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The influence of inorganic salts with chaotropic properties on the chromatographic behavior of ropinirole and its two impurities

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ABSTRACT

Chaotropic agents recently gained popularity as interesting and useful mobile phase additives in liquid chromatography due to their effect on analytes retention, peak symmetry and separation efficiency. They mimic the role of classical ion-pairing agents, but with less drawbacks, so their use becomes attractive in the field of pharmaceutical analysis. In this paper, the influence of sodium trifluoroacetate and sodium perchlorate on the chromatographic behavior of ropinirole and its impurities is examined. By the extended thermodynamic approach, it was shown that the separation in the given system was predominantly governed by electrostatic interactions between the protonated analytes and the charged surface of the stationary phase, but the ion-pair complex formation in the eluent also proved to be significant. Further, the employment of face-centered central composite design enabled the understanding of the effect of chaotropic agent concentration and its interactions with other factors (acetonitrile content and pH of the water phase) that influence the given chromatographic system. Finally, the same data was used for multi-objective optimization based on the grid point search method. After the method validation, the adequacy of the suggested approach in development of methods for routine pharmaceutical analysis was proven.

1. Introduction

The interest for simple inorganic chaotropic agents was renewed in recent years due to their ability to increase the retention of the oppositely charged analytes, also improving the peak symmetry and separation efficiency when used as mobile phase additives in liquid chromatography [1–8]. They mimic the role of classical ion-pairing agents, but with fewer drawbacks. Unlike classical ion-pairing agents that stick strongly to the stationary phase, chaotropic additives can be easily dissolved in mobile phase, so their impact on the initial column properties is reversible thus enabling longer column life.

These attractive properties of chaotropic agents can be of a great value in the pharmaceutical method of development strategies. However, the complexity of the chaotropic system requires special attention and detailed understanding of chaotropic agents' effects on chromatographic systems behavior, so in order to rationalize this approach it is necessary to understand the mechanism that underlies it. Therefore, the aim of this paper was to present detailed strategy for the examination of different chaotropic agents' influence and the thorough approach in selection of the proper chromatographic conditions on the example of mixture consisted of ropinirole (4-[2-(dipropylamino)ethyl]-1,3-dihydro-2H-indol-2-one) and its structurally related impurities A (4-[2-(propylamino)ethyl]-1,3-dihydro-2H-indol-2-one) and C (4-[2-(dipropylamino)ethyl]-1H-indol-2,3-dione) (Fig. 1). Ropinirole is a non-ergoline dopamine agonist specific for D2 and D3 dopamine receptors approved in the therapy of Parkinson's disease and restless leg syndrome. Several reversed-phase high-performance liquid chromatography (RP-HPLC) methods were developed for the analysis of ropinirole in bulk and dosage forms [9–11] and for the stability profiling [12,13]. The simultaneous separation and quantification of ropinirole and some impurities were achieved by capillary liquid chromatography [14] and by RP-HPLC using sodium alkylsulphonates as ion-pair reagents [15,16].

In this study, the examined agents with chaotropic properties were sodium trifluoroacetate (NaTFA) and sodium perchlorate (NaClO4). The description of the separation phenomena was achieved through the retention modeling from the theoretical and empirical aspects. The extended thermodynamic approach suggested by Cecchi et al. [17,18] enabled a comprehensive consideration of both the ion-pair complex formation and the double layer development. Further, the method development of the examined mixture was governed by the design of experiments methodology. This approach allowed the understanding of additional chromatographically relevant parameters as well as their interactions with chaotropic agents. Finally, the grid point search

