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Anja Tumpa

Department of Drug Analysis, University of Belgrade – Faculty of Pharmacy, Belgrade, Serbia

Tamara Miladinović

Pharmanova, Belgrade, Serbia

Tijana Rakić*

Department of Drug Analysis, University of Belgrade – Faculty of Pharmacy, Belgrade, Serbia

Ana Stajić

Department of Drug Analysis, University of Belgrade – Faculty of Pharmacy, Belgrade, Serbia

Biljana Jančić-Stojanović

Department of Drug Analysis, University of Belgrade – Faculty of Pharmacy, Belgrade, Serbia

*Address correspondence to Tijana Rakić. E-mail: tijana.rakic@pharmacy.bg.ac.rs

Abstract

A liquid chromatography method is reported for the determination of diclofenac potassium and its impurities using Quality by Design criteria. Central composite design was used for the investigation of the influence of critical parameters on performance that included the methanol concentration in the mobile phase, the pH of the aqueous phase, and the potassium dihydrogen phosphate concentration in the aqueous phase. Mathematical models enabled theoretical examination of experimental space to achieve maximal separation in minimal analysis time. A Monte Carlo simulation was used to evaluate the risk of uncertainty in model predictions, to adjusting process parameters, and to identify design space. Fractional factorial design was employed for robustness testing and method was fully validated. Optimal conditions were a C18 150 mm × 4.6 mm, 5 μm particle size column; a methanol – 68.3 mmol L^{-1} potassium dihydrogenphosphate (68.7:31.3, v/v) mobile phase at pH 3.0, a flow rate of 1 mL min^{-1}, a column temperature of 25°C, and ultraviolet detection at 254 nm.

Keywords: design space, diclofenac potassium, impurities, liquid chromatography, Monte Carlo simulation, quality by design

INTRODUCTION

Nowadays pharmaceutical method development can hardly be imagined without the application of Quality by Design concept. Beginning with Food and Drug Administration guidance (Food and Drug Administration's Good Manufacturing Practice for 21st Century), a new era in product development in pharmaceutical industry began. This guidance, followed by International Conference on Harmonization Q8, gave a new dimension to product development and consequently, caused many changes in product development and control. A literature survey has shown that there are many papers dealing with Quality by Design approach in method

development (Lebrun et al. 2008; Molnár, Rieger, and Monks 2010; Debrus et al. 2011; Monks et al. 2012; Cela et al. 2013; Furlanetto et al. 2013; Kormány, Molnár, and Rieger 2013; Mbinze et al. 2013; Orlandini, Pinzauti, and Furulanetto 2013; Rozet et al. 2013; Schmidt and Molnár 2013; Hubert et al. 2014). However, despite the availability of previous studies, development of a new method demands separate attention because each mixture possesses special characteristics and requires an adjustment of investigation strategy.

Here the development and validation of a liquid chromatography (LC) method for determination of diclofenac potassium and its four impurities is presented. Structures of the analytes are presented in **Figure 1**. The impurities include N-chloroacetyl-N-phenyl-2, 6-dichloroanilin, 1-(2, 6-dichlophenyl)-2-indolinon, N-phenyl-2,6-dichloranilin, and 2-indolinon (1-H-indol-2-ol).

Many reports have described LC methods for the determination of diclofenac sodium or diclofenac potassium and other drugs in pharmaceutical formulations (Ye and Zhang 2000; Krzek and Starek 2002; Bisas and Basu 2010; Elkady 2010; Gowramma et al. 2010; Khatal et al. 2010; Rele et al. 2011; Ambekar, Choudhari, and Ingale 2012; Chaple et al. 2012; Panda, Patanaik, and Ravi Kumar 2012; Rubim et al. 2013; Shaalan and Belal 2013; Belal et al. 2014; Rubim et al. 2014). Here these impurities were investigated together for the first time. Previous papers have considered 1-(2, 6-dichlophenyl)-2-indolinon and 2-indolinon (Krzek and Starek 2002), while others examined N-chloroacetyl-N-phenyl-2, 6-dichloroanilin and N-phenyl-2, 6-dichloranilin (Shaalan and Belal 2013; Belal et al. 2014). The Quality by Design approach with Monte Carlo simulations allows development of better methods than those without the implementation of these approaches. In addition, high-performance thin layer chromatography was used for the determination of diclofenac potassium (Ali et al. 2012; Mohite, Potawale, and

