



Dedicated to the memory of  
Professor Eugen Segal (1933-2013)

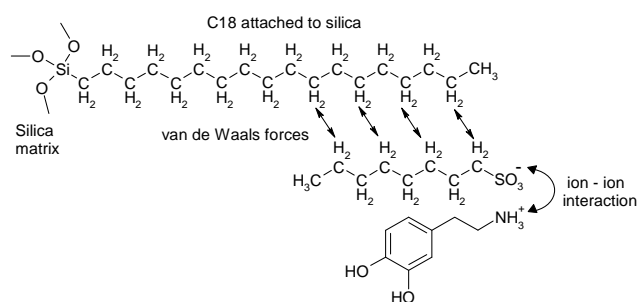
## RETENTION STUDY FOR SOME NEUROTRANSMITTERS UNDER ION-PAIRING LIQUID CHROMATOGRAPHIC MECHANISM

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The study is focused on the chromatographic behaviour of several compounds with an important neuronal role under ion-pairing mechanism. Among these compounds, epinephrine, norepinephrine, dopamine and serotonin are known neurotransmitters, while L-dopa is a neuronal precursor and dopac is a metabolite of dopamine. Sodium alkylsulphonates with five to eight carbon atoms added to mobile phase were used as ion-pairing agents. This study included the investigation of some major experimental parameters, such as the organic modifier content in mobile phase, the hydrophobicity and the concentration of the ion-pairing agent added to the aqueous phase, temperature, ionic strength and pH value of the mobile phase on the retention process. Two complementary theoretical models (adsorption and partition) were used to explain the functional dependences obtained for the retention data.



### INTRODUCTION

Generally, the biogenic amines are widespread in plants and animals where they have important metabolic and physiological roles, such as growth regulation (putrescine, spermidine, spermine), control of blood pressure (indoleamines and histamine), and neural transmission (catecholamines and serotonin).<sup>1</sup> Several analytical methods were used to investigate the biogenic amines content in foods and beverages because of their potential toxicity<sup>2</sup> among which we can mention capillary electrophoresis,<sup>3,4</sup> micellar electrokinetic capillary chromatography,<sup>5</sup> HPLC with electrochemi-

cal,<sup>6</sup> fluorimetric,<sup>7</sup> ionization mass spectrometric<sup>8,9</sup> detections or UHPLC.<sup>10</sup> The majority of assays employs fluorimetric detection with precolumn or postcolumn derivatization techniques. Particularly, a great number of papers concerned with developing selective and sensitive procedures for determination of catecholamines (and their precursor and metabolites).<sup>11,12</sup> Techniques such as microdialysis are routinely employed to measure neurotransmitter levels in living tissue systems.<sup>13</sup>

Several studies used ion-pairing mechanism in order to improve the determination of some biogenic amines.<sup>14,15</sup> It is the aim of this work to study the influence of the main elution parameters

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upon chromatographic behaviour for several compounds with neuronal importance under ion-pairing (IP) mechanism. This could be useful either for analytical purposes, or for a better understanding the interaction of these compounds with biochemical compounds during their absorption and transport.

The analytes investigated in this study are neurotransmitters like epinephrine (E), norepinephrine (NE), dopamine (DA) and serotonin (S), but also a neuronal precursor (L-Dopa – LD) and a metabolite (Dopac – D) of dopamine were studied. Levodopa is the “gold standard” for the treatment and management of Parkinson’s disease worldwide,<sup>16</sup> while DOPAC exhibits a considerable antiproliferative effect in LNCaP prostate cancer and HCT116 colon cancer cells.<sup>17</sup> Although DOPAC is a dihydroxyphenylacetic acid and unable to form ion-pairs with the alkylsulphonates, it has been used as neutral species for studying this chromatographic mechanism.