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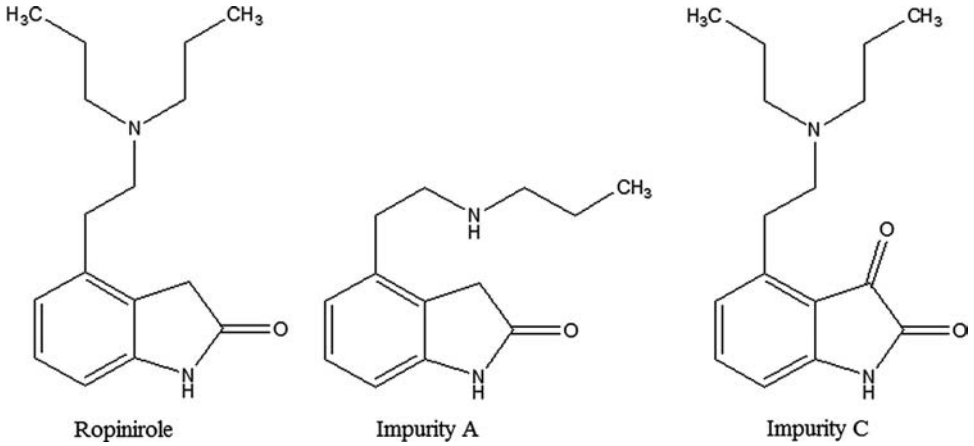

Fig. 1. Chemical structures of the analyzed substances.

method enabled the identification of the optimal chromatographic conditions and the method was fully validated.

2. Material and methods

2.1. Chemicals

All chemicals used were of the analytical grade. Acetonitrile (Fluka, Sigma-Aldrich, Steinheim, Germany), sodium perchlorate monohydrate (Fluka, Sigma-Aldrich, France), sodium trifluoroacetate (Aldrich, Sigma-Aldrich, USA), *ortho*-phosphoric acid (J.T. Baker, Deventer, Holland) and water (HPLC grade) filtered through Simplicity 185 (Millipore, Billerica, MA) were used for the preparation of the mobile phases. Standards of ropinirole and its impurities A and C were obtained from LGC GmbH, Luckenwalde, Germany. The dosage form used for method validation was Requip™ tablets (GlaxoSmithKline, UK).

2.2. Solutions

Stock solutions were prepared by dissolving the respective amounts of standard substances in the mixture acetonitrile–water (50:50, v/v) to obtain the concentrations of 500 μg/mL for ropinirole and 100 μg/mL for its impurities A and C.

Stock solutions were further diluted to obtain a mixture of 50 μg/mL for ropinirole and 1 μg/mL for the impurities. This mixture was used to obtain the chromatographic data for thermodynamic retention modeling and for the method optimization.

2.3. Solutions for the selectivity estimation

A mixture of the excipients–blank sample was prepared in the concentration ratio corresponding to the content in tablets. It was treated in the same manner as the tablet mass used for precision estimation. A standard solution mixture containing 200 μg/mL of ropinirole and 1 μg/mL of each impurity was used to prove the method selectivity.

2.4. Solutions for the linearity estimation

For the calibration curve, six solutions containing ropinirole (100–350 μg/mL) and seven solutions for the impurities A and C (0.1–1.2 μg/mL) were prepared from the corresponding standard solutions.

2.5. Solutions for the accuracy estimation

The laboratory mixtures containing blank sample and ropinirole, and blank sample and the impurities were prepared in the mixture of acetonitrile–water (50:50, v/v) and sonicated in the ultrasonic bath for 30 min. For the accuracy analysis of ropinirole, three series of three solutions in 80%, 100% and 120% concentration levels were prepared. For the analysis of the impurities accuracy, three series of three solutions in the limit of quantification (LOQ), 100% and 120% concentration levels were prepared.

2.6. Solutions for the precision estimation

For the precision estimation, a quantity of pulverized tablet mass corresponding to 25 mg of ropinirole was placed into a 50 mL volumetric flask and extracted with the mixture acetonitrile–water (50:50, v/v) using the ultrasonic bath for 30 min. The volumetric flask was filled to the mark with the same solvent, and the solution was filtered. From that stock solution, six solutions containing 200 μg/mL of ropinirole were prepared. Since the present impurities were below the limit of quantification, the precision was estimated from the replicates of laboratory mixture prepared for the accuracy testing.