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Gabhe 2013). Also, methods were reported for diclofenac sodium and diclofenac potassium determination in biological samples (Lee et al. 2000; Su et al. 2006; BhanuPraksah, VijayaSri, and Rama Krishna 2009; Sarfraz, Sarfraz, and Ahmad 2011; Prasad et al. 2012; Sahoo et al. 2015). A method for diclofenac potassium is available in the European Pharmacopoeia Seventh Edition using isocratic conditions with an analysis time of 40 minutes for the determination of diclofenac potassium and its impurities. N-Chloroacetyl-N-phenyl-2,6 dichloroanilin and N-phenyl-2,6-dichloranilin are not presented in the Seventh Edition European Pharmacopoeia, although 1-(2, 6-dichlophenyl)-2-indolinon and 2-indolinon(1-H-indol-2-ol) are included. The goal of this study was to develop a liquid chromatography method for the determination of diclofenac potassium and these four impurities using Quality by Design principles. This is the first report involving N-chloroacetyl-N-phenyl-2, 6 dichloroanilin and N-phenyl-2, 6-dichloranilin with diclofenac potassium and two other impurities. This approach allowed the development of reliable and reproductive chromatographic conditions.

EXPERIMENTAL

Chemicals and Reagents

The analytes diclofenac potassium and the impurities (Dipharma Francis s.r.l., Italy) were of analytical grade. The mobile phase and the solvents were prepared from methanol (J. T. Baker, Neatherlands), potassium dihydrogen phosphate (Sigma; Aldrich, Germany), *o* - phosphoric acid (Carlo Erba, Italy), and liquid chromatography grade water. Diclofenac potassium tablets were obtained from the local markets.

Chromatographic Conditions

The chromatographic system Waters Breeze included a Waters 1525 Binary HPLC Pump, Waters 2487 ultraviolet visible spectroscopy dual absorbance detector, and Breeze Software. Separations were performed on the Zorbax Extend XDB−C18 150 mm × 4.6 mm, 5 μm particle size column (Agilent Technologies, St. Clara, USA). The samples were introduced through a Rheodyne injector valve with a 20 μL sample loop. The flow rate of the mobile phase was 1 mL min^{-1} at 25°C with ultraviolet detection at 254 nm. A mixture of methanol−water (70:30 v/v) was employed as the solvent.

Mobile Phase

The mobile phase consisted of methanol with aqueous potassium dihydrogsen phosphate. The mobile phase pH was adjusted with phosphoric acid. The amount of organic solvent, the concentration of potassium dihydrogen phosphate in the aqueous phase, and the pH of the mobile phase were varied according the experimental design procedure. The mobile phase for the optimal chromatographic conditions included methanol and 68.3 mmol L^{-1} potassium dihydrogenphosphate in water at 68.7:31.3 (v/v) at pH 3.0.

Optimization and Robustness

The stock solution was prepared by dissolving diclofenac potassium and the impurities in methanol. The concentration of diclofenac potassium was 1 mg mL^{-1} and of impurities were 200 μg mL^{-1}. In order to obtain working standards, the stock solutions were diluted to 100 μg mL^{-1} for diclofenac potassium and 10 μg mL^{-1} for the impurities with solvent.

Selectivity

In order to evaluate the selectivity of the LC method, a placebo mixture was prepared in a ratio corresponding to the concentration in the tablets. A standard solution, containing 100 μg mL^{-1} of diclofenac potassium and 2 μg mL^{-1} of each impurity, was utilized to characterize the selectivity.

