Multi-criteria decision making approach for- the optimization of atorvastatin and its impurities separation by micellar liquid chromatography

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Summary

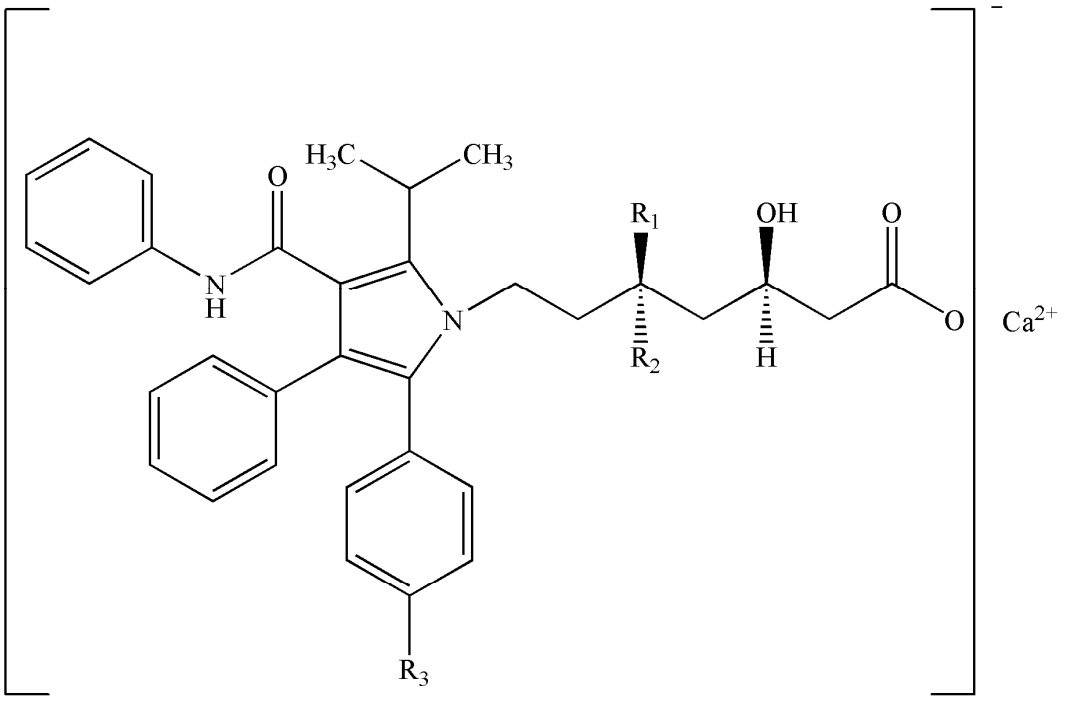
In the current paper, multi-criteria decision making (MCDM) approach was applied to optimize micellar liquid chromatography (MLC) intended for the pharmaceutical analysis of atorvastatin and its impurities, *trans*-atorvastatin and desfluoro-atorvastatin. MLC is a reversed-phase liquid chromatographic (RPLC) mode where the modification of mobile phase leads to the stationary phase modification. This results in the diverse interactions (hydrophobic, ionic and steric) significantly affecting retention and selectivity. For that reason double chained surfactant sodium dioctyl sulfosuccinate - AOT (Aerosol OT), with oxygen atoms in its tails, was used for the first time in such kind of separation. As the most efficient way to investigate a high number of factors, and simultaneously optimize defined antagonistic objectives (minimization of run time and maximization of atorvastatin and *trans*- atorvastatin resolution) MCDM approach was employed. Central composite design (CCD) with fractional factorial design, ± 0.5 star design and four replications in central point was used to define plan of experiments. Five responses selected during method development were optimized simultaneously using Derringer's desirability function. The predicted optimum was: 32 % acetonitrile, 2 % ethylene glycol, 66 % 6.4 mmol L^{-1} AOT in 20 mmol L^{-1} ammonium acetate, pH of the water phase 5.50 adjusted with acetic acid, flow rate 1.15 mL min^{-1} and column temperature of 10 °C.

Key words: Multi-criteria decision making; Derringer's desirability function; Micellar liquid chromatography; Atorvastatin and its impurities

Introduction

When one needs to optimize more than one response at a time and if the optimal values for each response are localized in different regions the application of multi-criteria decision making (MCDM) approach would be the best choice. In MCDM different approaches can be used, e.g. path of steepest ascent, constrained optimization procedure, Pareto-optimality, and Derringer's desirability function (1). In the presented study Derringer's desirability function was used for the optimization of atorvastatin (A), *trans*-atorvastatin (TA) and desfluoro-atorvastatin (DFA) separation in micellar liquid chromatography mode. Micellar liquid chromatography (MLC) is the modification of classical RP-HPLC mode of separation based on the studies of organized solutions. When micelles or microemulsion are used as mobile phases, solutes present in a mixture are separated on the basis of their differential partitioning between the bulk aqueous phase and the micellar aggregates or microemulsion droplets in the mobile phase, as well as between the bulk aqueous phase and the surfactant-coated stationary phase (2 - 4). This secondary partitioning enables better separation of complex mixtures especially those composed of analytes of different polarity.