2.7. Equipment

The experiments were performed on the chromatographic system Finnigan Surveyor Thermo Scientific consisted of HPLC Pump, Autosampler Plus and UV/VIS Plus Detector. ChromQuest was used for data collection and analysis. The volume of the injector sample loop was 25 μL, while the partial loop injection volume was 5 μL. Chromatographic separations were performed on XBridge® C18, 150 mm × 3 mm, 3.5 μm particle size column (Waters, Ireland). Column surface area was 99.1 m^2/column. Full details of the column properties are given in [19].

2.8. Chromatographic conditions

To collect the data necessary to build the thermodynamic model, the experiments were performed in isocratic mode. The mobile phase consisted of acetonitrile and aqueous phase (containing different amounts of NaTFA or NaClO4 and pH adjusted to 2.5 with *ortho*-phosphoric acid) 15:85 (v/v). Column temperature was set at 30 °C, while the flow rate was 0.7 mL/min. Detection was carried out at 250 nm.

For the determination of the adsorption isotherms of chaotropic agents, frontal chromatograms were performed using a gradient

delivery system with two pumps. Detection was carried out at 215 nm and 250 nm. The retention times of breakthrough curves were corrected for the system delay time obtained as the breakthrough time of acetone when the column was replaced with a zero-volume unit. The equation for the calculation of stationary phase concentration (C_s) was derived from Eq. (9) in [20].

$$C_s = \text{flowrate}(t_r - t_0) \times C_m/A \quad (1)$$

where t_r is a breakthrough retention time diminished for the system delay time, $t0$ is a void volume time, Cm is the concentration of the chaotropic agent in the eluent, and A is the surface area of the applied column.

The chromatographic conditions for the method optimization are defined by the face-centered 2^3 full factorial experimental design.

2.9. Software

Thermodynamic model fittings were performed using STATISTICA (StatSoft Inc., Tulsa, OK, USA). The experimental plan and data analysis were performed using Design-Expert® 7.0.0. (Stat-Ease Inc., Minneapolis, MN, USA) and Microsoft Excel (Microsoft, USA). Grid point search was processed in MATLAB® 7.10.0. (The MathWorks, Inc., USA).

3. Results and discussion

Ropinirole and its impurities A and C are organic basis. They differ in hydrophobicity which affects their retention in the reversed-phase LC system. In order to achieve the analytes protonation, the pH of the water phase was set at 2.5. The influence of added chaotropic agents was followed by monitoring the analytes retention factors (k), factors of asymmetry (As) and the number of theoretical plates (N). The increasing values of retention factors k are in accordance with the expected behavior and the effect of perchlorate anion is more prominent than the effect of trifluoroacetate anion. Also, the susceptibility of analytes to be affected by the increasing concentration of chaotropic additives increased with analytes hydrophobicity [21]. In liquid chromatography, peak tailing is a common issue when analytes are protonated basic compounds. The addition of chaotropic agents to the mobile phase and their subsequent adsorption on the stationary phase may suppress some unwanted secondary interactions and thus improve the peak asymmetry. Since the loading concentrations of the impurities A and C were small, this effect was not noted. However, the influence on the symmetry of ropinirole peak was evident – with the increasing concentration of the chaotrope, the factor of asymmetry As tended to one. When relatively high sample load is needed, as in the assays for the impurity profiling, the addition of chaotropic additives may be employed to improve the peak shape [22]. The increase of chaotrope concentration also led to the increase in separation efficiency seen as a number of theoretical plates N. This effect was observed regardless the analytes loading concentrations. Again, perchlorate showed stronger influence than trifluoroacetate.

