SIMVASTATIN ASSAY AND DISSOLUTION STUDIES BY FEASIBLE RP-HPLC IN TABLETS

Flávia Dias Marques-Marinho*, Amanda Leão dos Santos and Cristina Duarte Vianna-Soares
Departamento de Produtos Farmacêuticos, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Av. Pres. Antônio Carlos, 6627, 31270-901 Belo Horizonte - MG, Brasil
Ilka Afonso Reis
Departamento de Estatística, Instituto de Ciências Exatas, Universidade Federal de Minas Gerais, Av. Pres. Antônio Carlos, 6627, 31270-901 Belo Horizonte - MG, Brasil
José Carlos da Costa Zanon
Clínica Ouro Cordis, Hospital Santa Casa, R. José Moringa, 620, 35400-000 Ouro Preto - MG, Brasil
Angélica Alves Lima
Departamento de Análises Clínicas, Escola de Farmácia, Universidade Federal de Ouro Preto, R. Costa Sena, 171, 35400-000 Ouro Preto - MG, Brasil

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Commonly used HPLC acetonitrile solvent has been through a worldwide shortage with a cost increase in 2008 and 2009. In order to get around this situation, a method by RP-HPLC employing methanol and aqueous acid mobile phase was developed and validated to evaluate simvastatin. The quality control assay and dissolution studies of this lipid-lowering drug were performed in diluents methanol and 0.01 M phosphate buffer with 0.5% SDS, pH 7, respectively. Dissolution test aliquots did not go through sample treatment, as described in USP SIM tablets monograph by ultraviolet spectrophotometry. The proposed method is fast, simple, feasible and robust.

Keywords: Simvastatin tablets; dissolution studies; RP-HPLC.

INTRODUCTION

Simvastatin (SIM, Figure 1a), 2,2-dimethyl-1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethy]-1-naphthalenyl ester [15-[1a,3a,7b,8b (2S*,4S*),8ab]] butanoic acid is one of the most used statins as lipid-lowering agents, for the treatment of hypercholesterolemia. It reduces the morbidity and mortality associated with chronic heart disease. SIM is commercially available as tablets. Similarly to lovastatin (LOV, Figure 1b) SIM is administered in lactone form as a pro-drug, which is enzymatically hydrolyzed in vivo to active β-hydroxy acid form (Figure 1c), particularly in liver. The active form competitively inhibits 3-hydroxy-3-methylglutarylcoenzyme A (HMG-CoA) reductase, which converts HMG-CoA to mevalonate, a rate-limiting step in cholesterol biosynthesis.

SIM is semi-synthetically produced from LOV, which is a natural fermentation product of Aspergillus terreus. Hence, LOV can be found in SIM raw material as impurity. SIM quantitation in bulk or finished products samples has been reported using ultraviolet spectrophotometry and liquid chromatography (LC-UV/DAD). LC ion-pair coupled to UV detector and visible spectrometric method have also been reported. Furthermore, LC-MS/MS was employed to identify SIM impurities or degradation products in bulk drug or tablets dosage form. UV spectrometry, LC-UV and LC-MS have been reported to quantify SIM associated with other drugs such as ezetimibe, gemfibrozile or in multi-drugs formulation. A micellar electrokinetic chromatographic (MEKC) method has been employed to quantify SIM and LOV in tablets. Several other studies, not here considered because of its focus on SIM quantitation in biological samples describe LC coupled with UV or fluorescence detectors. LC is the most commonly reported method due to its feasibility to

couple with several detection techniques. Acetonitrile (ACN) widely used in combination with buffer solution in mobile phases, mainly in SIM official monographs of solid dosage forms. Methanol in a binary mobile phase has been reported for SIM determination in nanoparticles (specific pharmaceutical dosage form) in the concentration range 20-80 μg mL⁻¹. ACN worldwide shortage has reached a very high cost in the market between years 2008 and 2009. In order to get around this situation, an alternative lower cost methanolic mobile phase, not only to assay SIM, but also, to determine SIM in dissolution studies was applied for tablets routine analysis.

