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*Original Scientific Article*

# Determination of Simvastatin in Pharmaceutical Dosage Forms by Optimized and Validated Method Using HPLC/UV

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*Abstract.* Simvastatin belongs to the group of anticholesterol agents used in the treatment of hypercholesteraemia. Analytical methods used to determine the concentration of this active pharmaceutical ingredient (API), in dosage forms in the quality tests, are commonly based on high performance liquid chromatography (HPLC) and should be fast and reliable. The purpose of this study was to compare and validate two methods of analysis of simvastatin using HPLC and different eluent mixtures: acetonitrile/water *vs.* methanol/water in gradient elution. Several columns were tested at different temperatures. However satisfied peak shapes and validation parameters were obtained for both methods. The one using methanol as an eluent was chosen for the determination of simvastatin in dissolution tests, mostly due to lower price of the eluent.

*Keywords:* simvastatin, HPLC/UV, gradient elution, methanol/water, dissolution tests

## INTRODUCTION

Statins are a group of 3-hydroxy-3-methylglutaroyl-coenzyme A (HMG-CoA) reductase inhibitors used in heterozygotic hypercholesteraemia and hyperlipidemia.[1–3] Simvastatin, lovastatin and atrovastatin are the most used but only the first one is a prodrug.[3,4] Prodrug form is better absorbed in comparison to non-modified form. The chemical structure of simvastatin, (1S,3R,7S,8S,8aR)-8-[2-[(2R,4R)-4-hydroxy-2H-pyran-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a hexahydronaphthalen-1-yl 2,2 dimethylbutanoate.[5] Biotransformation into an active form of simvastatin (β-hydroxyacid) takes place in the liver by ring-opening reaction of the lacton. The inhibition of the HMG-CoA causes a decrease in LDL, low-density lipoprotein (20–40 %), triglycerides (10–20 %), while it increases HDL, high-density lipoprotein (5–15 %) and LDL receptor expression.[3,6] Due to this fact these compounds are the most commonly prescribed drugs for the prevention of atherosclerosis and heart disease, both as a prodrug or non-modified form. The fact that they can be used after heart attack and in co-existence of diabetes as well as in kidney dysfunction gives statins the status of a first choice drug. However, overdose of statins causes an increase of aminotransferases concentration which can lead to myopathy.[3]

Several methods have been used to determine statins in dosage forms[2,7–11] and human plasma[1,4,12] in bioavailability examination. In the majority of these studies direct spectrophotometric methods,[8] micellar capillary electrophoresis,[2] chromatographic techniques like liquid chromatography tandem with mass spectrometry (LC-MS),[1,6] high performance liquid chromatography with spectrophotometric detection (HPLC-UV)[10–13] or mass spectrometry[4] were applied. Among the HPLC-UV methods the isocratic elution based on acetonitrile/phosphate buffer/methanol mixture[11] or micro-emulsions[13] as a mobile phase is used. None of these methods was based on gradient elution with methanol/water mobile phase.

Acetonitrile is the most common and efficient solvent in drug analysis techniques. In view of production limitation of this chemical by the leading world producers in China a global shortage became. The amount of a HPLC grade acetonitrile falls down and its price has broken the record. This situation demands searching for an alternative solvent with similar properties. One of the alternative choice is methanol. The idea of possible substitution of acetonitrile by methanol is known, however so far, there were no studies concerning such comparison.

The aim of this study was to optimize, validate and compare two procedures for the determination of simvastatin using HPLC/UV with gradient elution of methanol/water *vs.* acetonitrile/water.

Second aim of our study was to use a more suita-

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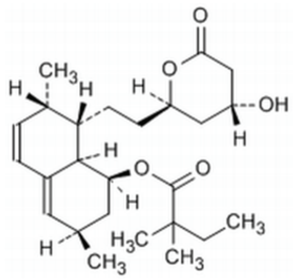

**Figure 1.** Simvastatin chemical formula.

ble procedure for the determination of simvastatin to carry out dissolution tests of commercially available drugs in tablets.

