Available online at www.sciencerepository.org

Science Repository

Research Article

New Validated Analytical Method for Determination of Simvastatin in Tablet Formulations by RP–HPLC

Safwan Ashour * ***and Soulafa Omar***

Department of Chemistry, Faculty of Science, University of Aleppo, Aleppo, Syria

ARTICLE INFO

Article history:
Received: 10 June, 2020
Accepted: 8 July, 2020
Published: 29 July, 2020

Keywords:
Simvastatin
high–performance liquid chromatography
tablets

ABSTRACT

RP–HPLC method has been developed and validated for the determination of simvastatin (SVS) in pure form and in tablets. Fluvastatin sodium was used as internal standard. The determination was performed on Nucleodur column C8 (250×4.6 mm i.d., 5 µm particle size); the mobile phase consisted of a mixture of phosphate buffer solution (KH_2PO_4 0.05 M, pH 4.83) and methanol (20:80, *v/v*), pumped at a flow rate 1.0 mL min^{-1}. Analyte was monitored by UV detection at 230 nm. The mean retention times for fluvastatin and simvastatin were about 4.40 and 9.00 min, respectively. The method was proved linear in the range of 3.5–550.0 µg mL^{-1} and exhibited good correlation coefficient (r>0.9998) and excellent mean recovery (100.71–102.18%). Very good limit of detection of 0.63 µg mL^{-1} was found for SVS. The method was validated statistically and by recovery studies for linearity, precision, repeatability, and reproducibility. This method was successfully applied to the determination of simvastatin content in four marketed brands from Syria. A good agreement between this method with the pharmacopoeial method for the determination of simvastatin in some real samples demonstrates that the proposed method is suitable to quantify simvastatin in pharmaceutical formulations.

Introduction

Simvastatin, 2,2'-dimethylbutanoic acid (1S,3R,7S,8S,8aR-(1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-[(2R,4R–(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl]ethyl]-1-naphthalenyl ester is used to reduce the amount of fatty substances such as low-density lipoprotein (LDL) cholesterol and triglycerides in the blood and to increase the amount of high-density lipoprotein (HDL) cholesterol in the blood. Simvastatin is also used to decrease the risk of heart attacks, strokes, and death, and to decrease the need for surgery to improve blood flow in people who have medical conditions that put them at high risk of developing heart and blood vessel problems [1]. The USP describes HPLC method for the estimation of simvastatin [2]. Spectrophotometric, colorimetric, modified glassy carbon electrode, RP-HPLC methods and various voltammetric techniques are reported for determination of simvastatin in pharmaceutical products [3-9]. Simvastatin with other drugs are determined by HPTLC, HPLC with UV detection and spectrophotometry in combined dosage forms [10-32]. Spectrophotometry and liquid chromatography–UV detection are used for determination of simvastatin either alone or in combination with other drugs in biological samples [32-35].

This manuscript deals the development and validation of RP-HPLC method serve as a reliable and rapid method for the determination of simvastatin in pharmaceutical preparations. In the proposed method, the separation and elution of simvastatin and internal standard were during 9 min run time. The precision of the described method has been checked regarding F-test using a pharmacopeia method as reference. The developed method can be serves as an alternative to the methods described in pharmacopeias.

Correspondence to: Safwan Ashour, Department of Chemistry, Faculty of Science, University of Aleppo, Aleppo, Syria; E-mail: profashour@hotmail.com

http://dx.doi.org/10.31487/j.DDA.2010.01.02

Experimental

I Instrumentation

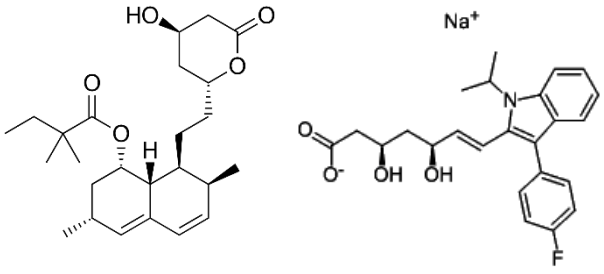
The L–2000 HPLC system composed of L-2130 binary pump, degasser, L-2350 column oven, autosampler and photodiode array (PDA) detector containing a quartz flow cell (all from Hitachi, Japan). Chromatograms were analysed and integrated automatically using the EzChrom Elite Hitachi Software. Metrohm compact titrator, Sartorius analytical balance, WTW pH meter and Dihan sonicator were used for all preparations.