To understand more deeply the processes governing the analytes retention upon the addition of chaotropic agents, the retention data were fitted to the thermodynamic model suggested by Cecchi et al. [17,18]. First, the adsorption profiles of examined chaotropes were experimentally determined via the breakthrough method of frontal analysis. The obtained data was perfectly described by Freundlich adsorption isotherms

$$[\text{LH}] = a[\text{H}]^b \quad (2)$$

where a and b are the constants, [LH] is a specific surface excess (μmol/m^2) of the additive, and [H] is its concentration in the mobile phase (mM). The measurement of the adsorption isotherms enabled the calculation of surface potentials according to the following Gouy–Chapman equation [18]:

$$\Psi^\circ = \frac{2RT}{F} \ln\left\{ \frac{[\text{LH}]|z_H|F}{\left(8\varepsilon_0\varepsilon_r RT \sum_i c_{0i}\right)^{1/2}} + \left[\frac{([\text{LH}]z_H F)^2}{8\varepsilon_0\varepsilon_r RT \sum_i c_{0i}} + 1 \right]^{1/2} \right\} \quad (3)$$

where R and F are the gas and the Faraday constants, respectively, T is the absolute temperature, ε_0 is the electrical permittivity of vacuum, ε_r is the dielectric constant of the mobile phase and Σc_i is the mobile phase concentration (mM) of singly charged electrolyte ion. According to the charge status z_H of the most adsorbophilic ion in the additive H, surface potential Ψ° is considered positive or negative. The curve parameters and their statistical estimation along with the surface potentials calculated for the highest concentrations of the tested chaotropic additives are given in Table 1. Higher surface potential developed upon the addition of perchlorates in comparison to trifluoroacetates is in accordance with the ion rank in the Hofmeister series.

Further, the obtained retention data were fitted to the model that takes into account the Gouy–Chapman expression for the potential and the Freundlich equation for the adsorption isotherm of the chaotropic additive [17]

$$k = \frac{c_1\{a[\text{H}]^b f + [(a[\text{H}]^b f)^2 + 1]^{1/2}\}^{\pm 2|z_E|} + c_2[\text{H}]}{(1 + c_3[\text{H}])\{1 + c_4[\text{H}]\{a[\text{H}]^b f + [(a[\text{H}]^b f)^2 + 1]^{1/2}\}^{-2}\}} \quad (4)$$

where

$$f = \frac{|z_H|F}{8\varepsilon_0\varepsilon_r RT \sum_i c_{0i}} \quad (5)$$

f (m^2/mol) can be evaluated from the eluent composition and operative temperature. In this study the ionic strength of the mobile phase was not kept constant and its change with the concentration of the added chaotrope was taken into account (see Eqs. (4) and (5) in [23]). Constants a and b are related to the Freundlich isotherm, [H] is the mobile phase concentration of the chaotrope, z_E and z_H are the charges of the analyte and the chaotropic ion, respectively, and c_1–c_4 are the fitting parameters with the appropriate physical meaning [18]. Parameter c_1 is generally not considered adjustable since it equals k_0, the retention factor of the analyte without chaotropic additive in the eluent, which can be experimentally obtained. However, in this investigation c_1 was left as a fitting parameter, because the model showed somewhat better statistics. Nevertheless, the predicted values are in the physically and chromatographically reasonable agreement with the experimental values. Parameters c_2, c_3 and c_4 are related to the thermodynamic equilibrium constants for the ion-pair formation in the stationary phase, for the ion-pair formation in the eluent and for the adsorption of the ion-interaction reagent onto the stationary phase, respectively. When the ionic surface potential determining additive is not strongly adsorbophilic, like chaotropic reagents, the right factor of the denominator could be considered not very different from one, hence c_4 was omitted from the fitting [17,24]. The estimated parameters and their statistical

Table 1
Freundlich constants (a and b) with standard errors (SE), correlation coefficients (r) and surface potentials (Ψ°) for the selected chaotropic agents [H].

[H]	a [mM^{-b}]	SEa	b	SE_b	r	Ψ° max [mV]
NaTFA	5.19E−03	6.39E−04	8.86E−01	2.85E−02	0.9976	−39.4
NaClO4	7.02E−03	4.29E−04	9.81E−01	1.41E−02	0.9991	−68.4

evaluation are presented in Table 2. The ANOVA test and the obtained *F*-values proved the significance of the models.