Stock Solutions

Stock solutions were prepared by dissolving diclofenac potassium and its impurities in methanol to obtain 1 mg mL^{-1} for diclofenac potassium and 100 μg mL^{-1} for the impurities.

Evaluation of the Linear Dynamic Range

For the construction of the calibration curve, eight solutions containing diclofenac potassium from 25 μg mL^{-1} to 250 μg mL^{-1} were prepared. For impurities, the linearity was evaluated using seven solutions across the following concentrations: N-chloroacetyl-N-phenyl-2, 6-dichloroanilin from 0.5 μg mL^{-1} to 15 μg mL^{-1}, 1-(2, 6-dichlophenyl)-2-indolinon from 0.15 μg mL^{-1} to 10 μg mL^{-1}, for N-phenyl-2, 6-dichloranilin from 0.5 μg mL^{-1} to 6 μg mL^{-1}, and for 2-indolinon (1-H-indol-2-ol) from 0.15 μg mL^{-1} to 5 μg mL^{-1}.

Evaluation of Accuracy

A solution containing placebo, diclofenac potassium, and its impurities were prepared in methanol and sonicated for 10 minutes. This solution was then used for the preparation of solutions at 80%, 100% and 120% levels.

Evaluation of Precision

The precision was determined using diclofenac potassium pharmaceutical products containing 50 mg of active substance per tablet. The concentrations of impurities were below the limit of detection, so the sample was fortified. A tablet mass containing 25 mg of diclofenac potassium was fortified with impurities; solvent was added and the solution was sonicated for 15 minutes. The solution was filtered to obtain 1 mg mL^{-1} and 20 μg mL^{-1} for diclofenac potassium and the impurities, respectively. This solution was diluted to provide 200 μg mL^{-1} and 4 μg mL^{-1} for diclofenac potassium and impurities. This procedure was repeated six times.

Analysis of Tablets

A pulverised tablet mass containing 25 mg of diclofenac potassium was extracted with 25 mL solvent in an ultrasonic bath during 15 min and filtered. This stock solution was used to prepare six solutions containing 200 μg mL^{-1} of diclofenac potassium.

Software

The experiments for optimization and robustness testing, as well method optimization and Design Space definitions were performed in Modde 10.1 (Umetrics, Umea, Sweden).

RESULTS AND DISCUSSION

Quality by Design is well-defined in International Conference on Harmonization Q8 and is largely complete in the International Conferences on Harmonization Q9 and Q10. General characteristics of this approach mean that risk management, scientific knowledge, and improved process understanding lead to more efficient and robust response in defined design space. Hence, an analytical method was developed that fulfills the criteria defined with this approach. Regardless of whether drug is generic or newly synthesized, this approach should be

incorporated in method development. Here an LC method was developed for diclofenac potassium and its impurities in line with Quality by Design principles.

Analytical Target Profile and Critical Quality Attributes

Quality by Design method development starts with definition of analytical target profile which is a set of criteria that defines what is measured and the required performance criteria of the method denoted as critical quality attributes (Orlandini, Pinzauti, and Furulanetto 2013; Rozet et al. 2013). In order to define critical quality attributes, preliminary studies were conducted. Taking into account the nature of investigated substances, a C_{18} column was chosen as stationary phase. Some chromatographic factors were set at defined levels at the beginning of the study, while other factors such as the organic modifier, aqueous phase composition, and pH of the mobile phase were varied. This study has shown that the elution order, 2-indolinon (1-H-indol-2-ol) before 1-(2, 6-dichlophenyl)-2-indolinon before N-chloroacetyl-N-phenyl-2, 6-dichloroanilin before diclofenac potassium before N-phenyl-2, 6-dichloranilin, was not affected by these changes. Also, methanol concentrations less than 60% caused long run times while values higher than 70% influenced the quality of the separation. Consequently, the methanol concentration was varied from 60% to 70%. Buffer concentrations from 25 mM to 75 mM were employed to evaluate the influence on the separation because small variations did not significantly affect the separation. Considering properties of investigated substances, pH values from 2.5 to 3.5 were selected for further investigation.