II Chromatographic Conditions

Nucleodur C8 column (250×4.6 mm, 5 µm particle size, Macherey–Nagel Germany) was used to achieve the separation. The mobile phase was a mixture of a KH_2PO_4 solution (0.05 M–pH 4.83) and methanol (20:80, *v/v*), filtered through a nylon 0.45 µm membrane filter and degassed by ultrasonic agitation before use. The mobile phase was prepared weekly and was delivered at a flow rate of 1.0 mL min^{-1}. The injection volume was 10 µL with ambient column oven temperature. Isocratic elution of all analytes was monitored at 230 nm.

III Chemicals and Materials

Analytical reagent grade KH_2PO_4 and HPLC grade methanol and water were purchased from Merck (Darmstadt, Germany). Simvastatin (SVS) was supplied by Cipal Ltd, India, and its purity was found to be 100.3%. Fluvastatin sodium (FVS) was used as the internal standard, and obtained from Zhejiang Materials Industry Chemical Group Ltd, China. The chemical structure of simvastatin and fluvastatin sodium is shown in (Figure 1). Tablets were purchased from Syrian market, containing simvastatin 5, 10, 20 and 40 mg per tablet.



A **B**

Figure 1: The chemical structure of **A)** simvastatin ($C_{25}H_{38}O_5$=418.56 g/mole) and **B)** fluvastatin sodium ($C_{24}H_{25}FNO_4$.Na=433.45 g/mole).

IV Standard Solutions

Standard stock solutions of SVS (1.0 mg mL^{-1}) and FVS (1.0 mg mL^{-1}) were prepared by direct weighing of standard substance with subsequent dissolution in methanol. From these stock solutions, the working standard solutions were prepared by further diluting of stock solution using methanol. These solutions were stored in the dark at 2–8 °C and were found to be stable for two days.

V Assay Procedure for Dosage Forms

Twenty tablets containing SVS were weighed and finely powdered. Five accurately weighed quantities of the powder equivalent to 40 mg of SVS were transferred into 100 mL separated volumetric flasks. A 80 mL of methanol was then added to each flask and the mixture was sonicated for 10 min. Then, the volume of each mixture was adjusted to 100 mL with methanol. The sample solutions were filtered, and a suitable concentration was prepared in 10 mL volumetric flasks containing 1 mL of the internal standard FVS. Finally, 10 µL of each diluted sample was injected into the column. Peak area ratios of SVS to that of FVS were then measured for the determination. SVS concentrations in the samples were then calculated using peak data and standard curve.

VI Method Validation

The method was validated in accordance with the International Conference on Harmonization (ICH) guidelines [36]. The following validation characteristics were addressed:

i Linearity

A series working standard solutions of SVS (3.5–550.0 µg mL^{-1}) were prepared by diluting the stock standard solution with the methanol. In each sample 1 mL of FVS was added (100 µg mL^{-1} in the final volume). Standard solutions were found to be stable during the analysis time. To construct the calibration curve five replicates (10 µL) of each standard solution were injected immediately after preparation into the column and the peak area of the chromatograms was measured. The calibration graph was constructed for the determination of SVS. Graph of the mean peak area ratio of SVS to that of FVS ($R_{SVS/FVS}$) versus the corresponding concentration of SVS (C_{SVS}) is described by regression equation, $R_{SVS/FVS}=mC_{SVS}+b$ (where m is the slope, b is the intercept and C is the concentration of the cited drug in µg/mL) obtained by least-squares method [37].

ii Sensitivity

The sensitivity of the method was determined on the limit of detection (LOD) and limit of quantification (LOQ). LOD) and LOQ of the SVS assay were determined experimentally by calibration curve method. LOD was expressed as the concentration of drug that generated a response to three times of the signal-to-noise (S/N) ratio, and LOQ was 10 times of the S/N ratio, thus, a calculation by using the following equations [38]:

$$\text{LOD} = \frac{3.3 \times SD\ of\ \ y - intercept}{Slope\ of\ calibration\ curve}$$

$$\text{LOQ} = \frac{10 \times SD\ of\ \ y - intercept}{Slope\ of\ calibration\ curve}$$

iii Precision

The intra-day precision was determined by measuring SVS samples of 3.5, 15, 55, 210 and 550 µg mL^{-1} and of FVS at the concentration used in the assay (100 µg mL^{-1}), from bulk or formulations, injected five times

on the same day. The percent relative standard deviation (RSD) of the predicted concentrations from the regression equation was taken as precision (Table 3).