## EXPERIMENTAL

### Chemicals

Simvastatin (SIM), shown in Figure 1, was obtained from Sigma Aldrich. Acetonitrile and methanol were HPLC grade, disodium phosphate and hydrochloric acid (pure grade) were purchased from POCh, Gliwice, Poland. Deionized water was obtained from a Milipore System. Ultrapure sodium dodecyl sulphate (SDS) was supplied by Merck. The orginal drug product containing simvastatin (Zocor) and the generic products (Simvacard, Simvasterol, Vasilip, Simratio) all containing 20 mg of simvastatin per tablet were purchased on the local pharmaceutical market.

### Instrumentation and chromatographic procedure

Chromatographic analyses were performed on HPLC Varian System with Galaxie Chromatographic Data System v. 1.9.302.530. Column oven Jetstream 2 Plus was used. Several columns including Beckmann Ultrasphere ODS 250 mm × 4.6 mm I.D. particle size 5 µm; Phenomenex Jupiter $C_{18}$ 150 mm × 4.6 mm I.D. particle size 5 µm; Phenomenex ODS Luna column 250 mm × 4.6 mm I.D. particle size 5 µm; Hypersil ODS 250 mm × 4.6 mm I.D. particle size 5 µm; Varian Microsorb MV $C_{18}$ 250 mm x 4.6 mm I.D. particle size 5 µm were tested.

A Genesis 10S spectrometer was used for maximum of absorption tests and dissolution tests were performed on ERWEKA DT 720.

*Procedure I*

A Phenomenex ODS Luna column (250 mm × 4.6 mm I.D. particle size 5 µm) was conditioned at 50 °C and the analyte was eluted with a mobile phase consisting of acetonitrile/water (40:60 *v*/*v*) at 0 min to acetonitrile/water (100:0 *v*/*v*) at 10 min at a flow rate of 1.5 mL $min^{-1}$. Simvastatin was determined by UV detection at the 238 nm. The injection volume was 20 µL.

*Procedure II*

A Hypersil ODC column (250 mm × 4.6 mm I.D. particle size 5 µm) was conditioned at 40 °C and the analyte was eluted with a mobile phase consisting of methanol/water (70:30, *v*/*v*) at 0 min to methanol/water (97:3 *v*/*v*) at 15 min at a flow rate 1.5 mL $min^{-1}$. The analytical wavelength was 238 nm and the injection volume was 20 µL.

### Conditions of dissolution testing

A European Pharmacopea dissolution apparatus 2 (paddle)[5,14] was used for tests with a paddle speed of 50 rpm. Tablets were placed in the dissolution medium consisting of 0.01 M phosphate buffer with 0.5 % SDS. The volume of the dissolution medium was 900 mL at the 37 ± 0.5 °C. 10 mL samples were taken from the vessel using syringe capped with a 0.45 µm filter at every 5, 10, 20, 30 minutes of the dissolution test and the same volume (10 mL) of the dissolution medium was rapidly added.[5]

### Preparation of standard solutions

A standard stock solution was prepared by dissolving 10 mg of simvastatin in methanol in a 10 mL volumetric flask and the volume was made up to the mark with methanol. Solution II was prepared by transferring 500 µL of stock solution to a 10 mL volumetric flask and the volume was made up to the mark with methanol. Tests solutions with concentration of 1, 5, 10, 20, 30 and 50 µg $mL^{-1}$ were prepared by taking 20, 100, 200, 400, 600 and 1000 µL respectively and made up to 1 mL in Eppendorf tubes.

## RESULTS AND DISCUSSION

### Optimization of the method

*The spectrum of simvastatin absorption*

The spectrum of simvastatin was recorded over the range of 200–320 nm. The maximum of absorption was measured at 238 nm.

*The Column Type*

Several columns were tested during the experiment. The peak shape and the resolution between peaks was taken under consideration. The best result were obtained for Phenomenex ODS Luna column, which was choosen for procedure I (Figure 2); and for Hypersil ODS column for procedure II (Figure 3).