The main objective of this paper was the investigation of improvement in selectivity promoted by MLC stationary and mobile phase modification (4, 5), as well as the application of MCDM approach to the optimization of the developed MLC method. The authors wanted to study all the presumed benefits of MLC applying it to the separation of A and its two impurities separation (Figure 1). According to the manufacturer (Krka, Novo Mesto, Slovenia) these two impurities have to be evaluated during the shelf-life and for that reason they were selected for this analysis. The therapy with statins is very long and thus the purity of these drugs is of a great significance.



atorvastatin	R_1=OH;	R_2=H;	R_3=F
trans-atorvastatin	R_1=H;	R_2=OH;	R_3=F
desfluoro-atorvastatin	R_1=OH;	R_2=H;	R_3=H

Figure 1. Chemical structures of atorvastatin, *trans*-atorvastatin and desfluoro-atorvastatin
Slika 1. Hemijske strukture atorvastatina, *trans*-atorvastatina i desfluoro-atorvastatina

In the literature, RP-LC method has been reported for the separation of atorvastatin (A), *trans*-atorvastatin (TA) and desfluoro-atorvastatin (DFA) (6). Very recently, a rapid resolution RP-LC method for the simultaneous quantitation of A and related substances in bulk drug has been developed (7). For the simultaneous quantitation of atorvastatin and its related impurities, micellar electrokinetic capillary chromatography (MEKC) was used (8). Liquid chromatography – tandem mass spectrometry was applied for separation, characterization and quantification of atorvastatin and its imputities, including *trans*-atorvastating and desfluoro-atorvastatin (9). Recently the authors have published the paper dealing with the optimization of artificial neural networks which can be used as a tool in modelling of A and its impurities retention when separated by micellar liquid chromatography (10). For the simultaneous determination of atorvastatin, aspirin and their major degradation products, salicylic acid, salicylsalicylic acid and the lactone form of atorvastatin, ultra

HPLC method was developed and validated (11). An ultra performance liquid chromatographic metod with UV detection was applied as stability indicating method for metoprolol, atorvastatin and ramipril in capsules (12). Also, review of HPLC methods for the determination of simvastatin and atorvastatin in bioanalytical assays, pharmaceutical assays and environmental applications is published (13).

In this paper the novelty was introduced by the application of Derringer's desirability function to simultaneously optimize the five chosen responses (retention factor of DFA − k_{DFA}, retention factor of TA − k_{TA}, retention factor of A − k_A, resolution between DFA and TA − $R_{DFA/TA}$ and $1-v/p$ value for A and TA) which improved and facilitated method optimization step. According to Ph. Eur. 6 (14) in the test for related substances when baseline separation between two peaks of very different high is not reached, the peak-to-valley ratio (*p/v*) may be employed for the assessment of the separation. However, as the better approach calculation of valley-to peak ratio (*v/p*) was proposed (15). For the sake of easier comparison of *v/p* with the resolution factor Rs, the subtracting of the ratio *v/p* from 1 was suggested ($1-v/p$) (16). The resulting value of 1 corresponds to baseline separation, but value of 0 to unresolved peaks. For the optimization purpose, it is advantageous to use the height of the peak that will result in the most sensitive *v/p* ratio. When there is a big difference in height (separation of active substance and its related impurity), the *v/p* ratio for smaller peak is much more sensitive to changes than the *v/p* ratio of large peak and hence is the most useful value to use for the calculation (15). So, in this case for the evaluation of A and TA separation equation $1-v/p$ was used, where *v* stands for the height of the valley between TA and A peak and *p* is TA peak height.

Experimental

Chemicals. All reagents used were of an analytical grade. Sodium dioctyl sulfosuccinate - AOT (Aerosol OT; docusate sodium), tetrahydrofuran dried (THF), dimethyl sulfoxide (DMSO), ethylene glycol and acetonitrile - HPLC gradient grade were obtained from Sigma (St. Louis, MO, USA). Water - HPLC grade, ammonium acetate (J. T. Baker, Deventer, Netherlands), ammonium hydroxide and glacial acetic acid (Sigma, St. Louis, MO, USA) were used to prepare a water phase. Reference substances of atorvastatin (batch ATS1-2542/1), *trans*-atorvastatin (batch ATN1-2042) and desfluoro-atorvastatin (batch ATN2-2429-C) were kindly donated by Krka (Novo Mesto, Slovenia).

Determination of AOT critical micellar concentration (CMC). For the determination of AOT CMC procedure proposed in (17) was used. Extrapolation of the decreasing and horizontal part of the plot of surface tension against the surfactant concentration results in a change in gradient at the CMC value (18). Surface tension was measured by weighting 50 drops of solutions containing different concentrations of AOT or AOT and acetonitrile. The weighting was done on analytical balance, precision ± 0.0001 g (Sartorius, Göttingen, Germany).