iv Accuracy

To determine the accuracy, assay the same different levels of drug concentration mentioned above were analysed by the proposed method. The percentage relative error and mean percentage recovery of SVS were calculated. Absolute recoveries at each concentration were measured by comparing the response of pretreated standards with the response of standards which had not been subjected to sample pretreatment.

v Specificity

The specificity of the method was established through the study of resolution factor of drug peaks from nearest resolving peak and also among all other peaks. Peak purity of SVS was assessed to evaluate the specificity of the method.

vi System Suitability

The system suitability test was performed to confirm that the LC system to be used was suitable for intended application. A standard solution containing 100 µg mL^{-1} of SVS in the presence of 100 µg mL^{-1} of internal standard were injected six times. The parameters peak area, resolution (R_s), capacity factor (k'), number of theoretical plates (N), tailing factor (peak symmetry, T) and % RSD were determined.

vii Robustness

Robustness of the proposed method was determined by estimating the effect of a small variation of certain parameters like the percentage of methanol in the mobile phase, the concentration of the aqueous component in the mobile phase and the flow rate. When the effect altering one set of conditions was tested, the other conditions were held constant at optimum values.

Results and Discussion

I Optimization of Chromatographic Conditions

FVS was used as internal standard, to improve the analytical performance and thus control undetermined changes in active pharmaceutical ingredient concentration and instrument response fluctuations, and also to reduce the problem of the many-fold dilution required in the classical batch procedures. The effect of the composition of the mobile phase on the retention time of SVS and the internal standard, FVS, was investigated. Drug peak was eluted fast with the solvent front when many solvents and water was used. When methanol and water were used as mobile phase drug eluted late and had broadened. Results of the effect of methanol percentage in the mobile phase are presented in (Figure 2A). An increase in the percentage of methanol decreases the retention of SVS and FVS. Increasing methanol percentage to more than 85% FVS peak is eluted with the solvent front, while at methanol percentage lower than 75% the elution of SVS peak is seriously delayed. The optimum methanol percentage was found to be 80%.

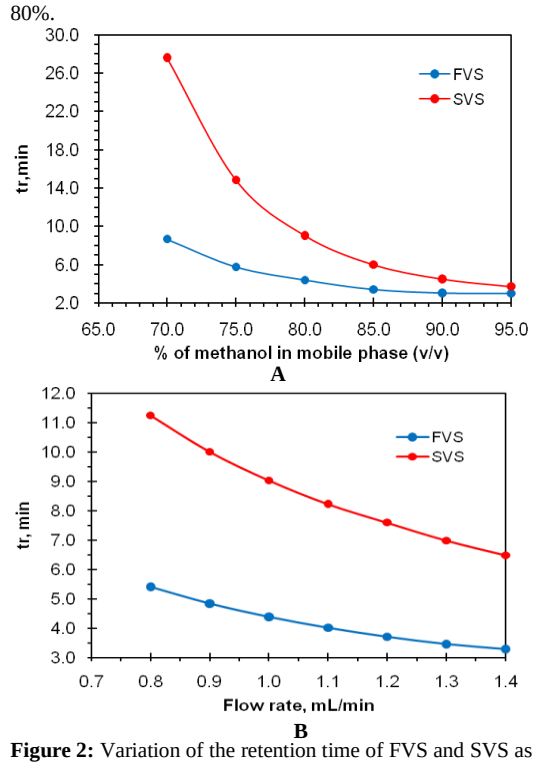


Figure 2: Variation of the retention time of FVS and SVS as a function of **A)** methanol percentage in the mobile phase and **B)** flow rate of the mobile phase.

