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Evaluation of the Solubility Profiles of Hypolipidemic Drugs

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Abstract

Hypolipidemic agents represent an important class of drugs used in the treatment of hyperlipoproteinemia. Statins are reversible, competitive inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, ezetimibe selectively inhibits intestinal and biliary absorption of cholesterol by blocking Niemann-Pick C1-like protein, while fibrates act as agonists of the peroxisome proliferator-activated receptor alpha nuclear receptor. Drug solubility is a critical parameter influencing bioavailability and therapeutic efficacy, particularly in orally administered formulations. This is of special importance for hypolipidemic agents such as simvastatin, atorvastatin, and fenofibrate, which are widely used in the treatment of hyperlipidemia.

In this study, an HPLC method was developed and validated for determining the solubility of these three drugs. Method validation confirmed specificity, linearity, and precision. Solubility was assessed in four media of varying pH values (1.2, 4.5, 6.8, and 7.4) at 37 °C, employing a Zorbax Eclipse C18 column (5 µm, 150 × 4.6 mm), with a mobile phase consisting of acetonitrile and 0.1 % phosphoric acid, and UV detection at 242 nm.

The results enabled solubility characterisation based on the dose number, which, in combination with literature-derived logP (partition coefficient) values, facilitated the Biopharmaceutics Classification System (BCS) categorisation of the investigated drugs. These findings provide valuable guidance for the design of new pharmaceutical formulations containing simvastatin, atorvastatin, or fenofibrate, as well as the optimisation of existing ones.

Keywords

Hypolipidemic drugs, solubility, HPLC, BCS classification, bioavailability

1 Introduction

Hypolipidemic drugs are used to treat hyperlipoproteinemia. This pharmacotherapeutic group includes statins, ezetimibe, fibrates, and others.[1] Statins are reversible, competitive inhibitors of HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase, the enzyme that converts HMG-CoA to mevalonate in the cholesterol biosynthetic pathway.[2] The hydrophilic part of the statin structure corresponds to the structure of the endogenous substrate of the enzyme and represents the pharmacophore of this group of drugs. Lovastatin (LV), simvastatin (SV), atorvastatin (AV), and rosuvastatin (RV) are among the hypolipidemic agents from the statin group most frequently used in clinical practice (Fig. 1).[3,4] Ezetimibe (EZ) (Fig. 1) selectively inhibits intestinal and biliary absorption of cholesterol by blocking the NPC1L1 (Niemann-Pick C1-like) protein, which is responsible for the transport of cholesterol into enterocytes or hepatocytes of the hepatobiliary system.[5,6] Fibrates are agonists of the PPARα (peroxisome proliferator-activated receptor alpha) nuclear receptor. By activating PPARα, fibrates regulate the expression of genes involved in various lipid metabolic pathways, thus lowering the level of TG and increasing the level of HDL in the circulation. Activation of PPARα leads to increased expression of lipoprotein lipase and decreased expression of apolipoprotein III (apoIII) in the liver. Fenofibrate (FF) is a key representative of fibrates (Fig. 1).[7,8]

Solubility is the maximum amount of a substance that dissolves in a given volume of solvent at a given temperature. For substances that undergo ionisation with changes in the pH of the medium, the ionised form typically exhibits greater solubility in polar solvents, whereas the opposite is true in non-polar solvents. Solubility is also a key parameter considered in the Biopharmaceutical Classification System (BCS), which classifies drug substances into four groups based on solubility and permeability.[9,10] This classification is highly important to pharmaceutical development, as it highlights potential problems that may arise in drug formulation. Understanding and determining the solubility of hypolipidemic drugs is essential not only for drug formulation purposes but also for predicting bioavailability upon administration, both of which ultimately influence a drug's efficacy and safety.