The main objective of the study was the development of a method for the determination of diclofenac potassium and four impurities with maximal separation and minimal analysis time. Therefore, the selectivity factor of the critical peak pair, diclofenac potassium and N-phenyl-2, 6-

dichloranilin, was defined as a critical quality attribute and the goal was to maximize its value. The analysis time was characterized as the retention factor of the last eluting peak as the retention factor for N-phenyl-2, 6-dichloranilin. Hence this parameter was the second critical quality attribute and the goal was to minimize this response. Finally, apart from achieving satisfactory values of defined critical quality attributes, the optimal point should be surrounded with sufficient design space. In this case, the methanol concentration in the mobile phase, the concentration of buffer in mobile phase, and the pH of the mobile phase were defined as critical process parameters and were further investigated.

Knowledge Space and Critical Quality Attribute Modeling

Critical quality attributes modeling is one of the key steps in Quality by Design development and definition of design space. The establishment of mathematical relationships between process parameters and important responses allows simulation of chromatographic processes and many theoretical experiments without the necessity for real experiments. During preliminary experiments, factors and their levels were defined. For deep understanding and investigating the influence of those selected factors on important responses, face centered central composite design was selected (Massart et al. 1997). The experimental plan defined by the applied design is presented in **Table 1**.

Experiments were performed randomly and the retention factor for N-phenyl-2, 6-dichloranilin and the selectivity factor for diclofenac potassium and N-phenyl-2,6-dichloranilin were monitored as critical quality attributes. Multiple linear regression and least squares methods were applied for creation of mathematical models. The best models for responses were investigated. The values of coefficient of determination, adjusted coefficient of determination,

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and predicted coefficient of determination were applied as indicators of model quality. Optimization of the values was done by removal of insignificant model terms. High values of coefficient of determination (higher than 0.776) and adjusted coefficient of determination (higher than 0.776) suggested that the models adequately fitted the investigated responses. In order to visualize the dependence of selected optimization, critical quality attributes on contour plots were constructed and presented in **Figure 2**.

Design Space and Working Point

Design space is the most important component of Quality by Design concept and its definition is a key part of method development. Design space provides definition of edges of failure, outside of which the method performances are not acceptable (Orlandini, Pinzauti, and Furulanetto 2013). These edges are defined graphically or by appropriate mathematical equations and ensure that the theoretical robustness is acquired within marked region. The method operating conditions are not defined as discrete points, but as a working space which decreases the chances for method failure (Mbinze et al. 2012).

The threshold of acceptable values for the selectivity factor of diclofenac potassium and N-phenyl-2, 6-dichloranilin was 1.12; for the retention factor of N-phenyl-2, 6-dichloranilin, a value of 10 was selected. Sweet spot regions were constructed by overlay of contour plots for analyzed responses and are presented in **Figure 3**. The green area shows where both critical quality attributes defined criteria are met, while blue region shows where only one of the criteria is met. The large green area provides the surface where the changes of critical process parameters do not give variation in critical quality attributes. However, this region does consider possible variation in precision of the investigated critical process parameters and model error.

Therefore, design space was created applying Monte Carlo simulations and mathematical models. This approach allows calculation of the risk of obtaining undesired results for each point in knowledge space.

Once the design space was established, each point within it may be a possible working point. It can be selected as point with highest separation quality, or on the basis of practical considerations. Also, the optimal point may be selected with the highest probability that critical quality attributes are within specifications, i.e., as the most robust point. Here, the most robust set point was identified. Therefore, the robust optimization was performed and the working point was selected as the one surrounded with the greatest design space. The optimum was at a methanol concentration of 68.7% in the mobile phase, a pH value 3.0, and potassium dihydrogen phosphate at 68.3 mmol L^{-1}. The resulting design space is presented in **Figure 4**.