*The Influence of Column Oven Temperature*

The effect of temperature on retention time, peak height, number of theoretical plates, tailing and asymmetry factor were calculated according to United States Pharmacopea:[15]

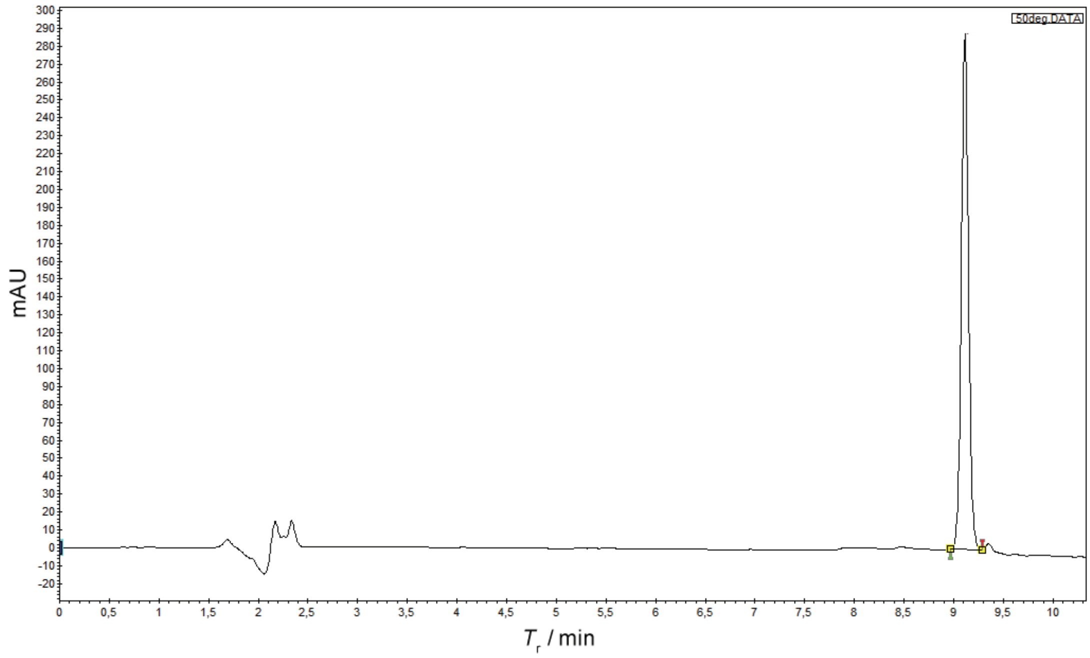


**Figure 2.** Simvastatin sample analysis using procedure I at 50 °C.

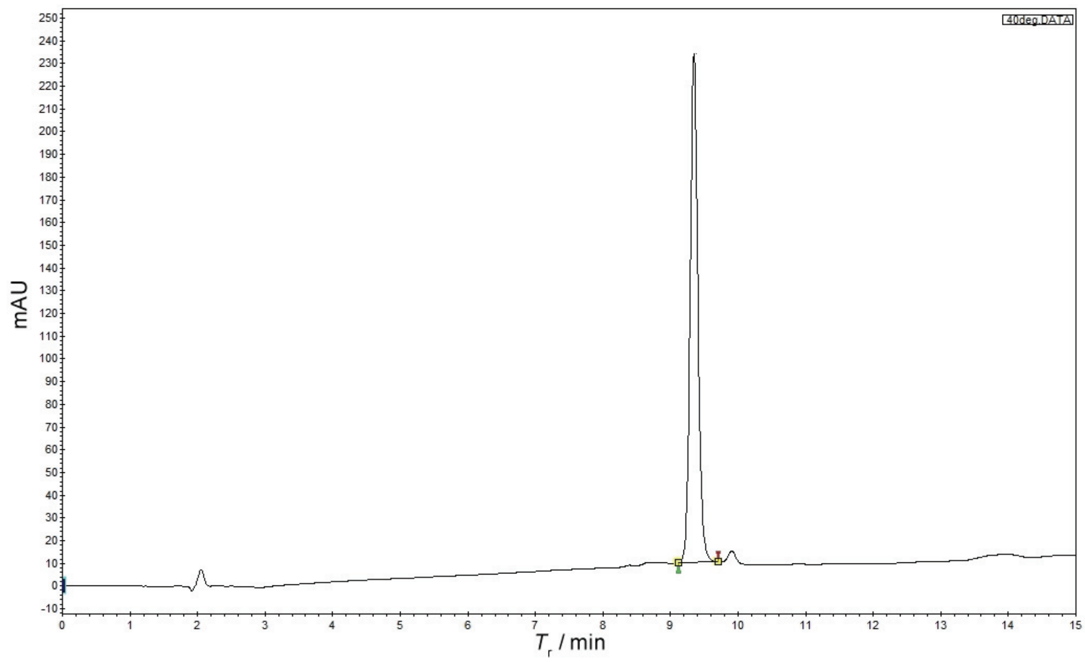


**Figure 3.** Simvastatin sample analysis using procedure II at 40 °C.

Tailing factor: $T_F = \dfrac{w_{5\,\%}}{2f_{5\,\%}}$

$w_{5\,\%}$ - width of peak at 5 % height
$f_{5\,\%}$ - distance from the peak maximum to the leading edge of the peak, the distance being measured at a point 5 % peak height from the baseline.

Asymmetry factor: $A_S = \dfrac{w_{10\,\%}}{2f_{10\,\%}}$

$w_{10\,\%}$ - width of peak at 10 % height
$f_{10\,\%}$ - distance from the peak maximum to the leading edge of the peak, the distance being measured at a point 10 % peak height from the baseline.

**Table 1.** Results of method optimization

| *T* / °C | $t_R$ / min[(a)] | H[(b)] | $T_F$[(c)] | $A_s$[(d)] | *N*[(e)] |
|---|---|---|---|---|---|
| | | | PROCEDURE I | | |
| 25 | 9.74 | 233.8 | 1.25 | 1.21 | 65500 |
| 35 | 9.49 | 270.8 | 1.28 | 1.14 | 73800 |
| 40 | 9.37 | 282.2 | 1.13 | 1.09 | 75850 |
| 45 | 9.23 | 281.7 | 1.13 | 1.05 | 76000 |
| 50 | 9.11 | 287.7 | 1.06 | 1.05 | 76200 |
| | | | PROCEDURE II | | |
| 25 | 10.57 | 193.0 | 1.18 | 1.17 | 29400 |
| 35 | 9.73 | 221.2 | 1.15 | 1.14 | 31850 |