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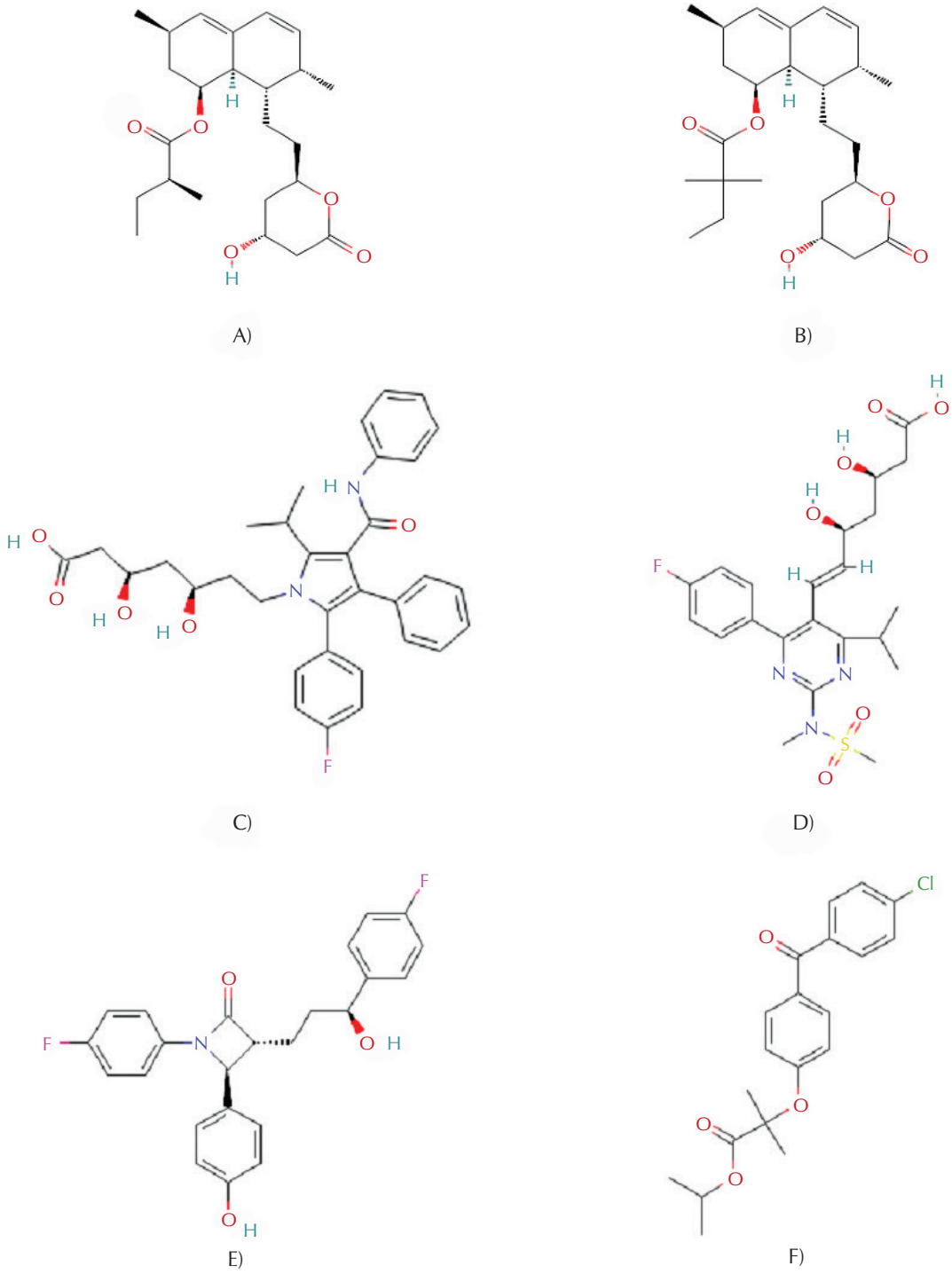

Fig. 1 – Chemical structures of hypolipidemic drugs: A) lovastatin (LV), B) simvastatin (SV), C) atorvastatin (AV), D) rosuvastatin (RV), E) ezetimibe (EZ), and F) fenofibrate (FF)

Slika 1 – Kemijske strukture hipolipidemika: A) lovastatin (LV), B) simvastatin (SV), C) atorvastatin (AV), D) rosuvastatin (RV), E) ezetimib (EZ) i F) fenofibrat (FF)

Numerous studies in the literature have reported on the application of various physicochemical methods for determining the content of hypolipidemics. *S. Bozhanov* and *V. Maslarska*[11] provided an overview of different HPLC methods for the separation and quantitative determination of statins, both individually and in combination with other active substances. *H. Sirén et al.*[12] developed and optimised a gas chromatography-mass spectrometry (GC-MS) method for the determination of statins. *T. Silva et al.*[13] developed and validated a simple and fast HPLC method for the determination of lovastatin, simvastatin, and pravastatin in the presence of degradation products and auxiliary substances from the formulations. *K. Kokilambigai et al.*[14] provided an overview of analytical methods for the determination of atorvastatin as an active substance, in pharmaceutical dosage form and in biological fluids. *A.*

Patel et al.[15] developed and validated an HPLC method for the simultaneous determination of the content of atorvastatin, ezetimibe, and fenofibrate in a tablet formulation. *A. Karunakaran et al.*[16] developed a UV spectrophotometric and HPLC method for the determination of rosuvastatin and fenofibrate in binary mixtures. *S. El-Moghazy et al.*[17] developed and validated an HPLC, TLC, and derivative spectrophotometric methods for the analysis of ezetimibe in the presence of its degradation products and in commercially available formulations. The aim of this study was to develop and validate an appropriate RP HPLC method for the identification, separation, and determination of hypolipidemic drugs. The validated method was used to test the solubility of SV, AV, and FF in different media spanning physiological pH values, with the objective of simulating the behaviour of these hypolipidemic drugs in different regions of the gastrointestinal tract, where pH values range from 1 to 8. Based on the results obtained, the solubility of these hypolipidemic drugs was defined, which, along with literature data on permeability, was used to classify SV, AV, and FF into the appropriate groups according to the BCS.