Chromatographic conditions. The chromatographic system consisted of Finnigan Surveyor Thermo Scientific, Finnigan Surveyor LC Pump Plus, Finnigan Surveyor UV/VIS Plus Detector, Finnigan Surveyor Autosampler Plus and ChromQuest for data collection. The samples were introduced through partial loop in 5 μL volume. For the separation the X-Terra™ Phenyl 4.6 mm × 150 mm, 5 μm particle size column was used. UV detection was performed at 254 nm.

Software. Design−Expert® 7.0.0. (Stat−Ease Inc., Minneapolis) was used for the definition of the plan of experiments, data analysis and desirability function calculations.

Standard solutions. For the optimization solutions containing 100 μg $mL^{-1}$of A and 0.3 μg mL^{-1} of TA and DFA were prepared in the mixture of acetonitrile-water (30:70 V/V).

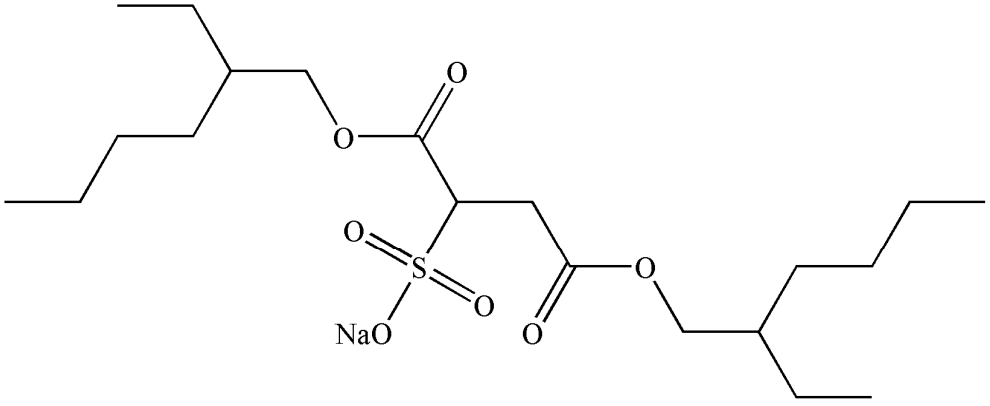
Results and discussion

Method development

In MLC eluents the micelles are formed as the surfactant is added in the concentration above its CMC. When the organic modifier is added to pure micellar mobile phase, elution strength is modified and the chromatographic behavior of analyzed substances significantly changes.

Surfactant selection

In this case, the necessity of the separation of two structurally very similar substances imposed AOT as surfactant in MLC separation of A, TA and DFA. The authors presumed that double chain, anionic and hydrophobic surfactant (Figure 2) can be important ingredient in the mobile phase intended for isocratic mode separation of A and TA. The structures of A and TA (Figure 1) differ only in the configuration at one C-atom (epimers with the different orientation of secondary alcohol group), which consequently leads to very similar, almost the same, chromatographic behavior for these two substances. It was expected that the presence of double chained surfactant and oxygen atoms (acceptors) in its tails may lead to intermolecular hydrogen bond formation with alcohol groups (donors) of A and TA. Hydrogen bond is an electrostatic dipole-dipole interaction varying in strength and length, as well as in bond angle which mostly depends on the nature of the hydrogen bond donor. As the orientation of alcohol groups in A is *cis* and in TA *trans*, it was expected that the formed hydrogen bonds will differ in length and angle resulting in improvement of A and TA separation performance in the presence of AOT.



sodium salt bis(2-ethylhexyl)sulfosuccinate (AOT)

Figure 2. Chemical structure of AOT (sodium bis(2-ethylhexyl)sulfosuccinate)
Slika 2. Hemijska struktura AOT-a (natrijum bis(2-etilheksil)sulfosukcinat)

The first step in this investigation was determination of AOT CMC in 20 mmol L^{-1} ammonium acetate applying the procedure published in the literature (17). Orientation of surfactant molecules at the air-water interface reduces surface tension and, therefore, the solvent drop size depends on the free surfactant monomers (17). An experimental study was performed by weighting 50 drops of solutions containing AOT (0.1 mmol L^{-1} to 2.0 mmol L^{-1}) in 20 mmol L^{-1} ammonium acetate, in order to monitor changes in the surface tension that could provide information about micelle formation, i.e. reaching and exceeding CMC. The determined CMC value was 0.4 mmol L^{-1} (Figure 3a).