EXPERIMENTAL

Chemicals and reagents

* e-mail: flaviadmar@hotmail.com

United States Pharmacopeia (USP) simvastatin reference standard

Figure 1. Chemical structure of (a, SIM) simvastatin, (b, LOV) lovastatin and (c) SIM β-hydroxy acid
of standard solutions at 2, 10, 18, 26, 34 µg mL⁻¹. All injections were in triplicate. The calibration plots behavior, normality and homoscedasticity (α = 0.05) were verified by weighted least squares method using a linear regression model to determine the weights, Shapiro-Wilk and Levene tests, respectively. Residues greater or equal than 3.0 were removed by using studentized residuals model. Correlation coefficients, r, were calculated and results were considered significant if correspondent p-value were less than 0.05. All statistical analyses were performed using the R software (R Foundation for Statistical Computing, Vienna, Austria).

Limits of quantitation (LOQ) and detection (LOD) were determined in triplicate (for methanolic solutions only) by successive dilutions of the lowest calibration curve point. The concentration for which the maximum precision remained below the acceptance limit (RSD 2.0%) was evaluated for LOQ. The minimum detectable area in the greatest dilution was considered for LOD.²³,²⁴

The method selectivity was evaluated by comparison of SIM chromatogram standard solution in methanol (40 µg mL⁻¹) with that of tablets excipients mixture (ascorbic acid, citric acid, hydroxypropylcellulose, butylated hydroxyanisole, hydroxypropylmethylcellulose, iron oxides, lactose, magnesium stearate, microcrystalline cellulose, starch, talc, titanium dioxide) in methanol. In dissolution medium, SIM standard solutions (22.2 µg mL⁻¹) were compared with that obtained by addition of a placebo mass, equivalent to one average weight in three dissolution vessels, agitated at 150 rpm for 30 min.²²

The intraday and inter-day precision for SIM tablets sample solutions (20, 40, 80 µg mL⁻¹ in methanol, triplicate) was performed in three days.²³,²⁴ A different analyst performed the procedure at the third day. SIM standard solutions (22.2 µg mL⁻¹ in dissolution medium, triplicate) were used to evaluate intra-day precision. Tablets dissolution test (30 min) inter-day precision was evaluated by different analysis in two days each.²³ The results were calculated by the common plot equations.

The standard addition method was employed for accuracy studies. Sample tablet solution in methanol was prepared as in sample preparation, except that, it was at 15 µg mL⁻¹ (37.5% of working concentration). SIM standard solution aliquots were added, so that, 50, 100 and 200% of working concentration (40 µg mL⁻¹) were obtained. The equation %R = [(Cₚ - Cₛ)/Cₛ] x 100 was employed to determine SIM recovery, in which, Cₛ is concentration in nonspiked solution, Cₚ is total concentration in spiked solution and Cₛ is concentration of standard solution. Accuracy in the dissolution medium (900 mL per vessel) was obtained by addition of SIM RS in three different amounts (2, 20, 30 mg) to a tablet placebo mixture, equivalent to one average weight. Each concentration was tested in triplicate.²³

Robustness was verified for SIM determination (40 µg mL⁻¹ in methanol, n = 6), by varying organic solvent ± 2.0%, flow rate ± 0.1 mL min⁻¹ and temperature ± 10 °C. Equivalent SIM standard solutions (n = 3) were run in the same conditions. For SIM dissolution medium solutions (n = 6), molarity ± 0.005 M, pH ±0.5 and C₈e (endcapped) column were tested. SIM concentrations were calculated by the calibration plot equation. All data were evaluated by analysis of variance (ANOVA) in a significance level α = 0.05 using R statistical software.

In a short term stability study SIM RS methanolic solution (40 µg mL⁻¹) was kept at room controlled temperature (22-24 °C) and was analyzed after 20 h. Dissolution stability study was evaluated by the addition of 20 mg SIM (one tablet) to dissolution medium (37 °C, 900 mL, 50 rpm, 30 min). This solution (22.2 µg mL⁻¹) was transferred to vials (n = 2), one kept at room temperature and the other at under refrigeration (2-8 °C). Vials were analyzed every hour during 7 h for comparison. A change less than 2.0% in the response was considered acceptable.²³

Dissolution studies for SIM tablets (n = 6) release was performed in two days, using the previous described conditions until 45 min and,
for additional 15 min at 150 rpm. Aliquots (3 mL) were withdrawn at
the time intervals 3, 5, 8, 10, 12, 15, 30, 45, 60 min, without medium
replacement. Filtered aliquots were maintained at 2-8 °C previous
to injection in the chromatograph. C8e (endcapped) column (250 x
4 mm, 5 µm) was used.