2 Materials and methodology

2.1 Equipment, chemicals, reagents, and chromatographic conditions

The following equipment was used: liquid chromatograph: Dionex UltiMate 3000 UHPLC (detector: Dionex UltiMate 3000; quaternary pump: Dionex UltiMate 3000; autosampler: Dionex UltiMate 3000; column thermostat: column compartment Dionex UltiMate 3000; data processing: Cobra Visard and Microsoft Excel 7.0), analytical balance: Mettler Toledo XS-105; ultrasonic bath: Karl Kolbe Scientific Technical Supplies DS 1062; filtration system: Whatman 47 mm Glass-nech; membrane filter holder: 0.45 mm Whatman International Ltd., Maid Stone, England; membrane sample filters: Macherey-Nagel GmbH & Co Kb., Germany 640 ∅125 mm; apparatus for obtaining distilled water; water bath with a shaker attachment Grant LSB 18. Working standards of SV, AV, and FF were supplied by Supelco. Analytical grade reagents: acetonitrile, methanol, potassium dihydrogen phosphate, phosphoric acid, hydrochloric acid, and potassium hydroxide were purchased from Fischer Scientific. Optimal chromatographic conditions for the separation of SV, AV, and FF were: chromatographic column Zorbax Eclipse 5 µm C18 150 × 4.6 mm; mobile phase A: acetonitrile; mobile phase B: 0,1 % phosphoric acid (*v*/*v*) (Gradient elution of the mobile phase presented in the Table 1), flow rate: 1.0 ml min^{-1}, injected volume: 20 µl, column temperature: 30 °C, and UV detection: 242 nm.

2.2 Preparation of solutions and medium for solubility testing

2.2.1 Preparation of 0.1 % phosphoric acid

An aliquot of 1.2 ml of 85 % phosphoric acid was transferred into a 1000 ml volumetric flask, and filled to the mark with distilled water.

Table 1 – Gradient elution profile
Tablica 1 – Program gradijentne elucije

Time / min	Proportion of phase B / %
0	50
7.0	50
9.0	20
13.0	25
15.0	25
17.0	50

2.2.2 Preparation of 0.1M HCl solution

An amount of 100 ml of 1 M HCl was transferred into a 1000 ml flask and filled to the mark with distilled water.

2.2.3 Preparation of phosphate buffer solutions pH 4.5, 6.8 and 7.4

In three 1000 ml volumetric flasks, 27.22 g of potassium dihydrogen phosphate was transferred into each flask. An amount of 700 ml of distilled water was added, and the mixtures were shaken in an ultrasonic bath until fully dissolved. Each flask was then filled to the mark with water, and the pH of the solution in the first flask was adjusted to 4.5, in the second to 6.8, and in the third to 7.4 by adding phosphoric acid or 1 M potassium hydroxide solution.

2.3 Preparation of solutions for evaluating the specificity/selectivity of the method

2.3.1 Standard stock solution for SV, AV, and FF

An amount of 25 mg of SV, AS, and FF was transferred into three 10 ml volumetric flasks, followed by adding 5 ml of methanol. The mixtures were then shaken in an ultrasonic bath until fully dissolved. Each flask was then filled to the mark with the same solvent, and the contents were filtered through a 0.45 µm PDVF membrane filter (c = 0.5 mg ml^{-1}).

2.3.2 Preparation of working dilution for assessment of specificity/selectivity of the method

2.0, 0.8, 0.8, 2.0, 0.8 and 0.8 ml of the standard stock solutions of LV, SV, AV, RV, EZ, and FF (respectively) were transferred into a 10 ml volumetric flask and diluted to the mark with 0.1 % phosphoric acid.

2.3.3 Preparation of working dilutions for calibration curve construction

A dilution series of standard stock solutions of the test compounds were prepared in seven 10 ml volumetric flasks (Table 2).