As it could be seen, the parameter c_2 is missing. Its inclusion was not reasonable, because its negative estimate suggested that the influence of ion-pair formation in the stationary phase was negligible in this chromatographic system [17]. As expected, the values of c_3 parameter show that ion-pairing constants are lower for trifluroacetate anion compared to perchlorate. To understand the contribution of the pure electrostatic interactions and ion-pairing in the eluent to the retention of the analytes, a graphical assessment was done. For the sake of simplicity, Fig. 2 presents only the plot of ropinirole retention when $NaClO_4$ is used as a chaotropic additive. The plots of the other analytes in combination with NaTFA were alike. Curve A stands for the retention model (Eq. (4)) fitted to the experimental data, while curves B and C correspond to the individual terms of Eq. (4). It is evident that the electrostatic term (curve B) has the greatest contribution to the analytes retention. The adsorption of the chaotropic ions onto the stationary phase leads to the development of the electrostatic double layer creating a difference in electrostatic potential between the charged surface and the electroneutral bulk of the eluent. Consequently, this causes the modification of the analytes adsorption onto the stationary phase. Since the charge status of the analyte is opposite to the charge of the chaotrope determining ion, the electrostatic interactions are attractive thus increasing the analytes retention. However, it is obvious that if only the effect of electrostatic attraction would be taken into account, the proper modeling of analytes retention would not be possible. On the other hand, when the ion-pair formation in eluent is not neglected, even though it occurs in a small extent, the biparametric model fitting is satisfactory. The ion-pair formation in eluent causes the decrease of analyte retention, which is well demonstrated by the curve C. It also affects the shape of the curve A leading to its foldover [18].

Table 2
Estimates of the fitting parameters and statistical evaluation of the retention models.

		Model parameters				Model estimation
		c_1	Std. error	c_3	Std. error	R^2
NaTFA	k_A	0.54	1.04E−02	5.01E−03	5.00E−04	0.9880
	k_C	1.89	2.63E−02	6.46E−03	3.92E−04	0.9918
	k_R	2.20	2.88E−02	5.80E−03	3.54E−04	0.9935
$NaClO_4$	k_A	0.56	2.71E−02	2.81E−02	2.55E−03	0.9629
	k_C	1.95	7.94E−02	3.04E−02	2.28E−03	0.9706
	k_R	2.23	9.41E−02	2.74E−02	2.20E−03	0.9727

k_A, k_C, and k_R – retention factors of the impurity A, impurity C and ropinirole, respectively.

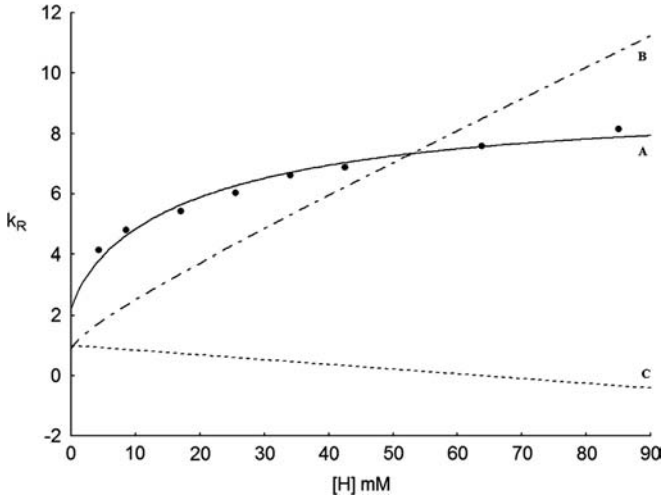

Fig. 2. Dependence of retention factor of ropinirole k_R on the mobile phase concentration of $NaClO_4$ – retention model (Eq. (4)) fitted to experimental data (curve A), contribution of the electrostatic interactions (curve B), contribution of the ion-pairing in the eluent (curve C).