The obtained borders of design space present the region where the changes of critical process parameters do not disturb the quality of the method with a probability of 90%. The identified critical process parameters ranges were: 65.3% to 72.0% for methanol concentration in the mobile phase, 29.83 to 106.83 mmol L^{-1} potassium dihydrogen phosphate in the aqueous phase, and pH from 2.5 to 3.5. However, the upper limits for potassium dihydrogen phosphate concentration were outside the investigated knowledge space. Therefore, they were corrected to the upper border of the knowledge space: 75 mmol L^{-1} for potassium dihydrogen phosphate. The optimal conditions were verified experimentally as shown in **Figure 5**.

Robustness

Although design space presents the region of theoretical robustness, robustness testing is required for method validation. Therefore, design of experiments methodology was used to

explore robustness close to the optimal point. The factors monitored in this phase were the three critical process parameters identified in optimization phase and two additional factors: column temperature and mobile phase flow rate. The ranges of factor variation were selected to be symmetrical around the nominal value. For evaluation of these five factors on robustness, fractional factorial design was chosen using 1/4 fraction of full factorial design. The experimental plan was set according to fractional factorial design 2^{5-3} and is presented in **Table 2**. In addition to these experiments, three measurements were performed at the central point. The same responses as in the optimization phase were followed. The experiments are performed randomly and the results are presented in **Table 2**.

The results were evaluated and coefficients for the primary effects are shown in **Table 3**. Statistical evaluation of results obtained from robustness testing was done by the algorithm of Dong (Heyden et al. 2001). Obtained $E_{critical}$ values are presented in **Table 3**. Values for factors effects for both responses are above the calculated $E_{critical}$, showing the insignificance of all factors in robustness testing.

Validation

The next step was to perform method validation. Under the optimal conditions, a placebo mixture, standards, and a solution for precision estimation were analyzed. There were no interferences, confirming the selectivity of the method. The linear dynamic range, accuracy, precision, and limits of detection (LOD) and quantification (LOQ) for the analytes were determined and obtained results are shown in **Table 4**. The results for all measurements were within the required values (Crowther 2001).

The method was employed for the analysis of tablets containing 50 mg of diclofenac potassium. The impurities were below the limit of quantification and the determined concentration of diclofenac potassium was 99.8% (49.9 mg per tablet).

CONCLUSIONS

A liquid chromatography method is reported for diclofenac potassium and four impurities in accordance with Quality by Design. The importance of this strategy in modern pharmaceutical analysis is emphasized and each step of the process is described in detail. Special attention was devoted to design of experiments for creation of reliable mathematical models. The study provided optimal chromatographic conditions for diclofenac potassium and its impurities in a well-defined, robust region. The method was validated and used for the analysis of real samples to verify the applicability of the method.

Acknowledgment

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Table 1. Face centered central composite design, retention factor for N-phenyl-2, 6-dichloranilin, and selectivity factor for diclofenac potassium and N-phenyl-2, 6-dichloranilin

Experiment	Factor			Response	
	A	B	C	Retention	Selectivity factor for
	Methanol concentration (%)	Mobile phase pH	Buffer concentration (mM)	factor for N-phenyl-2, 6-dichloranilin	diclofenac potassium and N-phenyl-2, 6-dichloranilin
1	60 (−1)*	2.5 (−1)	25 (−1)	17.21	1.1
2	70 (+1)	2.5 (−1)	25 (−1)	6.71	1.12
3	60 (−1)	3.5 (+1)	25 (−1)	19.56	1.1
4	70 (+1)	3.5 (+1)	25 (−1)	5.99	1.12
5	60 (−1)	2.5 (−1)	75 (+1)	23.37	1.07
6	70 (+1)	2.5 (−1)	75 (+1)	6.96	1.16
7	60 (−1)	3.5 (+1)	75 (+1)	18.41	1.11
8	70 (+1)	3.5	75 (+1)	6.29	1.17

		(+1)			
9	60 (−1)	3.0 (0)	50 (0)	23.22	1.07
10	70 (+1)	3.0 (0)	50 (0)	7.03	1.17
11	65 (0)	2.5 (−1)	50 (0)	12.5	1.14
12	65 (0)	3.5 (+1)	50 (0)	10.4	1.14
13	65 (0)	3.0 (0)	25 (−1)	11.92	1.12
14	65 (0)	3.0 (0)	75 (+1)	11.79	1.13
15	65 (0)	3.0 (0)	50 (0)	11.95	1.14
16	65 (0)	3.0 (0)	50 (0)	11.82	1.13
17	65 (0)	3.0 (0)	50 (0)	10.94	1.12

*Coded values are in parentheses.