Table 2 – Preparation of working dilutions for calibration curve construction
Tablica 2 – Priprema radnih razrjeđenja za izradu kalibracijskih pravaca

Concentration of working dilution / mg ml^{-1}	Measured volumes of standard stock solutions / ml						0.1 % phosphoric acid volume
	LV	SV	AV	RV	EZ	FF	
0.0055	0.25	0.10	0.10	0.25	0.10	0.10	up to the mark
0.0125	0.62	0.25	0.25	0.62	0.25	0.25	up to the mark
0.0250	1.25	0.50	0.50	1.25	0.50	0.50	up to the mark
0.0400	2.00	0.80	0.80	2.00	0.80	0.80	up to the mark
0.0600	3.00	1.20	1.20	3.00	1.20	1.20	up to the mark
0.0750	3.75	1.50	1.50	3.75	1.50	1.50	up to the mark
0.1000	5.00	2.00	2.00	5.00	2.00	2.00	up to the mark

Table 3 – Composition of the solutions for solubility testing
Tablica 3 – Sastav otopina za ispitivanje topljivosti

pH of medium	Weighed mass SV / mg	Weighed mass AV / mg	Weighed mass EZ / mg	Weighed mass FF / mg	Volume of solution / ml
1.2	8	8	10	25	10
4.5	8	8	10	25	10
6.8	8	8	10	25	10
7.4	8	8	10	25	10

2.4 Preparation of solutions for assessing method precision

2.4.1 Standard solution for evaluation of method reproducibility

In six 10 ml flasks, 1 ml of each standard stock solution of SV, AV, EZ, and FF, and 2.5 ml of each standard stock solution of LV and RV were transferred. The flasks were then filled to the mark with 0.1 % phosphoric acid (c = 0.055 mg ml^{-1}).

2.4.2 Preparation of solutions for testing the solubility of SV, AV, EZ, and FF

Preparation of solutions for solubility testing is shown in Table 3. All solutions were prepared in duplicate. The resulting supersaturated solutions were placed in a water bath with a shaker attachment at 37 °C for 24 h, after which they were filtered through membrane filters.

2.4.3 Dilutions of solutions for determination of SV, AV, EZ and FF

An amount of 1 ml of each solution of SV, AV, EZ, and FF prepared in pH 1.2 medium was transferred into four 100 ml flasks, and diluted to the mark with 0.1 M HCl. An amount of 1 ml of each solution of SV, AV, EZ, and FF prepared in pH 4.5 medium was transferred into four 20 ml flasks and diluted to the mark with 0.1 M HCl. An amount of 1 ml of each solution of SV, AV, EZ, and FF prepared in pH 6.8 and pH 7.4 medium was transferred into four 10 ml flasks and diluted to the mark with 0.1 M HCl.

3 Results and discussion

Lovastatin (LV), simvastatin (SV), atorvastatin (AV), rosuvastatin (RV), ezetimibe (EZ) and fenofibrate (FF) are non-polar compounds; therefore, the RP HPLC (Reversed-Phase High-Performance Liquid Chromatography) system was chosen for their analysis, in which the stationary phase was silica gel modified with octadecylsilyl groups. Lovastatin and simvastatin are structurally lactone forms of β-hydroxycarboxylic acid, so they are neutral molecules as a whole, while atorvastatin and rosuvastatin have a free carboxyl group, which determines them as weak acids (pKa (AV) = 4.3, pKa (RV) = 4.0).[18,19] Ezetimibe has a phenolic group in its structure, pKa = 9.73 and is in molecular form at physiological pH values.[20] Fenofibrate is a neutral molecule that hydrolyses *in vivo* to fenofibric acid with a pKa of 4.0.[21] The molecular forms of the tested compounds are dominant at pH values lower than 4.0; therefore, 0.1 % phosphoric acid was used as the aqueous component of the mobile phase. During the optimisation of the chromatographic conditions, the composition of the mobile phase was modified, given that the tested compounds differed in their lipophilicity. The logP values ranged from 0.13 for RV to 5.20 for FF.[19,22] 0.1 % phosphoric acid was used as the aqueous phase, and acetonitrile alone or in combination with methanol was used as the organic phase. Due to the differences in logP values, the set criteria for system suitability could not be met by isocratic elution: peak symmetry factor between 0.8 and 1.2; number of theoretical plateaus (N) greater than 2000, and retention factor value ($k´$) between 1 and 10, and a system in which the ratio of aqueous and organic phases changes over time, *i.e.,* a system with gradient elution, was chosen as the optimal chromatographic system. Under these conditions, the method for determining the

content of LV, SV, AV, RV, EZ, and FF was validated. Specificity, linearity, and precision were tested.

3.1 Specificity

To assess the specificity of the method, the following solutions of the solubility test medium and solutions of working standards of the LV, SV, AV, RV, EZ, and FF were injected into the chromatographic system. No peaks with retention times corresponding to the retention times of LV, SV, AV, RV, EZ, and FF were observed in the chromatograms of the solubility test medium, which confirmed that the components of the solubility test medium had not co-eluted with the test compounds (Figs. 2 and 3).