Further investigation of the chromatographic system that contains chaotropic additive and the method development of the examined mixture were governed by the design of experiments methodology. In this phase, the selected chaotropic agent was NaTFA. The aim was to examine the influence of the mobile phase composition, as well as the interactions of its components, on the chromatographic behavior of the analytes. Therefore, the selected factors were concentration of the chaotropic additive in the aqueous component of the mobile phase (x_1,10–50 mM), acetonitrile content in the mobile phase (x_2, 10–20%) and pH of the aqueous component of the mobile phase (x_3, 2–3). The face-centered central composite design (CCD) consisting of 2^3 full factorial design, ± 1 star design and four replications in central point was used to obtain data that describe the effects of the selected factors and also allow the definition of optimal conditions using the grid point search method. The retention factors of all the analytes and resolution factor between the impurity C and ropinirole were selected as the system outputs.

The experimental data was fitted to a quadratic model

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 \quad (6)$$

Table 3
Coefficients of quadratic models in terms of coded factors and statistical evaluation of the models.

	k_A	*p*-Value	k_C	*p*-Value	k_R	*p*-Value	R_{CR}	*p*-Value
b_0	1.20	–	4.00	–	4.75	–	2.89	–
b_1	0.33	< 0.0001	1.09	0.0001	1.39	< 0.0001	0.50	0.0008
b_2	−1.59	< 0.0001	−5.81	< 0.0001	−7.05	< 0.0001	−1.74	< 0.0001
b_3	0.054	0.2714	0.21	0.2186	0.25	0.2346	0.029	0.7672
b_{12}	−0.27	0.0007	−0.90	0.0009	−1.18	0.0006	−0.029	0.7927
b_{13}	−0.00125	0.9810	−0.00825	0.9635	−0.012	0.9576	0.00025	0.9982
b_{23}	−0.037	0.4853	−0.15	0.4264	−0.17	0.4675	0.033	0.7705
b_{11}	−0.044	0.6247	−0.19	0.5500	−0.26	0.5063	−0.093	0.6274
b_{22}	0.93	< 0.0001	3.54	< 0.0001	4.21	< 0.0001	−0.46	0.0383
b_{33}	0.006	0.9470	0.048	0.8759	0.10	0.7894	0.28	0.1697
Model *F*-value	164.67	< 0.0001	178.96	< 0.0001	186.72	< 0.0001	40.29	< 0.0001
Lack-of-fit *F*-value	1.42	0.4115	1.98	0.3047	1.51	0.3899	1.24	0.4571
R^2	0.9946	–	0.9951	–	0.9953	–	0.9784	–

k_A, k_C, and k_R – retention factors of the impurity A, impurity C and ropinirole, respectively.
R_{CR} – resolution factor between impurity C and ropinirole.

where y is the estimated response, x_1–x_3 are the variables (factors), b_1–b_3 are the coefficients of the linear parameters; b_{12}, b_{13} and b_{23} are the coefficients of the interaction parameters; and b_{11}, b_{22} and b_{33} are the coefficients of the quadratic parameters. The calculated coefficients in terms of coded factors are presented in Table 3. The model parameter is considered to be significant for $p < 0.05$ at 95% confidence level. The validity of the models was confirmed by the ANOVA test, the lack-of-fit test and determination coefficients.