RESULTS AND DISCUSSION

Very few reports describe the use of C8 column to determine
SIM in pharmaceutical samples. However, C8 has been chosen
because separation shows a slightly lower peak retention time (t<sub>R</sub>)
as fair as resolved as in a C18 column under the same conditions.
Most reported studies, use isocratic or gradient elution of ternary
or binary mobile phases containing acetonitrile (ACN) as organic
solvent, in a variable proportion from 60 to 85% v/v, usually with buffer.12,13
Because there is a need to address method validation for
SIM determination and dissolution studies, for which linear con-
centration ranges and diluents are usually different, a single feasible
isocratic liquid chromatographic method was proposed. Due to ACN
shortage and high cost in the market between years 2008 and 2009, an
alternative less expensive solvent was desirable.14 Different options
to reduce solvent cost are reported in the literature. A great effort has been done in order to
substitute ACN.25 Ribeiro et al. showed that low-cost ethanol can be a choice of mobile phase organic
modifier for many RP-HPLC applications. However, its use is not
worldwide allowed, because of the control regulations in some
countries, mainly in USA.26 A great effort has been done in order to
substitute ACN by methanol, what does not require modifications in
equipment nor in the column.27

A binary phase, methanol and water, has been reported for SIM
quantitation in nanoparticles and in human plasma.28,29 No report has
described the use of methanol binary phase in reverse column applied to SIM tablets.

Hence, solvent-strength nomograph for reverse-phase HPLC was
used to estimate a correspondent methanol percentage necessary to
substitute ACN.25 Considering 72.5% as an average ACN percentage
used in the reported mobile phases, a correspondent methanol per-
centage should be approximately 80%. In this work, different ratios of
methanol and 0.1% phosphoric acid (75:25, 80:20, 85:15, 90:10
v/v) in isocratic elution were evaluated for SIM. The 80:20 and 85:15
mobile phase ratios yielded SIM k values near 2.0 (3.54 and 1.79,
respectively) and were used to evaluate the selectivity of SIM and
LOV (SIM synthesis route precursor, 40 µg mL<sup>-1</sup> of each, in methanol
or 22.2 µg mL<sup>-1</sup> in dissolution medium) at room temperature imme-
diately after preparation and five days later. Superior chromatographic
separation and resolution between SIM, LOV and their degradation
products were observed for mobile phase 80:20 ratio than 85:15 one.

In the latter, nevertheless, peaks overlapping were observed for
SIM and LOV degradation products in methanol (Figure 2) and for

LOV and SIM degradation products in dissolution medium (Figure 3).
Interestingly, it should be noted that the products eluted with gre-
ter t<sub>R</sub> than SIM and LOV in methanol. The presence of methanol may
lead to lactone methanolysis and esterification, what is agreement with
previous report.30 On the other hand, the more polar products eluted at lower t<sub>R</sub> in dissolution medium because of lactone hydrolyses to the
 corresponding acid form in aqueous solutions (pH 7).31 The products
UV/DAD spectra (Figure 4) were identical to SIM and LOV original
spectra in both diluents; revealing that the chromophore moiety is not
affected by ring opening.41 For all these reasons, the 80:20 v/v ratio
mobile phase (1.5 mL min<sup>-1</sup>, 30 °C) was selected. Column temperatu-
re, set at 30 °C helped reducing methanol aqueous acid mobile phase
viscosity, decreasing backpressure, therefore avoiding fluctuations.
The backpressure was maintained around 125 bar.

System suitability showed %RSD values for peak area (0.47;
0.10) and t<sub>R</sub> (0.05; 0.02) less than 1.0% for methanol and dissolution
medium solutions, respectively. The results also demonstrate the sui-
tability of the system in terms of column efficiency greater than 2000
(N 5999; 6551), adequate peak shape less than 2.0 (T<sub>R</sub> 1.13; 1.15)
or asymmetry less than 1.2 (A 1.16; 1.19), appropriate SIM peak reten-
tion factor greater than 2.0 (k 2.67; 4.43) for solutions in methanol
and dissolution medium, respectively.33,34 No fluctuations in the baseline
was observed during the runs. Hence, the proposed method showed
system suitability for SIM determination in both diluents.