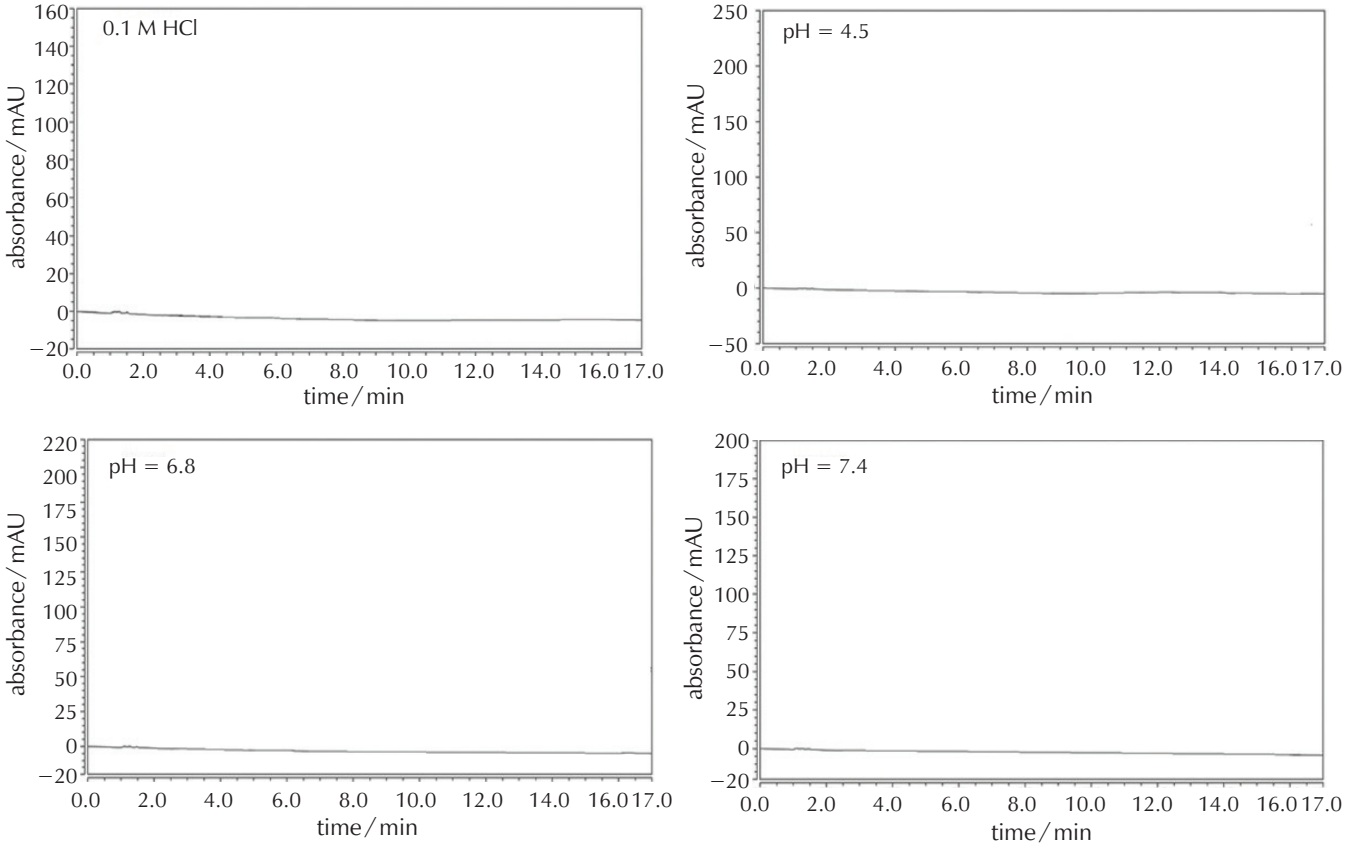

Fig. 2 – Chromatograms of the medium for solubility testing (pH = 1.2, pH = 4.5, pH = 6.8, pH = 7.4)
Slika 2 – Kromatogrami medija za ispitivanje topljivosti (pH = 1,2, pH = 4,5, pH = 6,8, pH = 7,4)

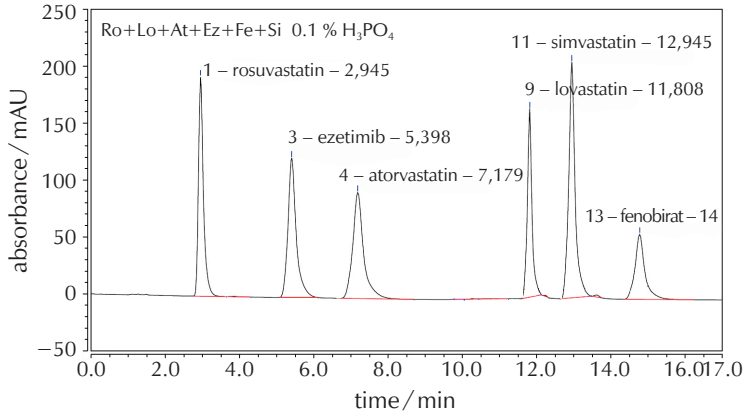

Fig. 3 – Chromatogram of working standard solutions of LV, SV, AV, RV, EZ, and FF
Slika 3 – Kromatogram radne standardne otopine koja uključuje LV, SV, AV, RV, EZ i FF

3.2 Linearity

The linearity of the method was assessed by constructing a calibration curve using the least square method. The calibration curve represents the dependence of the peak area of the standards on the concentration of the standard solutions. The results obtained are shown in Table 4 and Fig. 4. The parameters for the regression equations are shown in Table 5.