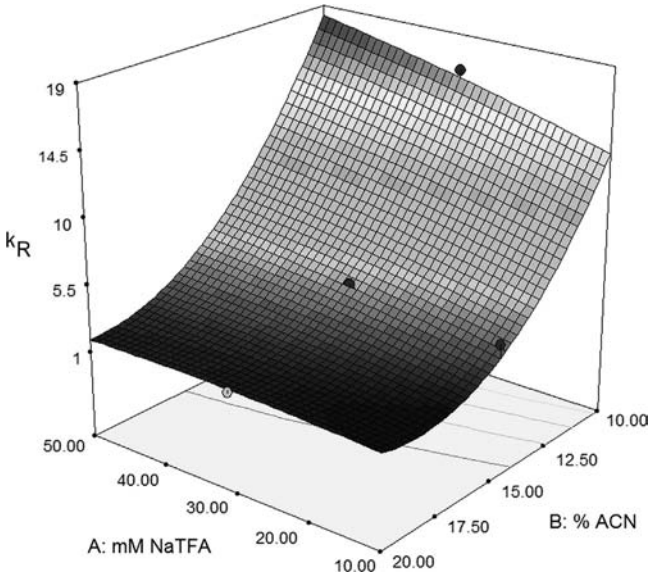

Fig. 3. Three-D response surface graph – dependance of the retention factors of ropinirole on the concentration of NaTFA in the water phase and acetonitrile content in the mobile phase.

Statistical analysis has shown that the influential factors for all the responses were the concentration of NaTFA and the content of acetonitrile. The pH value proved to be insignificant, which could be explained by the complete protonation of the analytes in the whole analyzed range. As the sign of the parameter suggests, the increase in NaTFA concentration led to the increase of retention factors, while the content of acetonitrile had the opposite effect, which is in accordance with the reversed-phase mechanism, and this dependence was quadratic. However, the most interesting is the interaction of these two factors. Namely, the increase of the retention factors caused by the increase of chaotrope concentration is more prominent on the lower levels of acetonitrile content and it could be seen that the total change of the retention factor in the examined range is five to six times greater at lower organic modifier concentration (for instance, at pH 2, at 10% of ACN for 50 and 10 mM NaTFA $\Delta k_R = 18.13 - 12.59 = 5.54$, while at 20% ACN for 50 and 10 mM NaTFA $\Delta k_R = 2.21 - 1.37 = 0.84$). This could be explained by the decrease of adsorption free energy of chaotrope when the higher percent of the organic modifier is used, which affects subsequent development of the surface potential. The effects of these two factors, as well as their interaction, can be easily visualized from the 3D-graph (Fig. 3). For the sake of simplicity, only the effect on ropinirole's retention factor is presented since the influence on the other analytes was analogous.

Finally, to optimize the method of practical value that could be used routinely, three goals were set: satisfactory retention of the first eluting peak ($k_A > 1.1$), good resolution between the critical pair impurity C–ropinirole ($R_{CR} > 1.3$), and the short total run time ($k_R < 4.5$). The grid point search method enabled the simultaneous optimization of these selected responses. The division of design space into a grid was achieved by discretization of the investigated factors. The increments for the concentration of NaTFA were 5 mM, for the acetonitrile content was 2%, and for the pH of the aqueous component of the mobile phase was 0.5 units since the previous

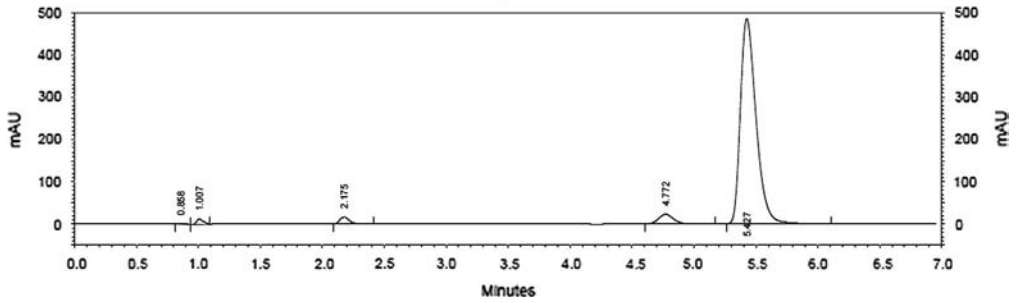

Fig. 4. Chromatogram obtained under the optimal chromatographic conditions acetonitrile–water phase (45 mM NaTFA, pH adjusted to 2.5 with *ortho*-phosphoric acid) 16:84 (v/v): impurity A (t_r 2.175 min), impurity C (t_r 4.772 min), and ropinirole (t_r 5.427 min).