| 40 | 9.35 | 223.7 | 1.08 | 1.10 | 32140 |
| 45 | 8.96 | 230.2 | 1.04 | 1.04 | 30400 |
| 50 | 8.62 | 246.7 | 0.96 | 1.00 | 27550 |

(a)retention time
(b)peak shape
(c)tailing factor
(d)assymetry factor
(e)number of theoretical plates

**Table 2.** Intra-day assay summary

| added SIM $\gamma$ / µg mL$^{-1}$ | found SIM[(a)] $\gamma$ / µg mL$^{-1}$ | Deviation / % | RSD / % | found SIM[(a)] $\gamma$ / µg mL$^{-1}$ | Deviation / % | RSD / % |
|---|---|---|---|---|---|---|
| | | PROCEDURE I | | | PROCEDURE II | |
| 1 | 1.0 | 2.0 | 1.1 | 1.1 | 8.0 | 2.9 |
| 5 | 5.2 | 3.8 | 3.2 | 5.0 | –0.1 | 0.4 |
| 10 | 9.5 | –5.0 | 3.2 | 10.1 | 0.6 | 0.9 |
| 20 | 20.4 | 1.8 | 5.0 | 19.3 | –3.4 | 1.1 |
| 30 | 28.9 | –3.7 | 1.3 | 29.0 | –3.3 | 0.9 |
| 50 | 47.9 | –4.2 | 0.1 | 50.0 | –0.1 | 1.2 |
| Overall *n* = 6 | | –0.9 | 2.3 | | 0.3 | 1.2 |

(a)mean values

Number of theoretical plates: $N = 16\left(\frac{t_R}{w}\right)^2$

$t_R$ - retention time
$w$ - peak width measured at the peak base

Results of the measurements by both procedures are given in Table 1. The best peak shape (height, tailing factor and asymmetry factor) along with the highest column efficiency was achieved at 50 °C in procedure I and at 40 °C in procedure II. An interesting effect was observed as the column efficiency in procedure II increased at 40 °C and than decreased at the upper temperature whereas the increase by procedure I followed the shape of a polynomial function of the 3rd degree.