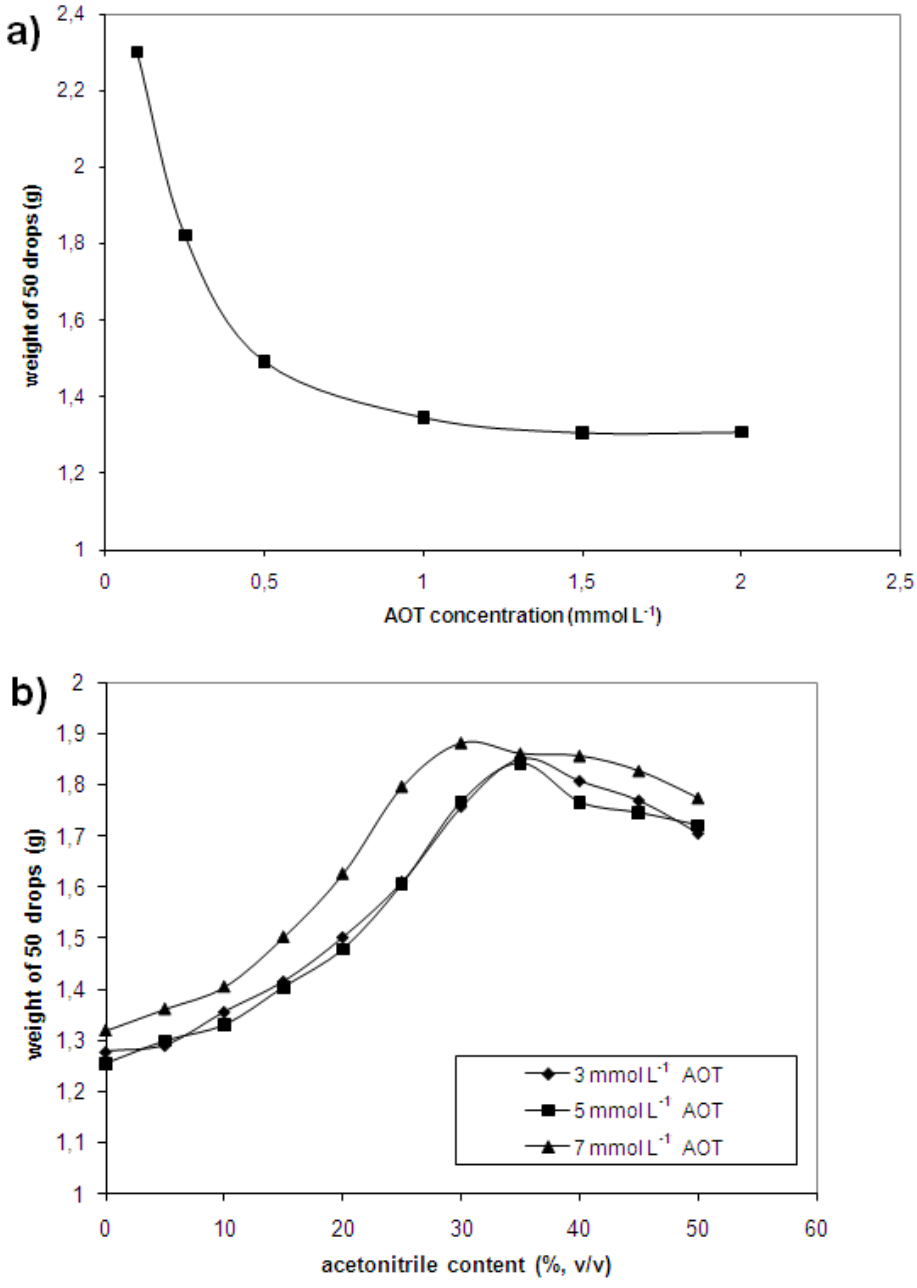


Figure 3. a) Determination of CMC for AOT in 20 mmol L^{-1} ammonium acetate
b) Weight of 50 drops of solutions containing AOT and acetonitrile
Slika 3. a) Određivanje CMC za AOT u 20 mmol L^{-1} amonijum-acetatu
b) Masa 50 kapi rastvora koji sadrži AOT i acetonitril

Organic modifier selection

As organic modifier acetonitrile was added so the influence of organic modifier on formed micelles should be acquired. For that reason the authors applied the same procedure used for the determination of AOT CMC in order to monitor changes in the surface tension that could provide information about micelle existence and disaggregation. It was proved that AOT micelles are not influenced by acetonitrile content up to 35 %, while the content higher than that is probably affecting micellar integrity as the mild decrease of the plot weight of 50 drops = f (acetonitrile concentration) is observed. However, the likely breakdown of micelles is not observed up to 50 % (Figure 3b).

Definition of other mobile phase constituents

As it could be concluded from the preliminary study, in the interest of adjusting elution strength, some additional organic modifier must be used. Tetrahydrofuran (THF), dimethyl sulfoxide (DMSO) and ethylene glycol were selected. THF and DMSO are dipolar aprotic solvents like acetonirtile, but with notable difference in structure. THF has a cyclic structure and completely different steric effects could be expected. Presence of the oxygen atom in its structure may also result in hydrogen bond formation and improved A and TA separation. On the other hand, DMSO has so called ″soft″ oxygen atom and ″hard″ sulfur atom that may lead to different interaction with A and TA and result in acceptable separation. As previously mentioned, the different orientation of alcohol groups in A and TA structure will definitely result in different interaction with ethylene glycol *via* hydrogen bonding with the two alcohol groups. Comparing the influence of 2 % THF, DMSO and ethylene glycol in the mobile phase containing 35 % acetonitrile and 63 % 5 mmol L^{-1} AOT in 20 mmol L^{-1} ammonium acetate (pH of the water phase was adjusted at 5.50 with acetic acid), the most significant and acceptable improvement of $1-v/p$ value could be noticed with ethylene glycol. When the content of ethylene glycol was reduced to 1 % or increased to 4 %, no substantial deterioration or improvement were observed. For that reason 2 % of ethylene glycol in the mobile phase was kept at the constant level during the method optimization.