Based on the obtained correlation coefficient values r (≥ 0.990) and the significance of the intercept on the ordinate ($t_b < t_{tab}$), it can be concluded that the degree of scatter of data points around the ideal line is minimal. Thus, the method demonstrated linearity over the tested concentration range for all six compounds.

3.3 Method precision

Method precision was evaluated at the levels of repeatability (intra-assay precision) and intermediate precision. To assess repeatability (intra-assay precision), standard solutions LV, SV, AV, RV, EZ, and FF were prepared at a concentration of 0.055 mg ml^{-1}. Based on the obtained peak area

Table 4 – Data for calculating the calibration curves
Tablica 4 – Podatci za proračun kalibracijskih pravaca

$C_{standard\ LV,\ SV,\ AV,\ RV,\ EZ,\ and\ FF}$ / mg ml^{-1}	As^a / mAU·min (LV)	As^a / mAU·min (SV)	As^a / mAU·min (AV)	As^a / mAU·min (RV)	As^a / mAU·min (EZ)	As^a / mAU·min (FF)
0.0055	2.98	4.56	2.97	3.82	3.09	1.73
0.0125	7.42	11.30	7.31	9.41	7.59	4.25
0.0250	14.84	22.42	14.86	18.78	15.14	8.56
0.0400	23.59	35.37	23.47	29.84	23.95	13.53
0.0600	34.10	52.64	34.77	44.58	35.56	20.33
0.0750	43.41	65.06	39.02	55.14	41.53	25.34
0.1000	57.70	86.52	45.84	73.38	54.23	33.70

[a] standard peak area

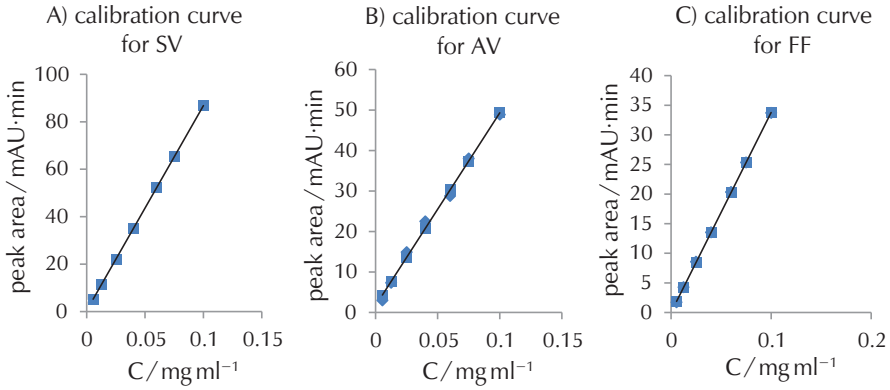

Fig. 4 – Calibration curves for assessing method linearity
Slika 4 – Kalibracijski pravci za procjenu linearnosti metode

Table 5 – Regression analysis parameters
Tablica 5 – Parametri regresijske analize

Concentration range / mg ml^{-1}	Compound	$y = ax + b$	r	t_b	t_{tab}
0.0055 – 0.1000	LV	$y = 576.87x + 0.2124$	0.99985	1.20	2.36
	SV	$y = 863.42x + 0.4728$	0.99982	1.66	2.36
	AV	$y = 476.18x + 1.6860$	0.99431	1.88	2.36
	RV	$y = 733.90x + 0.2237$	0.99987	1.10	2.36
	EZ	$y = 541.44x + 1.2741$	0.99647	1.59	2.36
	FF	$y = 337.59x + 0.0142$	0.99995	0.24	2.36

Table 6 – Data for calculating method repeatability
Tablica 6 – Podatci za analizu ponovljivosti metode

Injected concentration / mg ml^{-1}	No. of injections	*As*[a] / mAU·min (LV)	*As*[a] / mAU·min (SV)	*As*[a] / mAU·min (AV)	*As*[a] / mAU·min (RV)	*As*[a] / mAU·min (EZ)	*As*[a] / mAU·min (FF)
0.055	1	29.20	43.77	29.35	37.37	29.88	17.08
	2	28.87	43.27	28.98	36.91	29.52	16.81
	3	29.08	43.57	29.10	37.21	29.83	16.91
	4	29.11	43.57	29.21	37.29	29.80	16.97
	5	29.01	43.68	29.29	37.18	29.80	16.87
	6	29.30	44.11	29.49	37.55	30.19	17.08
Average value		29.09	43.66	29.24	37.25	29.84	16.95
Sd		0.15	0.28	0.18	0.21	0.21	0.11
RSD		0.51	0.63	0.62	0.57	0.72	0.65