## Method Validation

*Specificity and Selectivity*
The chromatograms of standard solution with the analyte concentration of 20 µg mL$^{-1}$ were compared with those of samples from dissolution tests and no interfering peaks were detected in the vicinity of the simvastatin peak in both methods. The retention times for simvastatin were 9.11 min and 9.51 min in procedure I and II, respectively.

*Linearity*
Linearity was examined by obtaining six point calibration curves over the concentration range of 1–50 µg mL$^{-1}$ in both methods which covers the concentration of samples during dissolution tests. Regression equations and correlation coefficients were $y = 0.53x - 0.08$, $r = 0.997$

**Table 3.** Inter-day assay summary

| | added SIM, $\gamma$ / μg mL$^{-1}$ | | | | |
|---|---|---|---|---|---|
| | 1 | 5 | 10 | 20 | 30 |
| found SIM (mean), $\gamma$ / μg mL$^{-1}$ Procedure I ($n$ = 6) | | | | | |
| Mean | 1.1 | 4.7 | 8.9 | 20.3 | 31.3 |
| Standard deviation | 0.1 | 0.2 | 0.1 | 0.3 | 1.3 |
| Deviation / % (accuracy) | 15.0 | –5.6 | –10.8 | 1.4 | 4.3 |
| RSD / % (precision) | 10.8 | 4.9 | 1.4 | 1.4 | 4.3 |
| Overall deviation / % | 0.86 | | | | |
| RSD / % | 10.8–1.3 | | | | |
| found SIM (mean), $\gamma$ / μg mL$^{-1}$ Procedure II ($n$ = 6) | | | | | |
| Mean | 1.1 | 5.2 | 10.2 | 19.8 | 29.2 |
| Standard deviation | 0.1 | 0.1 | 0.2 | 0.6 | 0.6 |
| Deviation / % (accuracy) | 14.0 | 3.4 | 1.8 | –1.0 | –2.7 |
| RSD / % (precision) | 7.6 | 2.0 | 2.1 | 3.1 | 2.1 |
| Overall deviation / % | 3.1 | | | | |
| RSD / % | 7.6–2.1 | | | | |

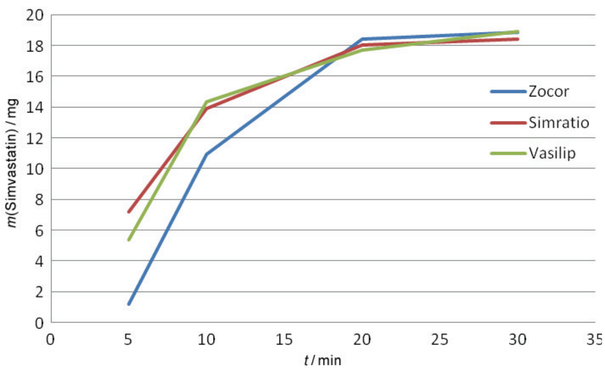


**Figure 4.** Dissolution graphs of chosen drugs.

and $y = 0.57x - 0.11$, r = 0.999 for method I and II respectively. The linearity was tested for five consecutive days. The RSD (%) values for slope factor and intercept of calibration curves were respectively 2.94, 60.98 (procedure I) and 2.62, 12.69 (procedure II).

*Precision and accuracy*

Precision and accuracy were measured by inter- and intra-day assays and the results are collected in Table 2. To determine the repeatability of the methods, six injections per each concentration (1, 5, 10, 20 and 30 μg mL$^{-1}$) under optimized conditions were tested. For intra-day assay RSD < 5.0 % with a acceptable accuracy (overall % deviation = –0.88) was calculated for procedure I and RSD < 2.9 % with a very good accuracy (overall % deviation = 0.28) for procedure II was obtained.