Finally the influence of water phase pH value was analyzed. During the preliminary experiments the authors concluded that in the optimization phase the pH of the mobile phase must be investigated in the range from 5.0 to 6.0 because from 4.0 to 5.0 the satisfactory separation of A and TA could not be reached (all the obtained values of $1-v/p$ were less than 0.2).

Method optimization

Independent and dependent variables selection

The first step in the method optimization was definition of the factors which were important for certain separation (independent variables). During the method development stage as the influential factors acetonitrile content and AOT concentration in 20 mmol L^{-1} ammonium acetate (constituents of MLC mobile phase), as well as the pH of the water phase, mobile phase flow rate and column temperature were extracted. Other factors like ammonium acetate concentration (20 mmol L^{-1}) and ethylene glycol content in the mobile phase (2 %) were kept constant. As dependent variables five responses were included in the investigation (retention factor of DFA − k_{DFA}, retention factor of TA − k_{TA}, retention factor of A − k_A, resolution between DFA and TA − $R_{DFA/TA}$ and $1-v/p$ value for A and TA).

Experimental design selection

The next step was the selection of the appropriate experimental design that will define which experiments should be carried out in the experimental region of interest. For this study experimental design must assure that all studied variables are examined at least in three factor levels, and as the most useful inscribed-CCD with 2^5 half-fractional factorial design, ±0.5 star design and four replications in central point was chosen. The plan of experiments is presented in Table I.

Table I Plan of experiment for inscribed CCD (coded values for factor levels are in brackets)
Tabela I Plan eksperimenta za *inscribed* CCD (kodirane vrednosti za nivoe faktora date su u zagradama)

	Factors levels Nivoi faktora									
№	x_1		x_2		x_3		x_4		x_5	
1	32	(-1)	3	(-1)	5.00	(-1)	1.00	(-1)	20.0	(+1)
2	40	(+1)	3	(-1)	5.00	(-1)	1.00	(-1)	10.0	(-1)
3	32	(-1)	7	(+1)	5.00	(-1)	1.00	(-1)	10.0	(-1)
4	40	(+1)	7	(+1)	5.00	(-1)	1.00	(-1)	20.0	(+1)
5	32	(-1)	3	(-1)	6.00	(+1)	1.00	(-1)	10.0	(-1)
6	40	(+1)	3	(-1)	6.00	(+1)	1.00	(-1)	20.0	(+1)
7	32	(-1)	7	(+1)	6.00	(+1)	1.00	(-1)	20.0	(+1)
8	40	(+1)	7	(+1)	6.00	(+1)	1.00	(-1)	10.0	(-1)
9	32	(-1)	3	(-1)	5.00	(-1)	1.50	(+1)	10.0	(-1)
10	40	(+1)	3	(-1)	5.00	(-1)	1.50	(+1)	20.0	(+1)
11	32	(-1)	7	(+1)	5.00	(-1)	1.50	(+1)	20.0	(+1)
12	40	(+1)	7	(+1)	5.00	(-1)	1.50	(+1)	10.0	(-1)
13	32	(-1)	3	(-1)	6.00	(+1)	1.50	(+1)	20.0	(+1)
14	40	(+1)	3	(-1)	6.00	(+1)	1.50	(+1)	10.0	(-1)
15	32	(-1)	7	(+1)	6.00	(+1)	1.50	(+1)	10.0	(-1)
16	40	(+1)	7	(+1)	6.00	(+1)	1.50	(+1)	20.0	(+1)
17	34	(-0.5)	5	(0)	5.50	(0)	1.25	(0)	15.0	(0)
18	38	(+0.5)	5	(0)	5.50	(0)	1.25	(0)	15.0	(0)
19	36	(0)	4	(-0.5)	5.50	(0)	1.25	(0)	15.0	(0)
20	36	(0)	6	(+0.5)	5.50	(0)	1.25	(0)	15.0	(0)
21	36	(0)	5	(0)	5.25	(-0.5)	1.25	(0)	15.0	(0)
22	36	(0)	5	(0)	5.75	(+0.5)	1.25	(0)	15.0	(0)
23	36	(0)	5	(0)	5.50	(0)	1.13	(-0.5)	15.0	(0)
24	36	(0)	5	(0)	5.50	(0)	1.38	(+0.5)	15.0	(0)
25	36	(0)	5	(0)	5.50	(0)	1.25	(0)	12.0	(-0.5)
26	36	(0)	5	(0)	5.50	(0)	1.25	(0)	17.0	(+0.5)
27	36	(0)	5	(0)	5.50	(0)	1.25	(0)	15.0	(0)
28	36	(0)	5	(0)	5.50	(0)	1.25	(0)	15.0	(0)
29	36	(0)	5	(0)	5.50	(0)	1.25	(0)	15.0	(0)
30	36	(0)	5	(0)	5.50	(0)	1.25	(0)	15.0	(0)

x_1 – content of acetonitrile (%), x_2 – content of AOT (mmoL L^{-1}) in 20 mmoL L^{-1} ammonium acetate, x_3 – pH of the water phase; x_4 – flow rate; x_5 – column temperature
x_1 – sadržaj acetonitrila (%), x_2 – koncentracija AOT-a (mmoL L^{-1}) u 20 mmoL L^{-1} amonijum-acetatu, x_3 – pH vrednost vodene faze; x_4 – protok mobilne faze; x_5 – temperatura kolone