Table 2. Robustness characterized by fractional factorial design 2^{5-2}, retention factor for N-phenyl-2, 6-dichloranilin, and selectivity factor for diclofenac potassium and N-phenyl-2, 6-dichloranilin

Experiment	Methanol concentration (%)	Buffer concentration (mM)	Mobile phase pH	Column temperature (°C)	Flow rate (mL min^{-1})	Retention factor for N-phenyl-2, 6-dichloranilin	Selectivity factor for diclofenac potassium and N-phenyl-2, 6-dichloranilin
1	66.7	63.3	2.8	27	1.1	5.886	1.177
2	70.7	63.3	2.8	23	0,9	7.565	1.18
3	66.7	73.3	2.8	27	1.1	6.517	1.146
4	70.7	73.3	2.8	23	0.9	6.429	1.179
5	66.7	63.3	3.2	27	1.1	9.728	1.161
6	70.7	63.3	3.2	23	0.9	6.104	1.142
7	66.7	73.3	3.2	27	1.1	11.672	1.17
8	70.7	73.3	3.2	23	0.9	5.23	1.186
9	68.7	68.3	3.0	25	1.0	7.272	1.151
10	68.7	68.3	3.0	25	1.0	6.997	1.155
11	68.7	68.3	3.0	25	1.0	7.022	1.157

Table 3. Coefficients from the mathematical models for robustness and their statistical

significance

Coefficient	Response	
	Retention factor for N-phenyl-2, 6-dichloranilin	Selectivity factor for diclofenac potassium and N-phenyl-2, 6-dichloranilin
Constant	3.9463	76.9865
Methanol content (%)	−0.57177	0.2728
Buffer concentration (mM)	0.03806	0.1736
pH of the mobile phase	0.4274	−0.1901
Column temperature (°C)	−0.3092	0.5374
Flow rate (mL min^{-1})	−0.7865	−0.3224
Ecritical calculated according to Heyden et al. (2001)	1.2727	0.7790

Table 4. Analytical figures of merit

Parameter		Diclofenac potassium	N-Chloroacetyl-N-phenyl-2, 6-dichloroanilin	1-(2, 6-Dichlophenyl)-2-indolinon	N-Phenyl-2, 6-dichloranilin	2-Indolinon (1-H-indol-2-ol)
	Linear dynamic range (µg mL^{-1})	25–250	0.5–15	0.15–10	0.5–6	0.15–5
	Slope	21.734	23.77	28.708	19.5755	72.29
	Intercept	47.9324	–0.3405	1.1594	4.3304	6.798
	Correlation coefficient	0.9997	0.9998	0.9991	0.9939	0.9991
	p-value	0.1375	0.813	0.6946	0.357	0.1302
	Limit of Quantification		0.5	0.15	0.5	0.13
	Limit of Detection		0.15	0.04	0.15	0.08
Accuracy as the	80%	98.9	98.8	101.3	103.9	101.2
	100%	99.7	96.7	103.7	100.1	98.9

Recovery (%)	120%	97.2	97.5	106.9	99.1	97.9
Precision	Relative Standard Deviation (%)	1.5	1.4	1.93	1.84	1.9

Figure 1. Analytes.

diclofenac potassium

N-chloroacetyl-N-phenyl-2.6-dichloroaniline
(impurity 1)

1-(2.6-dichlorophenyl)-2-indolinone
(impurity 2)

N-phenyl-2.6-dichloroaniline
(impurity 3)