[a] standard peak area

Table 7 – Data for calculating mean precision
Tablica 7 – Podatci za izračunavanje srednje preciznosti

Injected concentration / mg ml^{-1}	No. of injections	*As*[1] / mAU·min (LV)	*As*[a] / mAU·min (SV)	*As*[a] / mAU·min (AV)	*As*[a] / mAU·min (RV)	*As*[a] / mAU·min (EZ)	*As*[a] / mAU·min (FF)
0.055	1	27.58	41.30	28.53	36.31	29.11	16.43
	2	28.40	42.57	29.32	37.41	29.81	16.88
	3	28.46	42.64	29.27	37.38	29.85	16.86
	4	28.36	42.73	29.23	37.29	29.84	16.88
	5	28.36	42.75	29.28	37.28	29.80	16.86
	6	28.38	42.59	29.30	37.39	29.83	16.86
Average value		28.26	42.43	29.16	37.18	29.71	16.79
Sd		0.33	0.56	0.31	0.43	0.29	0.18
RSD		1.18	1.32	1.05	1.15	0.99	1.07

[a] standard peak area

values, the relative standard deviation (RSD) was calculated, and the repeatability of the method was determined. The results are shown in Table 6.

Based on the obtained RSD values (< 2 %), it can be concluded that the method meets the requirements for repeatability. Intermediate precision was evaluated by a second analyst on a different day, who independently prepared standard solutions of LV, SV, AV, RV, EZ, and FF at a concentration of 0.055 mg ml^{-1}. The results obtained are presented in Table 7.

The value for RSD is less than 3 %, which meets the requirements for intermediate precision of the method.

3.4 Determination of the solubility of SV, AV, EZ, and FF

The validated chromatographic method was used to determine the solubility of SV, AV, EZ, and FF, using the calibration curve method. Based on the equation $y = ax + b$, where y – peak area, x – unknown concentration, a – slope of the line, b – intercept on the ordinate, the concentrations of SV, AV, EZ, and FF in the tested media were calculated. Each determination was performed in duplicate for each solubility test medium. The results are presented in Tables 8 to 11.

Table 8 – Determination of SV solubility
Tablica 8 – Određivanje topljivosti SV

	pH 1.2	pH 4.5	pH 6.8	pH 7.4
Peak area / mAU·min	1.57	1.57	0.79	0.94
	2.09	1.27	1.28	0.19
Measured concentration / mg ml^{-1}	0.0013	0.0013	0.0003	0.0005
	0.0019	0.0009	0.0009	/ *
Average value / mg ml^{-1}	0.0016	0.0011	0.0006	0.0005

* The concentrations obtained were below the limit of quantification (LOQ), due to the low solubility of the test compounds in the solubility test media.

Table 9 – Determination of AV solubility
Tablica 9 – Određivanje topljivosti AV

	pH 1.2	pH 4.5	pH 6.8	pH 7.4
Peak area / mAU·min	11.89	50.10	231.47	252.23
	10.09	104.77	183.53	208.18
Measured concentration / mg ml^{-1}	0.0214	0.1017	0.4826	0.5261
	0.0176	0.2165	0.3819	0.4336
Average value / mg ml^{-1}	0.0195	0.1591	0.4322	0.4798

Table 10 – Determination of EZ solubility
Tablica 10 – Određivanje topljivosti EZ

	pH 1.2	pH 4.5	pH 6.8	pH 7.4
Peak area / mAU·min	0.50	0.44	0.62	0.26
	1.00	0.34	0.55	0.15
Measured concentration / mg ml^{-1}	/ *	/ *	/ *	/ *
	/ *	/ *	/ *	/ *
Average value / mg ml^{-1}	/ *	/ *	/ *	/ *

* The concentrations obtained were below the limit of quantification (LOQ), due to the low solubility of the test compounds in the solubility test media.

Table 11 – Determination of FF solubility
Tablica 11 – Određivanje topljivosti FF

	pH 1.2	pH 4.5	pH 6.8	pH 7.4
Peak area / mAU·min	0.06	0.05	0.03	0.09
	0.04	0.05	0.03	/ *
Measured concentration / mg ml^{-1}	0.1357	0.1060	0.0468	0.2245
	0.0764	0.1060	0.0468	/ *
Average value / mg ml^{-1}	0.2121	0.160	0.0468	0.2245

* The concentrations obtained were below the limit of quantification (LOQ), due to the low solubility of the test compounds in the solubility test media.