Optimization based on multi-criteria decision making approach

The experiments were conducted according to the defined plan and the resulting responses are presented in Table II. Design-Expert 7.0.0 was employed for the necessary calculations. For k_{DFA}, k_{TA}, k_A and $1-v/p$ quadratic response model was suggested as the most adequate and calculated coefficients of response model for coded factor levels are given in Table III. On the other hand, for $R_{DFA/TA}$, five-factor interaction response model was suggested as the most adequate. The coefficients of response model calculated for coded factor levels are given in Table IV.

Table II Experimentally obtained responses
Tabela II Eksperimentalno dobijeni odgovori

Exp. №	Responses				
	k_{DFA}	k_{TA}	k_A	$R_{DF/TA}$	$1-v/p$
1	22.64	26.18	26.57	2.81	0.54
2	8.39	9.20	9.62	1.32	0.07
3	17.64	20.50	21.43	2.80	0.43
4	6.37	7.02	7.36	1.18	0.16
5	8.02	9.46	9.96	1.78	0.53
6	5.19	5.74	6.13	1.24	0.24
7	6.08	7.01	7.41	1.65	0.21
8	2.61	2.85	3.08	0.62	0.33
9	27.80	32.70	34.08	3.40	0.13
10	7.10	7.90	8.27	0.76	0.24
11	18.69	21.43	22.31	2.58	0.28
12	6.04	6.68	7.03	1.02	0.15
13	12.67	14.47	15.32	1.35	0.35
14	3.19	3.54	3.74	0.91	0.14
15	10.03	11.43	12.20	1.39	0.35
16	2.14	2.36	2.50	0.51	0.09
17	9.60	10.88	11.61	1.92	0.53
18	5.81	6.44	6.88	1.25	0.59
19	6.32	7.10	7.60	1.74	0.51
20	6.39	7.16	7.67	1.45	0.56
21	8.97	10.09	10.71	1.59	0.34
22	7.09	8.08	8.64	1.94	0.42
23	10.09	11.53	12.37	1.95	0.60
24	6.70	7.57	8.09	1.51	0.53
25	8.37	9.44	10.16	1.76	0.56
26	5.55	6.25	6.67	1.44	0.73
27	5.73	6.45	6.93	1.57	0.61
28	6.07	6.73	7.28	1.69	0.55
29	5.86	6.53	7.00	1.69	0.55
30	6.09	6.75	7.28	1.56	0.53

k_{DFA} – retention factor for DFA; k_{TA} – retention factor for TA; k_A–retention factor for A;
$R_{DFA/TA}$ – resolution between DFA and TA; $1-v/p$ – measure of A and TA peak separation
k_{DFA} – retencioni faktor za DFA; k_{TA} – retencioni faktor za TA; k_A–retencioni faktor za A;
$R_{DFA/TA}$ – rezolucija između DFA i TA; $1-v/p$ – separacija između A i TA

Table III Coefficients of quadratic response model for k_{DF}, k_{TA}, k_A and $1-v/p$

$y = b_o + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_5x_5 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{14}x_1x_4 + b_{15}x_1x_5 + b_{23}x_2x_3 + b_{24}x_2x_4 + b_{25}x_2x_5 + b_{34}x_3x_4 + b_{35}x_3x_5 + b_{45}x_4x_5 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{44}x_4^2 + b_{55}x_5^2$

Tabela III Koeficijenti kvadratnog modela za odgovore: k_{DF}, k_{TA}, k_A i $1-v/p$

$y = b_o + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_5x_5 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{14}x_1x_4 + b_{15}x_1x_5 + b_{23}x_2x_3 + b_{24}x_2x_4 + b_{25}x_2x_5 + b_{34}x_3x_4 + b_{35}x_3x_5 + b_{45}x_4x_5 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{44}x_4^2 + b_{55}x_5^2$