SIM calibration plots were built in both diluents. The simplest mod-
el that adequately described the concentration-response relationship
was the weighted least squares method using a linear regression
model (variances versus mean responses in each concentration) to
determine the weights.42 The plots for SIM methanolic (Table 1) or
dissolution medium (Table 2) solutions showed a linear behavior.
No residues were removed for methanolic solutions plots, however,
the common plot for dissolution medium solutions had two residues
(6.6%) removed, less than 22% of the data.43
Plots in methanol, as well as, in dissolution medium showed no significant parallelism deviation by ANOVA (p-value > 0.05). Common regression plots were significant (p-value < 0.05), correlation coefficients (r) were above 0.999 and determination coefficients ($r^2$) were greater or equal 0.98. The regression RSD values were less than 1.0%. Intercept was not statistically different from zero for dissolution medium solutions (p-value > 0.05) and percentage of the intercept relative to 100% analyte level was less than 2.0% for methanolic solutions.

For both diluents, data analyses followed adequate normality and homoscedasticity (p-value > 0.05). The RSD value found (1.2%) for LOQ was lower than 2.0% for SIM methanolic solutions. The method selectivity is demonstrated in Figures 5 and 6 for methanolic and dissolution medium solutions, respectively. Tablet (b) placebo constituents did not exhibit interfering peaks over (a) SIM RS retention time. Tablet (c) placebo added of SIM RS methanolic solutions also did not interfere in (a) SIM RS retention time.

**Table 1. Results of adjusted regression model ($r > 0.9999$)$^a$ for SIM determination in methanolic solutions (0.04-0.80 µg, n=3) by HPLC$^b$**

<table>
<thead>
<tr>
<th>SIM curve data</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Common curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average area±SE</td>
<td>916.51 ± 7.83</td>
<td>922.84 ± 3.80</td>
<td>921.39 ± 6.23</td>
</tr>
<tr>
<td>Regression equation</td>
<td>$y = 2253.33x + 1.55$</td>
<td>$y = 2257.91x + 2.93$</td>
<td>$y = 2257.66x +1.88$</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.11</td>
<td>0.11</td>
<td>0.12</td>
</tr>
<tr>
<td>p-value</td>
<td>0.01 (0.17)$^c$</td>
<td>1.43x10$^{-3}$ (0.32)$^c$</td>
<td>6x10$^{-4}$ (0.21)$^c$</td>
</tr>
<tr>
<td>ShapiroWilk</td>
<td>0.40</td>
<td>0.74</td>
<td>0.36</td>
</tr>
<tr>
<td>Levene</td>
<td>0.97</td>
<td>0.92</td>
<td>0.59</td>
</tr>
</tbody>
</table>

a: p-value < 2.0 x 10$^{-16}$; b: chromatographic conditions C8 (250 x 4 mm, 5 µm), 30 °C, methanol: 0.1% phosphoric acid 80:20 v/v, 1.5 mL min$^{-1}$, λ 238 nm; c: intercept percentage relative to 100% analyte level that must be less than 2%.

**Table 2. Results of adjusted regression model ($r^2 > 0.999$)$^c$ for SIM determination (2-34 µg mL$^{-1}$, n = 3) in dissolution medium$^b$ by HPLC**

<table>
<thead>
<tr>
<th>SIM curve data</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Common curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average area±SE</td>
<td>387.77 ± 1.35</td>
<td>386.31 ± 1.41</td>
<td>387.21 ± 1.66</td>
</tr>
<tr>
<td>Regression equation</td>
<td>$y = 21.545x - 0.140$</td>
<td>$y = 21.453x - 0.178$</td>
<td>$y = 21.526x - 0.107$</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.29</td>
<td>0.17</td>
<td>0.29</td>
</tr>
<tr>
<td>p-value</td>
<td>0.97</td>
<td>0.60</td>
<td>0.68</td>
</tr>
<tr>
<td>ShapiroWilk</td>
<td>0.44</td>
<td>0.87</td>
<td>0.13</td>
</tr>
<tr>
<td>Levene</td>
<td>0.92</td>
<td>0.86</td>
<td>0.86</td>
</tr>
</tbody>
</table>

a: p-value < 2.0x10$^{-16}$; b: 0.5% SDS in monobasic sodium phosphate (pH 7, 0.01 M); c: conditions as in Table 1.