The effect of pH in the chromatographic elution of the compounds was also investigated by change the concentration values of the aqueous component of the mobile phase from 0.01 to 0.5 M. A concentration value of 0.05 M KH_2PO_4 (pH 4.83) was chosen for the optimum separation of the compounds, as at this concentration, a satisfactory separation and peak asymmetry for the drug was obtained with mobile phase consisting of methanol: 0.05 M KH_2PO_4 pH 4.83 (80:20, *v/v*), pumped at a flow rate 1.0 mL min^{-1} (Figure 2B) at 25 ºC. Nucleodur C8 column (250×4.6 mm, 5 µm particle size) gave the most suitable resolution between FVS and SVS peaks (>4) according to the pharmacopeial requirement while the other columns (Supelcosil C8, Hichrom 5 C8, Nucleodur C18, ODS Hypersil C18) cause the peaks of the FVS and SVS either to be overlapped or to have unsuitable resolution (<4).

The use of isocratic elution was proven to be short retention time for the SVS peak and helped in the separation of FVS and SVS. Figure 3 shows a typical chromatogram obtained by the proposed RP-HPLC method, demonstrating the resolution of the symmetrical peaks corresponding to FVS and SVS with a flow rate of 1.0 mL min^{-1}. The retention time of FSV and SVS was about 4.407 and 9.047 min, respectively. The retention time observed allows a fast determination of the drug, which is suitable for QC laboratories. Quantitation was achieved with UV detection at 230 nm based on peak area.

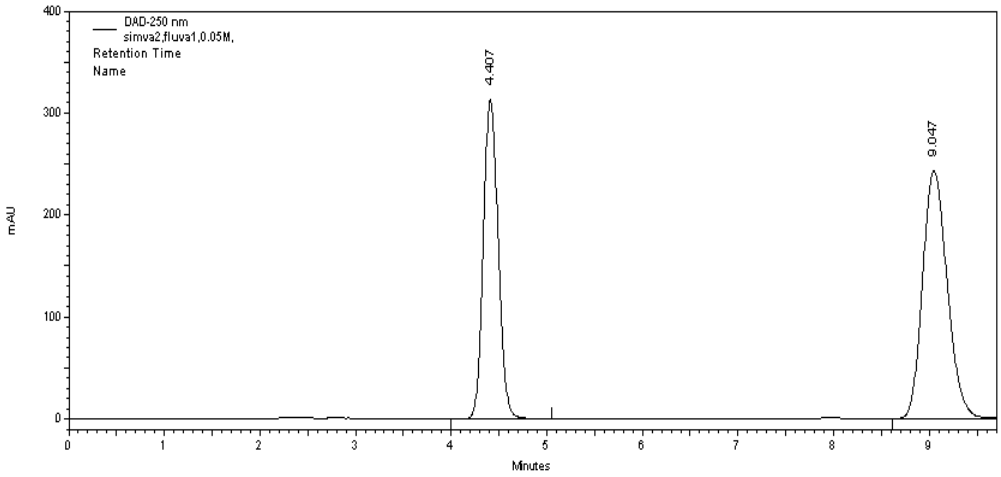


Figure 3: A typical chromatogram of a mixture of FVS (100 μg mL^{-1}) and SVS (100 μg mL^{-1}) at retention times 4.407 and 9.047 min, respectively, under optimal conditions.

II Method Validation

i System Suitability

The system suitability requirements for 100 μg mL^{-1} of SVS in the presence of 100 μg mL^{-1} of internal standard was a %RSD for peak area less than 1.30, a peak tailing factor less than 1.1 and R_s greater than 14 between adjacent peaks for all analytes. This method met these requirements. The results are shown in (Table 1).

Table 1: System suitability parameters.

Parameters	FVS	SVS	Preferable levels
Capacity factor (*k'*)	4.47	8.05	2–10
Selectivity (α)	–	1.8	1.0–2.0
Resolution (R_s)	–	14.75	> 1.5
Number of theoretical plates (*N*)	32753	37516	> 2500
Tailing factor (*T*)	1.21	1.04	< 1.5
% RSD for six injections	1.26	1.28	

ii Linearity

Under the optimal conditions for HPLC, the calibration curve obtained was linear over the concentration range of 3.5–550.0 μg mL^{-1}. Correlation coefficients (*r*) of the regression equations were greater than 0.999 in all cases. Characteristic parameters for regression equations and (*r*) were given in (Table 2). The linearity of the calibration graph was validated by the high value of (*r*) of the regression.

Table 2: Calibration data for the estimation of SVS by HPLC.