Intra-day precision and accuracy for intra-day assay were determined in five days in six successive injections per each of four concentrations (1; 5; 10; 20; 30 μg mL$^{-1}$). RSD (in %) was less than 10.8 for procedure I and less than 7.6 for procedure II this corresponding to acceptance criteria for analytical methods. Percent deviation was calculated to be less than 15 and 14 for procedure I and procedure II, respectively thus proving both methods to be reliable within analytic ranges.

*Limits of Detection and Quantitation*

Limit of detection (LOD) and limit of quantitation (LOQ) were measured for simvastatin (SIM) and it was calculated to be 250 ng mL$^{-1}$ and 1 μg mL$^{-1}$, respectively, for procedure I and II, as determined according to 3:1 and 10:1 signal/noise ratios. The LOQ value was the same as the first point of linearity range.

## Dissolution Tests

The second procedure was chosen for the determination of simvastatin in solid dosage forms (tablets). The main reason for the choice of the methanol/water eluent mixture was a better peak shape (tailing factor, asymmetry factor) and better accuracy and precision.

The results of the dissolution tests are shown in Figure4. All tablets released at least 80 % of simvastatin at the last time point (after 30 min of the test). However the difference between original drug (Zocor) and generic drugs (Simratio, Vasilip) has been noticed. The

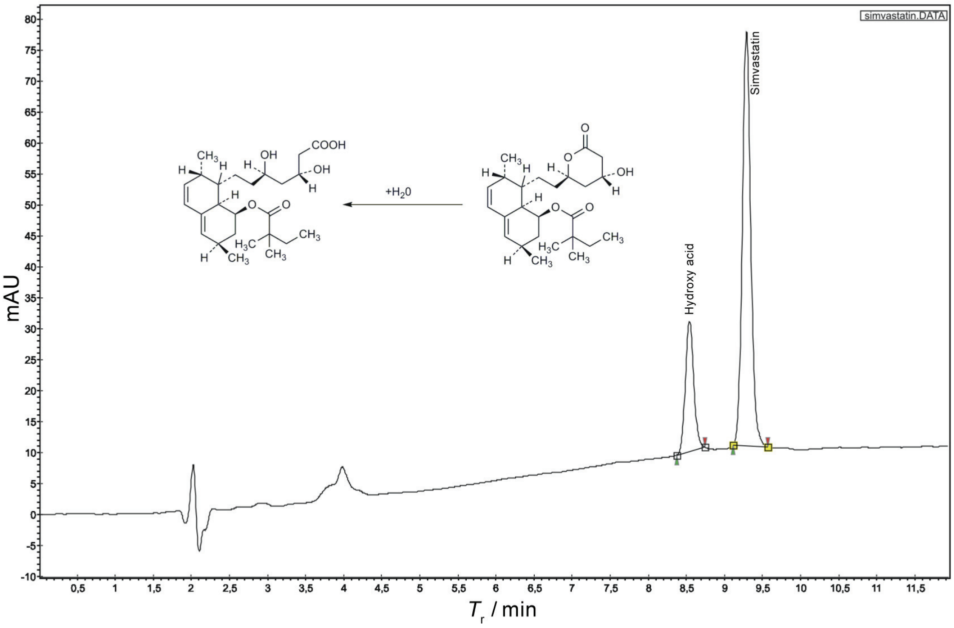


**Figure 5.** Chromatogram of Zocor analysis after 90 day storage.

composition of excipients in generic drugs causes the simvastatin to release more rapidly during the first five minutes of the test. Nevertheless, it does not have any influence on drug action. Zocor, Simratio and Vasilip at the last time point released on an average 18.83 mg (RSD = 0.8 %), 18.43 mg (RSD = 2.4 %), 18.92 mg (RSD = 1.5 %) respectively.