4 BCS classification

The BCS classification is a classification system that classifies drugs into four groups based on solubility and permeability.[9] The parameter describing solubility is the dose number (*Do*) of the substance, which is calculated using the following Eq. (1):

$$Do = Mo/Vo \cdot Cs \quad (1)$$

Do is the dose number of the substance, *Mo* is maximum single dose of the substance, *Vo* is 250 ml (volume of water with which a single dose is administered), and *Cs* refers to the lowest solubility of the substance in the physiological pH range.[9]

$$\text{SV: } Mo = 80 \text{ mg},[23]\ Cs = 0.0005 \text{ mg ml}^{-1};\ Do = (80 \text{ mg}/250 \text{ ml}) / 0.0005 \text{ mg ml}^{-1} = 640 \quad (2)$$

$$\text{AV: } Mo = 80 \text{ mg},[23]\ Cs = 0.0195 \text{ mg ml}^{-1};\ Do = (80 \text{ mg}/250 \text{ ml}) / 0.0195 \text{ mg ml}^{-1} = 16{,}41 \quad (3)$$

$$\text{FF: } Mo = 267 \text{ mg},[23]\ Cs = 0.0468 \text{ µg ml}^{-1};\ Do = (267 \text{ mg}/250 \text{ ml}) / (0.0468 \cdot 10^{-3} \text{ mg ml}^{-1}) = 22820.5 \quad (4)$$

Since the dose numbers (Do) of the tested substances were greater than 1, as calculated using Equations (1)-(3), they were classified as poorly soluble compounds.[10] The parameter used to describe the permeability of the tested compounds was the logP value. According to literature data, the logP values for SV, AV, and FF are 4.68,[24] 6.36,[18] and 5.2,[22] respectively. All three logP values are higher than the logP of metoprolol, which is commonly used as a standard (logP = 1.88).[25] Therefore, SV, AV, and FF can be classified as highly permeable substances. Based on both the dose number and logP values, SV, AV, and FF are poorly soluble yet highly permeable substances, and according to the BSC, they belong to Class II.

5 Conclusion

An HPLC method for determining the solubility of six hypolipidemic drugs – LV, SV, AV, RV, EZ, and FF – was developed and validated. The proposed method is rapid, efficient, sensitive, selective, and linear ($R \geq 0.9990$) over the concentration range of 0.0055 to 0.100 mg ml^{-1}, and precise ($RSD < 2$ %). The method was applied to determine the solubility of SV, AF, and FF in different media of varying pH values (1.2, 4.5, 6.8, and 7.4). Based on the concentrations obtained, their solubility classification was determined according to the BCS. SV, AV, and FF belong to Class II of the BCS classification system. The proposed method may also be used for quality control of pharmaceutical products and intermediates containing hypolipidemic drugs.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest to disclose, including financial, personal or other relationships.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author, Nemanja Turković, upon reasonable request.

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SAŽETAK

Procjena profila topljivosti hipolipidemijskih lijekova

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Hipolipidemici predstavljaju važnu klasu lijekova koji se upotrebljavaju za liječenje hiperlipoproteinemije. Statini su reverzibilni, kompetitivni inhibitori 3-hidroksi-3-metilglutaril-koenzima A reduktaze, ezetimib selektivno inhibira crijevnu i bilijarnu apsorpciju kolesterola blokiranjem Niemann-Pick C1-sličnog proteina, dok su fibrati agonisti peroksisomskog proliferatorom aktiviranog alfa nuklearnog receptora. Topljivost lijeka ključni je parametar koji utječe na bioraspoloživost i terapijsku učinkovitost, posebno u oralno primijenjenim formulacijama. To je od posebne važnosti za hipolipidemike poput simvastatina, atorvastatina i fenofibrata, koji se široko upotrebljavaju u liječenju hiperlipidemije.

U ovoj studiji razvijena je i validirana HPLC metoda za određivanje topljivosti tih triju lijekova. Validacija metode potvrdila je specifičnost, linearnost i preciznost. Topljivost je procijenjena u četiri medija različitih pH vrijednosti (1,2, 4,5, 6,8 i 7,4) pri 37 °C, uporabom Zorbax Eclipse C18 kolone (5 μm, 150 × 4.6 mm) s mobilnom fazom koja se sastojala od acetonitrila i 0,1 % fosforne kiseline te UV detekcijom na 242 nm.

Rezultati su omogućili karakterizaciju topljivosti na temelju broja doza, što je u kombinaciji s vrijednostima logP (koeficijent raspodjele) izvedenim iz literature, olakšalo kategorizaciju ispitivanih lijekova prema Biofarmaceutskom klasifikacijskom sustavu (BCS). Ti nalazi pružaju vrijedne smjernice za dizajn novih i optimizaciju postojećih farmaceutskih formulacija koje sadrže simvastatin, atorvastatin ili fenofibrat.

Ključne riječi

Hipolipidemijski lijekovi, topljivost, HPLC, BCS klasifikacija, bioraspoloživost

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