**Figure 5. Chromatograms of (a) simvastatin reference standard, (b) tablets placebo and (c) tablets placebo added of SIM RS in methanol. Chromatographic conditions: C8 (250x4 mm, 5 µm) 30 °C, methanol: 0.1% phosphoric acid (80:20 v/v), 1.5 mL min$^{-1}$, λ 238 nm, 10 µL.**

**Figure 6. Chromatograms of (a) simvastatin reference standard and (b) tablets placebo in dissolution medium. Chromatographic conditions: as in Figure 5. Dissolution conditions: 0.5% SDS in monobasic sodium phosphate (pH 7, 0.01 M), 900 mL, 37 °C, paddles, 150 rpm, 30 min.**

Accuracy found values ranged from 99.5 to 100.5% for SIM methanolic solutions, attesting the usual range of acceptance for pharmaceutical products (98.0 to 102.0%). Dissolution medium solutions found values (99.1 to 100.4%) ranged between 95.0 and 105.0% for tablets placebo added of 2.0, 20.0 and 30.0 mg of SIM, in accordance with the USP34 criteria. These results attest the accuracy.

Robustness statistical analysis (Table 5) showed no significant difference (α 0.05) between standardized (nominal) analytical conditions and deliberate variations. The method using HPLC can be applied in a temperature range of 20 to 40 °C with no strict column oven control and small variations in flow and organic solvent proportion do not strongly affect the results.

For dissolution medium solutions (Table 6), changes in the medium molarity showed greater influence (p-value closer to 0.05) than variations in medium pH. Furthermore, the solutions can be analyzed regardless of endcapped (C8e) or not (C8) columns.

A short-term stability data for SIM RS methanolic solution evaluated after 20 h at 22-24 °C showed a maximum variation of 0.55%. SIM dissolution medium solution kept at 22-24 °C showed variations equal to or greater than 2.0% after 3 h (2.0%) to 7 h (3.4%) standing in room controlled temperature. On the other hand, the maximum variation was 1.1% after 7 h under refrigeration (2-8 °C).
Simvastatin assay and dissolution studies by feasible RP-HPLC in tablets

Table 3. HPLC results of precision and accuracy (% R) for SIM tablets in methanol

<table>
<thead>
<tr>
<th>Conc.</th>
<th>Precision</th>
<th>SIM, µg mL⁻¹ mean (%RSD)</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=3)</td>
<td></td>
<td>Day 1 (n=3)</td>
<td>Day 2 (n=9)</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>19.56 (1.20)</td>
<td>19.76 (1.65)</td>
</tr>
<tr>
<td>40</td>
<td></td>
<td>39.63 (1.86)</td>
<td>38.77 (1.24)</td>
</tr>
<tr>
<td>80</td>
<td></td>
<td>78.01 (0.76)</td>
<td>77.79 (0.08)</td>
</tr>
</tbody>
</table>

a: conditions as in Table 1; b: analyst I; c: analyst II.

Table 4. HPLC results of precision and accuracy (% R) for SIM tablets in dissolution medium (900 mL, 37 °C, paddles, 50 rpm, 30 min)

<table>
<thead>
<tr>
<th>SIM, µg mL⁻¹ mean (%RSD)</th>
<th>Preciseion</th>
<th>Day 1 (n=6)</th>
<th>Day 2 (n=12)</th>
<th>Interday (n=3)</th>
<th>% R</th>
</tr>
</thead>
<tbody>
<tr>
<td>21.82 (1.50)</td>
<td>21.68 (1.43)</td>
<td>21.75 (1.44)</td>
<td>2.20 (1.75)</td>
<td>99.1</td>
<td></td>
</tr>
<tr>
<td>21.51 (1.50)</td>
<td>21.76 (1.32)</td>
<td>21.63 (1.47)</td>
<td>22.07 (1.12)</td>
<td>99.3</td>
<td></td>
</tr>
</tbody>
</table>

a: HPLC conditions and dissolution medium as in Table 1 and Table 2, respectively; b: analyst I; c: analyst II; d: average, n=24.