	k_{DF}	p-value	k_{TA}	p-value	k_A	p-value	$1-v/p$	p-value
b_0	6.90	<0.0001*	7.76	<0.0001*	8.32	<0.0001*	0.55	0.0007*
b_1	-5.12	<0.0001*	-6.07	<0.0001*	-6.30	<0.0001*	-0.083	0.0010*
b_2	-1.54	0.0030*	-1.81	0.0029*	-1.84	0.0037*	-0.013	0.4712
b_3	-3.98	<0.0001*	-4.59	<0.0001*	-4.69	<0.0001*	0.017	0.3529
b_4	0.55	0.1868	0.64	0.1865	0.71	0.1672	-0.049	0.0190*
b_5	-0.26	0.5202	-0.35	0.4509	-0.42	0.3949	0.005	0.7798
b_{12}	0.75	0.0862	0.94	0.0699	0.92	0.0870	0.020	0.2849
b_{13}	2.20	0.0003*	2.63	0.0003*	2.67	0.0004*	0.0075	0.6799
b_{14}	-1.18	0.0140*	-1.33	0.0172*	-1.45	0.0147*	0.026	0.1699
b_{15}	0.25	0.5382	0.36	0.4499	0.43	0.3968	0.0063	0.7306
b_{23}	0.56	0.1827	0.67	0.1724	0.65	0.2078	-0.02	0.2849
b_{24}	-0.15	0.7178	-0.22	0.6412	-0.27	0.5842	0.016	0.3797
b_{25}	-0.20	0.6150	-0.19	0.6870	-0.19	0.7013	-0.064	0.0055*
b_{34}	0.096	0.8100	0.058	0.9011	0.029	0.9527	0.0013	0.9449
b_{35}	0.46	0.2702	0.55	0.2550	0.63	0.2251	-0.056	0.0109
b_{45}	-0.63	0.1396	-0.76	0.1300	-0.75	0.1527	0.025	0.1890
b_{11}	1.15	0.7740	1.21	0.7980	1.17	0.8147	0.069	0.7077
b_{22}	-4.25	0.3070	-4.93	0.3118	-5.28	0.3053	-0.031	0.8633
b_{33}	2.45	0.5457	2.91	0.5415	2.88	0.5658	-0.065	0.0051
b_{44}	3.91	0.3431	4.75	0.3253	5.10	0.3194	0.089	0.6291
b_{55}	0.11	0.9773	0.067	0.9807	0.011	0.9804	0.24	0.1829

* significant model terms at 95% confidence level

* interval pouzdanosti 95 %

Table IV Coefficients of second-order response model for $R_{DFA/TA}$
$y = b_o + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_5x_5 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{14}x_1x_4 + b_{15}x_1x_5 + b_{23}x_2x_3 + b_{24}x_2x_4 + b_{25}x_2x_5 + b_{34}x_3x_4 + b_{35}x_3x_5 + b_{45}x_4x_5$

Tabela IV Koeficijenti modela drugog reda za odgovor $R_{DFA/TA}$
$y = b_o + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_5x_5 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{14}x_1x_4 + b_{15}x_1x_5 + b_{23}x_2x_3 + b_{24}x_2x_4 + b_{25}x_2x_5 + b_{34}x_3x_4 + b_{35}x_3x_5 + b_{45}x_4x_5$

	$R_{DFA/TA}$	p-value
b_0	1.610	<0.0001*
b_1	-0.640	<0.0001*
b_2	-0.120	0.0211*
b_3	-0.380	<0.0001*
b_4	-0.100	0.0413*
b_5	-0.080	0.1037
b_{12}	0.001	0.9790
b_{13}	0.280	<0.0001*
b_{14}	-0.052	0.2784
b_{15}	0.050	0.3010
b_{23}	-0.025	0.5997
b_{24}	-0.001	0.9790
b_{25}	0.084	0.0936
b_{34}	-0.049	0.3128
b_{35}	0.079	0.1129
b_{45}	-0.120	0.0243*

* significant model terms at 95% confidence level
* interval pouzdanosti 95 %

In the next stage, in order to discover global optimal conditions several dependent properties were simultaneously optimized using MCDM based approach. For that purpose, Derringer's desirability function (19) was selected to optimize five responses with different targets. Derringer and Suich (19) proposed three types of desirability functions depending on whether a particular response is to be maximized, minimized or assigned a target value. In order to emphasize certain goal assigned to the response, the weights (exponents) of adopted function have to be selected (15). It is important to know that weights lower than 1 give less emphasis to the goal, while weights greater than 1 give more emphasis to the goal. The restrictions adopted for the determination of the most appropriate global desirability in this investigation are graphically presented in Figure 4.

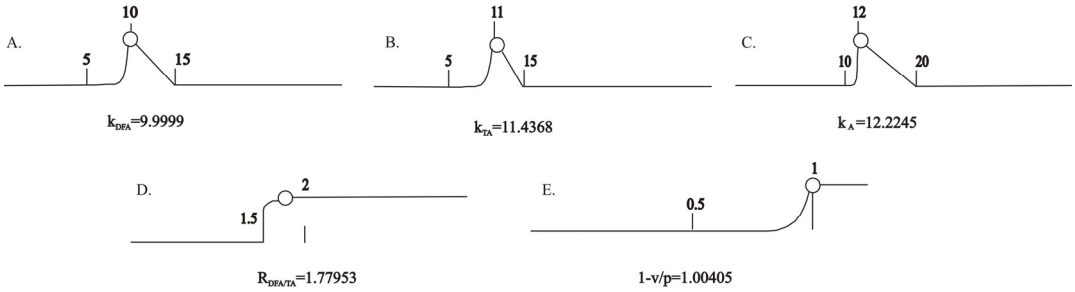


Figure 4 Graphical representation of the constraints adopted for the determination of global desirability (A – k_{DFA}; B – k_{TA}; C – k_A; D – $R_{DFA/TA}$ and E – $1-v/p$)