Parameters	Simvastatin
Retention time (min)	9.04
Optimum concentration range (μg mL^{-1})	3.5–550.0
Regression equation	$R_{SVS/FVS}$=0.0078C_{SVS}+0.0643
Correlation coefficient (*r*)	0.9999
Standard deviation of slope	7.07×10^{-5}
Standard deviation of intercept	0.00167
LOQ (μg mL^{-1})	2.11
LOD (μg mL^{-1})	0.63

iii Specificity

The specificity of the chromatographic method was determined to ensure separation of SVS and FVS as illustrated in (Figure 3) where complete separation was noticed. The HPLC chromatogram recorded for the analyte in tablets (Figure 4) showed almost no peaks within a retention time range of 10 min. The Figures show that SVS are clearly separated, and the peak of analyte was pure and the excipients in the formulation

did not interfere the analyte. Thus, the HPLC method presented in this study is selective for SVS.

\

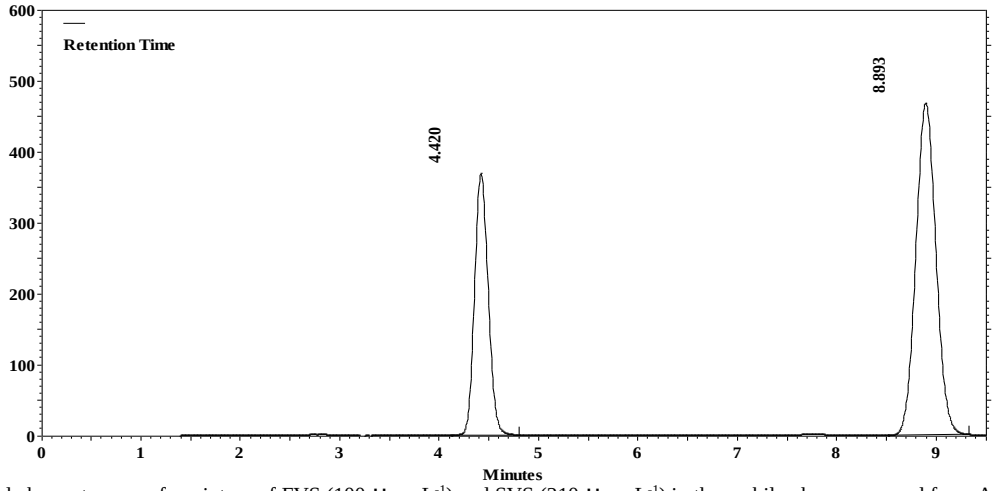


Figure 4: A typical chromatogram of a mixture of FVS (100 μg mL^{-1}) and SVS (210 μg mL^{-1}) in the mobile phase, prepared from Adacor 20 mg tablets, under optimal conditions.

iv Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantification (LOQ) were determined experimentally. LOD was found to be 0.63 μg mL^{-1}, and LOQ was 2.11 μg mL^{-1} for SVS showed a good sensitivity of the proposed method.

v Accuracy and Precision

The precision and accuracy of the method were evaluated by analysis of five samples for the drug. The proposed method was successfully applied for the analysis of the drug by intra-day (analysis of standard solutions of SVS in replicates of six in the same day), and the recovery experiments were carried out by spiking the already analysed samples with five different concentrations of standard SVS. The percent recoveries obtained were from 100.71 to 102.18%. The standard deviation, relative standard deviation and relative error % of different amounts tested were determined from the calibration curve. Acceptable repeatability of the results within one day was observed. The accuracy of the method is indicated by the excellent recovery, and the precision is supported by the low relative standard deviation, as recorded in (Table 3).

Table 3: Accuracy and precision of the determination of simvastatin by HPLC.

Nominal concentration (μg mL^{-1})	Intra–day *(n=6)*			
	Mean±SD (μg mL^{-1})	RSD (%)	Recovery (%)	Relative error (%)
3.50	3.55±0.09	2.53	101.43	1.43
15.00	15.23±0.28	1.84	101.53	1.53
55.00	55.39±0.77	1.39	100.71	0.71
210.00	214.57±2.76	1.29	102.18	2.18
550.00	558.28±5.97	1.07	101.51	1.51

vi Robustness

The robustness of the method was studied by using five replicates at SVS concentration level of 100 μg mL^{-1}. The obtained results are shown in (Table 4). The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust.