Table 5. HPLC results of robustness for SIM tablets in methanol

<table>
<thead>
<tr>
<th>SIM Robustness (n=6)</th>
<th>Parameters</th>
<th>Mean µg mL⁻¹(%)</th>
<th>%RSD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol ratio (%)</td>
<td>78</td>
<td>38.80 (97.00)</td>
<td>0.37</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>38.91 (97.28)</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td></td>
<td>82</td>
<td>38.82 (97.04)</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>Flow rate (mL min⁻¹)</td>
<td>1.4</td>
<td>38.98 (97.44)</td>
<td>0.42</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>38.91 (97.28)</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>38.98 (97.46)</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>20</td>
<td>38.86 (97.15)</td>
<td>0.56</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>38.91 (97.28)</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>38.96 (97.39)</td>
<td>0.52</td>
<td></td>
</tr>
</tbody>
</table>

a: standardized (nominal) HPLC conditions as in Table 5; b: dissolution test nominal conditions: 0.5% SDS in monobasic sodium phosphate (pH 7, 0.01 M), 900 mL, 37 °C, paddles, 50 rpm, 30 min.

Table 6. HPLC results of robustness for SIM tablets in methanol and in dissolution medium

<table>
<thead>
<tr>
<th>SIM Robustness (n=6)</th>
<th>Parameters</th>
<th>Mean µg mL⁻¹(%)</th>
<th>%RSD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.5</td>
<td>21.78 (98.00)</td>
<td>1.46</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>21.82 (98.20)</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>21.85 (98.33)</td>
<td>1.35</td>
<td></td>
</tr>
<tr>
<td>Molarity</td>
<td>0.005</td>
<td>21.72 (97.75)</td>
<td>1.05</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>0.010</td>
<td>21.82 (98.20)</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.015</td>
<td>21.51 (96.78)</td>
<td>1.55</td>
<td></td>
</tr>
<tr>
<td>C8 column</td>
<td>endcapped</td>
<td>21.56 (97.04)</td>
<td>1.31</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>non-endcapped</td>
<td>21.82 (98.20)</td>
<td>0.93</td>
<td></td>
</tr>
</tbody>
</table>

a: standardized (nominal) HPLC conditions as in Table 5; b: dissolution test nominal conditions: 0.5% SDS in monobasic sodium phosphate (pH 7, 0.01 M), 900 mL, 37 °C, paddles, 50 rpm, 30 min.

fact can be explained by SIM temperature sensitiveness. These results indicate that SIM can be assayed under room temperature in methanolic solutions, as well as, for dissolution test. However, it is recommended that SIM solutions to be kept under refrigeration for release profile studies, which are, usually, longer tests.

Because the release profile is an important stage in the development and quality monitoring of the pharmaceutical products, C8e (As 1.00, %RSD 0.12) was preferred rather than a C8, (As 1.20, %RSD 0.32), since more symmetric peaks were obtained. In addition, a HPLC method was chosen for SIM tablets dissolution studies due to improved analytical sensitivity and reduced interference from excipients, without any sample treatment. Contrastingly, a method using UV spectrophotometric is described in international compendia for SIM tablets monograph, in which a sample preparation (centrifugation with pre-washed manganese dioxide) is required after SIM dissolution test, before drug quantitation.

Zocor® rapidly dissolving tablets release profile (Figure 7) was greater than 85% in 10 min. The RSD precision values between days for time points above 85% drug release (10, 12, 15, 30, 45 min) and below (8 min) were less than 5 and 10%, respectively. Time points 3 and 5 min were not considered because of high RSD values (> 20%) found for six units tested each day. These results attest that proposed method is adequate for SIM tablets in vitro release studies.

Figure 7. SIM 20 mg tablets (n = 6) release profiles in different days (n=2). Conditions: 0.5% SDS in monobasic sodium phosphate (pH 7, 0.01 M), 900 mL, 37 °C, paddles, 50 rpm for 45 min, 150 rpm for 15 min
In summary, the proposed RP-HPLC method advantages are the lower cost (50% less than ACN) and the absence of sample treatment after dissolution test compared to the UV method.\textsuperscript{2} In addition, it presented a low total run time (7.4 min) in isocratic elution, high selectivity towards SIM and LOV (SIM synthesis route precursor) degradation products in a wide concentration range (0.04-0.80 µg equivalent to 4-80 µg mL\textsuperscript{-1}), with similar backpressure regarding ACN use (125 versus 130 bar). Furthermore, a liquid chromatographic method had not yet been applied to SIM tablets using official dissolution test conditions.\textsuperscript{2,29}

CONCLUSION

An alternative validated method using RP-HPLC was successfully applied for SIM assay, as well as, for dissolution test and profile studies in tablets. The method demonstrated to be fast, simple, feasible and affordable when challenged for robustness either in assay conditions, especially for temperature, or in dissolution conditions, after buffer molarity and pH variations.

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