Slika 4 Grafički prikaz uslova definisanih za određivanje globalne *desirability* funkcije (A – k_{DFA}; B – k_{TA}; C – k_A; D – $R_{DFA/TA}$ i E – $1-v/p$)

It can be seen from Figure 4A, 4B and 4C that k_{DFA}, k_{TA} and k_A responses were set as *target* best kind and selected values for weights were 10 and 1, respectively. For $R_{DFA/TA}$ (Figure 4D) and $1-v/p$ (Figure 4E) the responses constraint was defined as *maximization*. In the former case weight was adjusted to 0.1 as any response value above 1.5 can be considered as acceptable. However, for $1-v/p$ s was set to 10, as the most desirable response would have value 1. In this way the significance of this response in the definition of global optimum was emphasized.

According to defined constraints the predicted optimal mobile phase composition was: 32 % acetonitrile, 2 % ethylene glycol, 66 % 6.4 mmol L^{-1} AOT in 20 mmol L^{-1} ammonium acetate, pH of the water phase 5.50 adjusted with acetic acid, flow rate 1.15 mL min^{-1} and column temperature of 10 °C and the corresponding global desirability 0.980.

Finally, the predicted optimal mobile phase was applied for the separation of investigated substances in order to check the concordance between predicted and experimentally obtained response values. The obtained experimental data for k_{DFA}, k_{TA}, k_A, $R_{DFA/TA}$ and $1-v/p$ were in a good agreement with predicted ones presented in Figure 4. Representative chromatogram obtained after injection of a mixture, in which the impurities TA and DFA had the maximum allowed concentrations in formulations, under optimal conditions can be found in Malenović *et al*. (10).

Conclusion

In this paper a novel, simpler and more efficient MLC method for the separation of A, TA and DFA was proposed. Application of MCDM approach improved method optimization step by the desirability estimation of several interrelated responses. Global optimal chromatographic conditions were defined using Derringer's desirability function. First of all five investigated responses were converted to desirability functions,

which were then modeled in the experimental domain with a good fit. Then, the global desirability was defined according to adopted restrictions and the optimal chromatographic conditions settled. Also, 3D-graphs of global desirability were constructed and their investigation enabled the evaluation of system behavior. It could be seen that all the parameters of the proposed method must be strictly controlled in order to maintain method performance, which could have been expected, due to the structural resemblance between A and TA that results in similar chromatographic behavior and problematic separation. Finally, it can be concluded that the proposed desirability-based MCDM approach proved to be valuable tool in the optimization of chromatographic methods, especially novel ones like MLC.

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Multikriterijumski pristup optimizaciji metode micelarne tečne hromatografije za analizu atorvastatina i njegovih nečistoća

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Kratak sadržaj

U ovom radu, za optimizaciju metode micelarne tečne hromatografije (MLC) namenjene za farmaceutsku analizu atorvastatina i njegovih nečistoća, *trans*-atorvastatina i desfluoro-atorvastatina primenjen je multikriterijumski pristup. MLC je oblik reverzno-fazne tečne hromatografije gde promene u mobilnoj fazi dovode i do modifikacije stacionarne faze. Ovo rezultuje različitim interakcijama (hidrofobnim, jonskim, sternim) sa značajnim uticajem na retenciju i selektivnost. Zbog toga je za separaciju odabran surfaktant koji sadrži dvostruki lanac i atom kiseonika, natrijum dioktil sulfosukcinat – AOT (Aerosol OT). Kao najefikasniji način za istraživanje velikog broja faktora i istovremenu optimizaciju suprotstavljenih ciljeva (minimizacija vremena trajanja razdvajanja i maksimizacija rezolucije između atorvastatina i *trans*-atorvastatina) primenjena je multikriterijumska optimizacija. Centralni kompozicioni dizajn (CCD) sa frakcionim faktorskim dizajnom, ± 0.5 zvezda dizajnom i četiri replikacije u centralnoj tački korišćen je za definisanje plana eksperimenta. Korišćenjem *Derringer* funkcije poželjnih odgovora, optimizirano je pet odgovora. Predviđeni optimalni uslovi bili su: 32 % acetonitrila, 2 % etilen-glikola, 66 % 6,4 mmol L^{-1} AOT u 20 mmol L^{-1} amonijum-acetatu, pH vodene faze 5,50 podešen sirćetnom kiselinom, protok 1,15 mL min^{-1} uz temperaturu kolone 10 °C.

Ključne reči: Multikriterijumska optimizacija, *Derringer* funkcija poželjnih odgovora, Micelarna tečna hromatografija, Atorvastatin i njegove nečistoće