2-indolinone
(impurity 4)
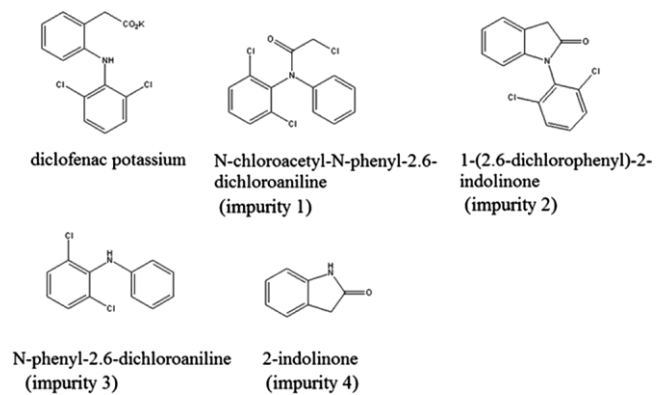
Figure 2. Contour plots for the (A) retention factor for N-phenyl-2, 6-dichloranilin obtained by plotting methanol concentration in the mobile phase as a f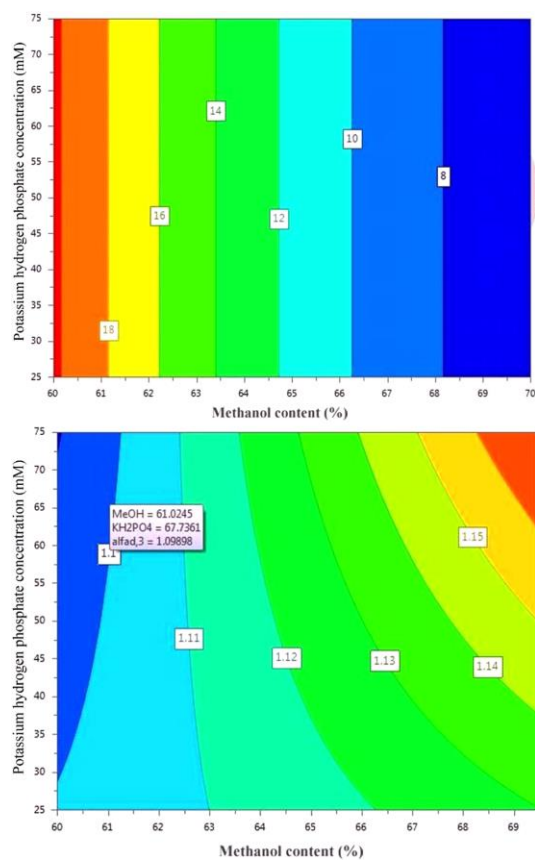unction potassium hydrogen phosphate concentration at pH 3.0 and (B) selectivity factor for diclofenac potassium and N-phenyl-2, 6-dichloranilin obtained by plotting methanol concentration in the mobile phase as a function of potassium hydrogen phosphate concentration at pH 3.0.