Table 4: Results of robustness study of SVS.

Parameter	SVS			
	Assay %	RSD %	t_r	*T*
Change in methanol percentage				
75%	100.92	1.29	14.853	1.05
80%	100.57	1.20	9.047	1.04
85%	99.89	1.33	6.0	1.03

Change in concentration of KH_2PO_4				
0.025 M	101.04	1.31	9.133	1.05
0.050 M	100.51	1.24	9.047	1.03
0.075 M	100.21	1.29	9.093	1.04
Change in flow rate				
0.9 mL/min	99.91	1.35	10.02	1.04
1.0 mL/min	100.54	1.22	9.047	1.02
1.1 mL/min	100.87	1.20	8.240	1.03

III Application of the Assay

The validity of the proposed method for the determination of simvastatin was assessed by measuring drug concentration of pharmaceutical dosage forms (Figure 4). The results obtained with the proposed method were compared with the pharmacopeial method and are shown in (Table 5) [2]. Mean values were obtained with a Student's *t*- and *F*-tests at 95% confidence limits for four degrees of freedom. The results showed comparable accuracy (*t*-test) and precision (*F*-test), since the calculated values of *t*- and *F*-tests were less than the theoretical data. The proposed method is simple, rapid, accurate, highly sensitive and suitable for the routine quality control without interference from the excipients and additives.

Table 5: Determination of SVS in tablets by the proposed and official methods.

Product [a]	Pharmaceutical company (country of origin)	%Recovery[b] ±SD	
		Proposed method	Official method [2]
Adacor 20mg	Adamco Pharma (Syria)	101.30±1.50 *t* =1.94 *F* = 3.79	100.87±0.77 *t* =1.96
Simvacor 40mg	Alfares (Syria)	101.84±2.07 *t* =1.99 *F* = 3.08	101.00±1.18 *t* =1.47
Zocorine 10mg	Asia (Syria)	101.67±1.62 *t* =2.92 *F* = 1.26	101.93±1.44 *t* =2.31
Zukol 5mg	K.C. pharma (Syria)	100.75±0.81 *t* =2.05 *F* = 1.60	99.46±0.73 *t* =1.28

[a] The dose is 5, 10, 20 and 40 mg expressed as simvastatin for all products.
[b]Five independent analyses. At 95% confidence level t-value is 2.776 and F-value is 6.26.

Conclusion

The proposed HPLC method was successfully applied to the determination of simvastatin in bulk powder and tablet dosage form. The suggested method was found to be simple, accurate, precise and robust. Moreover, the method is fast with respect to analysis time as compared to sophisticated chromatographic techniques. The method provided excellent specificity and linearity with LOQ of 2.11 µg mL^{-1} and LOD of 0.63 µg mL^{-1}. The sample recoveries from all formulations were in good agreement with their respective label claims, which suggested non-interference of formulations excipients in the estimation. Hence this method can be conveniently used for routine quality control analysis of simvastatin in its pharmaceutical formulation.

Conflicts of Interest

None.

References

1. Budavari S (2001) The Merck Index, An Encyclopedia of Chemicals, Drug and Biologicals,12th Ed.,Merck &Co., Inc., New Jersey, USA.
2. United States Pharmacopoeia (2007) United States Pharmacopeial Convention, Rockville, 30th ed.
3. Basavaiah K, Tharpa K (2008) The development and validation of visible spectrophotometric methods for simvastatin determination in pure and the tablet dosage forms. *Chemical Industry and Chemical Engineering Quarterly* 14: 205-210.
4. Arayne MS, Sultana N, Hussain F, Ali SA (2007) Validated spectrophotometric method for quantitative determination of simvastatin in pharmaceutical formulations and human serum. *J Anal Chem (Translation of Zhurnal Analiticheskoi Khimii)* 62: 536-541.
5. L Wang, M Asgharnejad (2000) Second-derivative UV spectrometric determination of simvastatin in its tablet dosage form. *J Pharm Biomed Anal* 21: 1243-1248. [Crossref]
6. Mohie M K Sharaf El-Din, Khalid A M Attia, Mohamed W I Nassar, Mohamed M Y Kaddah (2010) Colorimetric determination of

simvastatin and lovastatin in pure form and in pharmaceutical formulations. *Spectrochim Acta A Mol Biomol Spectrosc* 76: 423-428. [Crossref]

