separation and at 50:50%,
tailing of amlodipine was observed, at 30:70%,
again there was no good separation. Gradient
composition of mobile phase were tried in order
to get better separation and good resolution. At
time 0.5, 3.0, 3.0, 2.0, 7.0 min, acetonitrile (30,
40, 70, 30, 30) : (phosphate buffer 70, 60, 30, 70,
70)), good separation of amlodipine and
rosuvastatin was observed but the retention
time was more. At time 0.5, 2.0, 3.0, 3.0, 2.0 min,
acetonitrile (40, 55, 70, 40, 40) : (phosphate
buffer 60, 45, 30, 60, 60)), better resolution and
less retention time was observed. Different
values of pH of phosphate buffer were tried.
Phosphate buffer with pH 3.0 has been selected
for analysis. The isobestic wavelength 239 nm
has been found to be optimum (Figure 3). The
average retention time for amlodipine and
rosuvastatin was found to be 2.40±0.16 min and
4.28±0.04 min, respectively (Figure 4).

Linearity
A linear calibration graph was obtained over six
concentrations 10, 20, 30, 40, 50, 100 ppm
(Figure 5, 6).

Precision
Intra-day precision of the method was
determined by repeat analysis (three identical

Fig. 2. Structure of rosuvastatin

![Structure of rosuvastatin](image)
Accuracy
To ensure the accuracy of the analytical method, the recovery studies were carried out. Known amounts of amlodipine and rosuvastatin were added to a pre quantified sample solution of its dosage form and the amounts were estimated by measuring the peak area ratios and by fitting these values to the straight line equation of calibration curve. The recovery studies were carried out three times over the specified concentration range. Accuracy was evaluated at three different concentrations equivalent to 80, 100 and 120% of the active ingredient by calculating the recovery with %RSD (Table 3).

Repeatability
The peak area of 40 ppm drug solution was analyzed six times on the same day. The % RSD was calculated for the resultant peak area (Table 4).

Robustness
The HPLC method was found to be robust as the results were not significantly affected by slight variation in the extraction time, composition of mobile phase, flow rate and wavelength.

CONCLUSION
The proposed method is simple, accurate, rapid, economical and selective for the simultaneous estimation of Amlodipine and Rosuvastatin in bulk form without prior separation. The excipients of the commercial sample analyzed did not interfere in the analysis, which proved the specificity of the method for these drugs. The proposed method involves direct quantification of both the components. By HPLC method, analysis can be done within 10 min with the use of simple solvents. Hence, developed HPLC method can be conveniently adopted for the routine quality control analysis in the combination formulations.
Table 1. Precision of amlodipine

<table>
<thead>
<tr>
<th>Conc. (ppm)</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>Mean</th>
<th>±SD</th>
<th>%RSD</th>
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<tbody>
<tr>
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<td>297573.69</td>
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<td>2518405.27</td>
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<td>13238.06</td>
<td>0.53</td>
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</table>

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<tr>
<th>Conc. (ppm)</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>Mean</th>
<th>±SD</th>
<th>%RSD</th>
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<tbody>
<tr>
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Table 2. Precision of rosuvastatin

<table>
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<th>Conc. (ppm)</th>
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<th>III</th>
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<th>±SD</th>
<th>%RSD</th>
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<table>
<thead>
<tr>
<th>Conc. (ppm)</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>Mean</th>
<th>±SD</th>
<th>%RSD</th>
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Table 3. Recovery study

<table>
<thead>
<tr>
<th>Level of recovery</th>
<th>% Recovery found (Amlodipine)</th>
<th>% Recovery found (Rosuvastatin)</th>
<th>% RSD (Amlodipine)</th>
<th>% RSD (Rosuvastatin)</th>
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</thead>
<tbody>
<tr>
<td>80%</td>
<td>99.77</td>
<td>99.87</td>
<td>0.18</td>
<td>0.21</td>
</tr>
<tr>
<td>100%</td>
<td>99.52</td>
<td>99.34</td>
<td>0.41</td>
<td>0.25</td>
</tr>
<tr>
<td>120%</td>
<td>99.40</td>
<td>100.04</td>
<td>0.49</td>
<td>0.33</td>
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Table 4. Repeatability study

<table>
<thead>
<tr>
<th>Drug</th>
<th>Conc. (ppm)</th>
<th>% RDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amlodipine</td>
<td>40</td>
<td>0.92</td>
</tr>
<tr>
<td>Rosuvastatin</td>
<td>40</td>
<td>1.39</td>
</tr>
</tbody>
</table>

REFERENCES
European Pharmacopoeia 5.0, Council of Europe: Strasbourg, France, 2005; 236-970.
ICH Harmonised Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology Q2(R1),