Figure 3. Sweet spot plots obtained by plotting methanol concentration in the mobile phase as a function of potassium hydrogen phosphate concentration in the aqueous phase at pH 3.0 defined by the selectivity factor for diclofenac potassium and N-phenyl-2, 6-dichloranilin >1.12 and the retention factor for N-phenyl-2, 6-dichloranilin <10. Regions where only one criterion was met are blue; regions where both criteria were met are green.
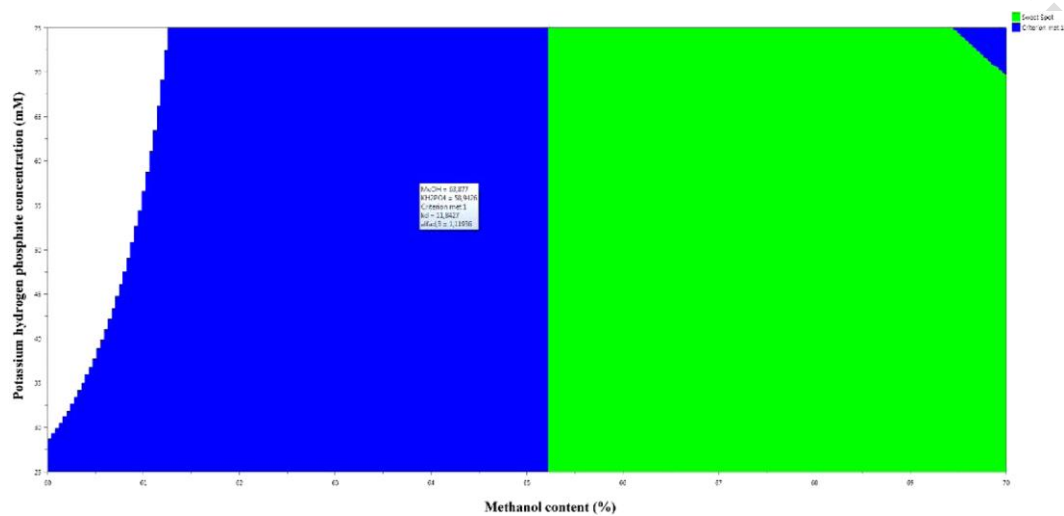
Figure 4. Design space for the separation of diclofenac potassium and its impurities for methanol and potassium hydrogen phosphate concentrations in the aqueous phase at pH 3.0.
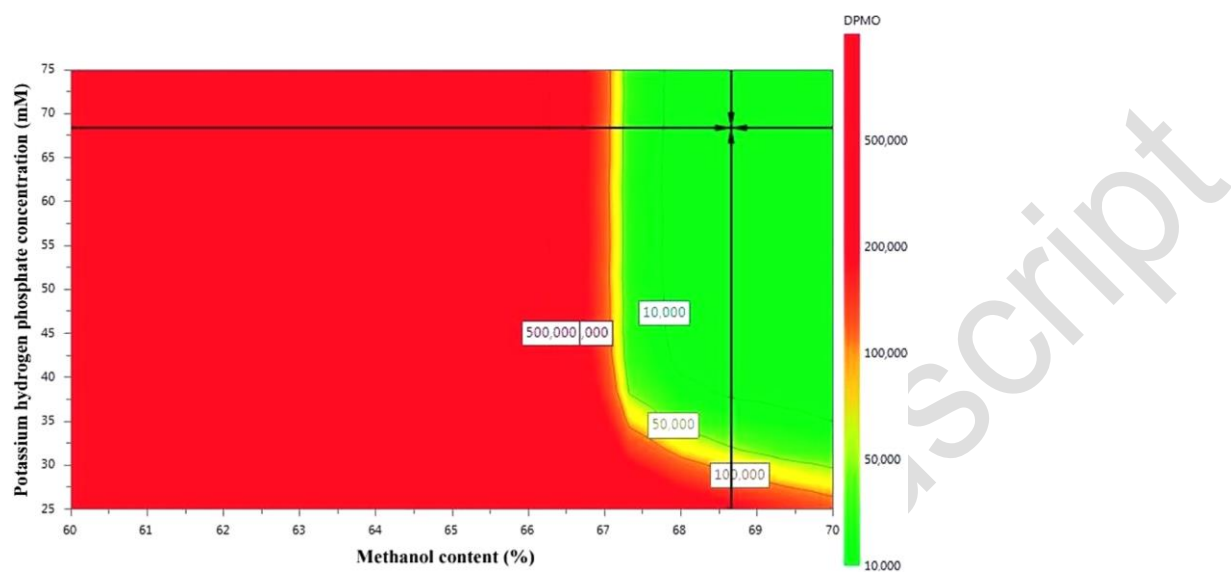
Figure 5. Chromatogram of diclofenac potassium and its impurities under the optimal conditions: (1) 2-indolinon (1-H-indol-2-ol), (2) 1-(2, 6-dichlophenyl)-2-indolinon, (3) N-chloroacetyl-N-phenyl-2, 6-dichloroanilin, (4) diclofenac potassium, and (5) N-phenyl-2, 6-dichloranilin.