### Stability

Stability of stock solution was carried out in closed volumetric flask at room temperature. Stability had been tested before each validation procedure during the whole experiment. None of interfering peaks of degradation substance were observed. In comparison, samples after dissolution test were storage in the freezer at −20 °C during 90 days. Presence of water in the sample caused transformation simvastatin into its active ingredient – hydroxy acid. Figure 5 gives the chromatogram received in procedure II of analysis Zocor sample and it clearly shows the advanced degradation process. It is the main reason why during storage the influence of water must be avoided.

## CONCLUSIONS

Both methods were optimized with satisfactory precision and accuracy. Absence of excipient peaks interferences and a good resolution ensure the specificity of both methods. Better peak parameters like peak asymmetry and tailing factor and low price promote the choice of methanol. Lower column efficiency in procedure II in comparison to procedure I do not influence on determination of simvastatin in poor matrix (excipiens of the tablet only). It might be sighnificant in case of biological samples. Results of the dissolution test show that all tablets used in the examination (Zocor, Simratio, Vasilip) fullfil to pharmacopean requirements.

## REFERENCES


1. H. Yang, Y. Feng, and Y. Luan, *J. Chromatogr. B.* **785** (2003) 369–375.
2. W. Kostowski and Z. S. Herman, *Farmakologia. Podstawy farmakologii*, 3rd ed. PZWL, Warsaw, 2005.
3. D. Anantha Kumar, D. P. Sujan, V. Vijayasree, and J. V. L. N. Seshagiri Rao, *E-J.Chem*. **6** (2009) 541–544.
4. B. Patel, N. Sharma, M. Sanyal, and P. S. Shrivastav, *J. Sep. Sci.* **31** (2008) 301–313.
5. A. Melanović, M. Medenica, D. Ivanović, and B. Jančić, *Chromatographia* **63** (2006) 95–100.
6. M. A. Veronin and N. T. Nguyen, *Ann. Pharmacother.* **42** (2008) 613–620.
7. B. Barrett, J. Huclová, V. Bořek-Dohalský, B. Nêmec, and I. Jelìnek, *J. Pharm. Biomed. Anal.* **41** (2006) 517–526.
8. The European Pharmacopea edition 6, Council of Europe, Strasbourg, (2007) 2881–2883.
9. B. Nigović, Ś. Komorsky-Lovrić, and D. Devčić, *Croat.Chem. Acta* **81** (2008) 453–459.
10. L. Wang and M. Asharnejad. *J. Pharm. Biomed. Anal.* **21** (2000) 1243–1248.
11. E. Abu-Nameh, R. Shawabkeh, and A. Ali *J.Anal.Chem.* **61** (2006) 63–66.
12. http://www.accessdata.fda.gov/scripts/cder/dissolution/dsp_SearchResults_Dissolutions.cfm
13. M. Srinivasu, A. Narasa Raju, and G. Om Reddy, *J. Pharm. Biomed. Anal.* **29** (2002) 715–721.
14. B. Kim, E. Ban, J. Park, Y. Song, and C. Kim. *J. Liq. Chromatogr. Relat. Technol.* **27** (2005) 3089–3102.

# SAŽETAK

## Određivanje simvastatina u dozama farmaceutskih oblika optimiziranom i validiranom metodom koristeći HPLC/UV

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Simvastatin spada u skupinu antikolesterolnih spojeva koji se koriste pri tretmanu hiperkolesteremije. Analitičke metode koje se koriste za određivanje koncentracije aktivnih farmaceutskih sastojaka u dozama za testove kvalitete najčešće su temeljene na visokodjelotvornoj tekućinskoj kromatografiji (HPLC). Testovi trebaju biti brzi i pouzdani. Cilj ove studije je usporedba i validacija dviju metoda HPLC analize simvastatina uz upotrebu različitih eluensa: acetonitril/voda odnosno metanol/voda (gradijentno eluiranje). Testirane su različite kolone i varirane temperature analize. Iako su za obje metode dobiveni zadovoljavajući oblici krivulja i validacijski parametri, metoda u kojoj se metanol koristi kao eluens odabrana je za određivanje simvastatina u testovima brzine otapanja uglavnom zbog niže cijene tog eluensa.