7. Zhang H, Hu CG, Wu SJ, Hu SS (2005) Enhanced oxidation of simvastatin at a multi-walled carbon nanotubes-dihexadecyl hydrogen phosphate composite modified glassy carbon electrode and the application in determining simvastatin in pharmaceutical dosage forms. *Electroanalysis (N.Y.)* 17: 749-754.
8. Marques-Marinho FD, Freitas BD, da Costa Zanon JC, Reis IA, Lima AA, Vianna-Soares CD (2013) Development and validation of a RP-HPLC method for simvastatin capsules. *Curr Pharm Anal* 9: 2-12.
9. O Coruh, S A Ozkan (2006) Determination of the antihyperlipidemic simvastatin by various voltammetric techniques in tablets and serum samples. *Pharmazie* 61: 285-290. [Crossref]
10. Chaudhari B, Patel N, Shah P (2007) Determination of simvastatin, pravastatin sodium and rosuvastatin calcium in tablet dosage forms by HPTLC. *Ind J Pharm Sci* 69: 130-132.
11. Komsta L, Skibinski R, Hopkala H, Winiarczyk-Serwacka M (2007) Comparative validation of densitometric and videodensitometric determination of lovastatin and simvastatin in pharmaceuticals. *Chemia Anal (Warsaw)* 52: 771-779.
12. Nováková L, Šatínskýand D, Solich P (2008) HPLC methods for the determination of simvastatin and atorvastatin. *Trends in Anal Chem* 27: 352-367.
13. Kurbanoglu S, Esim O, Ozkan CK, Savaser A, Ozkan Y, Ozkan SA (2019) Development and validation of RP-LC method for the simultaneous determination of simvastatin and ezetimibe in fixed-dose combination tablets and in rabbit serum. *Chromatographia* 82: 279-285.
14. Ali H, Nazzal S (2009) Development and validation of a reversed-phase HPLC method for the simultaneous analysis of simvastatin and tocotrienols in combined dosage forms. *J Pharm Biomed Anal* 49: 950-956.
15. Mohamed Hefnawy, Mohammed Al-Omar, Saeed Julkhuf (2009) Rapid and sensitive simultaneous determination of ezetimibe and simvastatin from their combination drug products by monolithic silica high-performance liquid chromatographic column. *J Pharm Biomed Anal* 50: 527-534. [Crossref]
16. Özaltin N, Uçaktürk E (2007) Simultaneous determination of ezetimibe and simvastatin in pharmaceutical formulations by dual-mode gradient LC. *Chromatographia* 66: S87-S91.
17. Hassan A Alhazmi, Ahmed M Alnami, Mohammed A A Arishi, Raad K Alameer, Mohammed Al Bratty et al. (2018) A fast and validated reversed-phase HPLC method for simultaneous determination of simvastatin, atorvastatin, telmisartan and irbesartan in bulk drugs and tablet formulations. *Sci Pharm* 86: 1-13. [Crossref]
18. Asmaa A El-Zaher, Ehab F Elkady, Hanan M Elwy, Mahmoud Abo El Makarim Saleh (2018) A versatile liquid chromatographic method for the simultaneous determination of metformin, sitagliptin, simvastatin, and ezetimibe in different dosage forms. *J AOAC Int* 101: 401-409. [Crossref]
19. P Ramalingam, V Udaya Bhaskar, Y Padmanabha Reddy, K Vinod Kumar (2014) Stability-indicating RP-HPLC method for the simultaneous determination of sitagliptin and simvastatin in tablets. *Indian J Pharm Sci* 76: 407-414. [Crossref]
20. Mohammed E A Hammouda, Mohamed A Abu El-Enin, Dina T El-Sherbiny, Dalia R El-Wasseef, Saadia M El-Ashry (2013) Microemulsion liquid chromatographic method for simultaneous determination of simvastatin and ezetimibe in their combined dosage forms. *J Anal Methods Chem* 2013: 132836. [Crossref]
21. Taízia D Silva, Marcelo A Oliveira, Renata B de Oliveira, Cristina D Vianna-Soares CD (2012) Development and validation of a simple and fast HPLC method for determination of lovastatin, pravastatin and simvastatin. *J Chromatogr Sci* 50: 831-838. [Crossref]