Table 4
Validation parameters: limits of detection, linearity and accuracy of the proposed LC method.

Substance	LOD (μg/mL)	Linearity				Accuracy	
		Concentration range (μg/mL)	*a*	*b*	*r*	Concentration level (μg/mL)	Recovery (%)[a]
Ropinirole	–	100–350	59.27	−75.65	0.9958	160	100.4
						200	98.9
						240	99.8
Impurity A	0.03	0.1–1.2	61.03	−2.96	0.9965	0.1	113.0
						1.0	93.2
						1.2	93.3
Impurity C	0.03	0.1–1.2	80.03	−1.27	0.9975	0.1	99.6
						1.0	95.8
						1.2	94.4

a – slope, *b* – intercept, and *r* – correlation coefficient (acceptance value > 0.99 for active ingredients and > 0.98 for related compounds).

[a] Recovery: acceptance value 98.0–102.0% for active ingredients, and 70.0–130.0% for related compounds.

results suggested that the influence of this factor was insignificant. Thus, the number of levels for factors x_1, x_2 and x_3 were 9, 6 and 3, respectively, so the total number of the investigated grid points was 162. According to the defined criteria, the point that corresponds to 16% of acetonitrile, 45 mM NaTFA and pH 2.5 were selected as optimal. The predicted values of the selected responses were $k_A=1.11$, $R_{CR}=2.84$ and $k_R=4.23$. The chromatogram acquired under the optimal conditions is presented in Fig. 4. High agreement between the predicted and the obtained response values ($k_A=1.16$, $R_{CR}=2.94$ and $k_R=4.39$) verified the adequacy of the selected optimum.

In order to prove the reliability and applicability of the developed method, it was further subjected to the validation process. The selectivity was proven by the absence of significant interfering peaks originating from the blank sample at the retention times of the analyzed compounds. The method sensitivity was determined by the definition of limit of detection (LOD) and limit of quantification (LOQ). These values for the impurities were experimentally determined based on the signal-to-noise (S/N) approach. An S/N of 3:1 and 10:1 is generally considered acceptable for the estimation of LOD and LOQ, respectively (Table 4). Linear relationships between the peak areas and the concentration ranges for ropinirole and impurities A and C were established. The calculated regression parameters are given in Table 4 and are within the linearity acceptance criteria [25]. The method precision was assessed by calculating the relative standard deviation (RSD): ropinirole (1.21%), impurity A (0.96%) and impurity C (2.27%). The obtained values fulfilled the required criteria (RSD 2% for active ingredients and 15% for related compounds) [25]. The accuracy of the proposed method was evaluated according to the obtained recovery values for laboratory mixture presented in Table 4. They are all within the acceptance criteria (recovery values: 98.0–102.0% for active ingredients, 70.0–130.0% for related compounds) [25].

4. Conclusion

A thorough explanation of chromatographic behavior of basic substances, ropinirole and its impurities A and C, in the liquid chromatographic system that contains chaotropic additives is given. The effect of the analyzed chaotropes (NaTFA and $NaClO_4$) on the retention factors of the analytes was in agreement with their rank in the Hofmeister series and was also concentration dependable. Moreover, the positive influence on peak symmetry and separation efficacy was proved. The susceptibility of analytes to be affected by the chaotropic agent was determined by their hydrophobicity.

The extended thermodynamic approach enabled the comprehension of the processes that underlie the separation in the given system. It suggested that the separation was predominantly governed by electrostatic interactions between the protonated analytes and charged surface of the stationary phase, while the ion-pair complex formation in the eluent also proved to be significant.

The experimental design governed the method development, but also enabled the analysis of factors interactions in the given chromatographic system. It was observed that the chaotropic effect is more conspicuous at the lower concentrations of the organic modifier. Furthermore, the identification of optimal chromatographic conditions was done applying the grid point search method, and after the method validation, the adequacy of the suggested approach in development of methods for routine pharmaceutical analysis was proven.

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