22. R P Dixit, C R Barhate, S G Padhye, C L Viswanathan, M S Nagarsenker (2010) Stability indicating RP-HPLC method for simultaneous determination of simvastatin and ezetimibe from tablet dosage form. *Indian J Pharm Sci* 72: 204-210. [Crossref]
23. Bharat G Chaudhari, Natvarlal M Patel, Paresh B Shah (2007) Stability-indicating reversed-phase liquid chromatographic method for simultaneous determination of simvastatin and ezetimibe from their combination drug products. *J AOAC Int* 90: 1242-1249. [Crossref]
24. Hayam Mahmoud Lotfy, Amal Mahmoud Aboul Alamein, Maha Abdel Monem Hegazy (2010) Quantitative analysis of the cholesterol-lowering drugs ezetimibe and simvastatin in pure powder, binary mixtures, and a combined dosage form by spectrophotometry, chemometry, and high-performance column liquid chromatography. *J AOAC Int* 93: 1844-1855. [Crossref]
25. Rajput S, Raj H (2007) Simultaneous spectroscopic estimation of ezetimibe and simvastatin in tablet dosage forms. *Ind J Pharm Sci* 69: 759-762.
26. Fernanda Heringer de Souza, Vítor Todeschini, Maximiliano da Silva Sangoi (2018) Chemometric-assisted spectrophotometric method for the simultaneous quantitative determination of ezetimibe and simvastatin in their combined dosage forms. *J AOAC Int* 101: 1015-1020.
27. Shoeb Alahmad, Hamed M Elfatatry, Mokhtar M Mabrouk, Sherin F Hammad, Fotouh R Mansour (2018) Development and validation of chemometric spectrophotometric methods for simultaneous determination of simvastatin and nicotinic acid in binary combinations. *Curr Drug Discov Technol* 15: 149-155. [Crossref]
28. Nancy Magdy, Miriam F Ayad (2015) Two smart spectrophotometric methods for the simultaneous estimation of simvastatin and ezetimibe in combined dosage form. *Spectrochim Acta A Mol Biomol Spectrosc* 137: 685-691. [Crossref]
29. Hayam Mahmoud Lotfy, Maha Abdel Monem Hegazy (2013) Simultaneous determination of some cholesterol-lowering drugs in their binary mixture by novel spectrophotometric methods. *Spectrochim Acta A Mol Biomol Spectrosc* 113: 107-114. [Crossref]
30. Hadir M Maher, Rasha M Youssef, Ekram M Hassan, Eman I El-Kimary, Magda A Barary (2011) Enhanced spectrophotometric determination of two antihyperlipidemic mixtures containing ezetimibe in pharmaceutical preparations. *Drug Test Anal* 3: 97-105. [Crossref]
31. Lotfy HM, Hegazy MA, Abdel-Gawad SA (2013) Simultaneous determination of simvastatin and sitagliptin in tablets by new univariate spectrophotometric and multivariate factor based methods. *European J Chem* 4: 414-421.
32. N Erk (2002) Rapid spectrophotometric method for quantitative determination of simvastatin and fluvastatin in human serum and pharmaceutical formulations. *Pharmazie* 57: 817-819. [Crossref]
33. Kim BC, Ban E, Park JS, Song YK, Kim CK (2004) Determination of simvastatin in human plasma by column-switching HPLC with UV detection. *J Liquid Chromatography and Related Tech* 27: 3089-3102.

34. L Tan, L L Yang, X Zhang, Y S Yuan, S S Ling (2000) Determination of simvastatin in human plasma by high-performance liquid chromatography. *Sepu* 18: 232-234. [Crossref]
35. G Carlucci, P Mazzeo, L Biordi, M Bologna (1992) Simultaneous determination of simvastatin and its hydroxy acid form in human plasma by high-performance liquid chromatography with UV detection. *J Pharm Biomed Anal* 10: 693-697. [Crossref]
36. ICH, Q2 (R1) (2005) Validation of Analytical Procedures: Text and Methodology, International Conference on Harmonization: Geneva.
37. Miller JN, Miller JC (2010) Statistics and Chemometrics for Analytical Chemistry, 6th ed., Ellis Horwood, Chichester : 110.
38. Long GL, Winefordner JD (1983) Limit of Detection. A Closer Look at the IUPAC Definition. *Anal Chem* 55: 712A-721A.