## THEORETICAL CONSIDERATIONS

Two main models are describing the retention process in ion-pairing liquid chromatography, namely the partition and electrostatic models, which are very useful in understanding this complex process and also for the analytical method

$$\ln k_B^{IPA} = \ln k_B^0 + \left(\frac{z_B}{2}\right) \left[ \ln \left( \frac{n_0 K_{IPA} C_{IPA}}{\kappa} \right) + \ln \left( \frac{F^2}{RT \epsilon_0 \epsilon_m} \right) + 1 \right] \quad (2)$$

In this equation the charge of the analyte is  $+z_B$ , the charge of IPA is  $z_{IPA} = -1$ , the concentration of  $IPA^-$  in mobile phase is  $C_{IPA}$ , the retention factor of B in absence of IPA is  $k_B^0$ ;  $\ln$  represents the natural base logarithm,  $n_0$  is a parameter related to the adsorption of  $IPA^-$  as a monolayer of the stationary phase surface (as mol/m<sup>3</sup>);  $\kappa$  - inverse Debye length (m<sup>-1</sup>);  $F$  – Faraday constant (C/mol);  $T$  – absolute temperature (in K);  $R$  – gas constant (J/mol K);  $\epsilon_0$  – permittivity of vacuum (F/m);  $\epsilon_m$  – dielectric constant of the mobile phase ( $\epsilon$  for vacuum is 1).  $K_{IPA}$  represents the equilibrium constant for the adsorption of  $IPA^-$  on the stationary phase surface and this is related to the hydrophobic character of this species. Experimentally, the major parameters affecting the ion-pairing mechanism are the hydrophobicity of

development. Reversed-phase liquid chromatography under ion pair-mechanism is usually applied to very polar analyte, with basic property (B). Under this particular mechanism, the protonated form of the analyte ( $BH^+$ ) interacts with an opposite charged ion-pairing agent ( $IPA^-$ ), added to mobile phase and forms an ion pair ( $BH^+IPA^-$ ) with an enhanced retention on the stationary phase. This ion-pair can be formed either in mobile phase and then will participate to the distribution process between mobile and stationary phase (partition model), or can be formed on the surface of stationary phase due to the electrostatic attraction of the adsorbed  $IPA^-$  (electrostatic model). Overall, the entire process is governed by the free energy  $\Delta G^0$ , which relates the retention factor  $k_B$ , the equilibrium constant of the adsorption  $K_{IPA}$ , and the column phase ratio  $\phi$ , according to the fundamental relationship:<sup>18</sup>

$$k_B^{IPA} = \phi K_{IPA} = \phi e^{-\frac{\Delta G^0}{RT}} \quad (1)$$

According to the electrostatic model of the ion-pairing mechanism, the dependence of the retention factor  $k$  for the analyte in the presence of  $IPA^-$ , denoted by  $k_B^{IPA}$ , can be described by the following equation:<sup>18,19</sup>

the ion-pair agent (IPA); its concentration in aqueous component, pH (when it influences the dissociation of the studied analyte), the ionic strength, the concentration of the organic modifier from mobile phase and temperature.

According to the partition model the dependence of retention factor of the analyte B in IP mechanism on pH value of the mobile phase has two contributions due to the partition between mobile and stationary phase of the uncharged species B ( $k_B^0$ ), and to the partition of  $B^+$  entirely found as ion pair  $BH^+IPA^-$  between the two phases ( $k_{BH^+IPA^-}$ ):

$$k_B^{IPA} = k_B^0 + k_{BH^+IPA^-} \frac{K_b \times 10^{-pH}}{K_w + K_b \times 10^{-pH}} \quad (3)$$

where  $K_b$  represents the basicity constant for analytes having basic character, as our case. Basically, this dependence should be a sigmoidal curve, with the inflexion point situated at the value of pH equal to the  $pK_b$  values of the basic compound.<sup>20</sup>

## EXPERIMENTAL

All experiments were performed on an Agilent 1100 liquid chromatograph (Agilent Technologies, Waldbronn, Germany), consisting in the following modules: degasser (G1379A), binary pump (G1312A), autosampler (G1313A), column thermostat (G1316A) and diode array detector (G1315A). The chromatographic system was operationally qualified before the study. Chromatographic data were acquired by means of Agilent Chemstation software rev. B.01.03. and the values were plotted by means of Origin program. pH measurements were performed at room temperature using a multi-parameter analyzer CONSORT C861, with pH glass electrode with integrated temperature sensor, CONSORT SP20T.

**Reagents:** Methanol was HPLC (gradient) grade from Merck (Darmstadt, Germany). Water (resistivity minimum 18.2 M $\Omega$  and TOC maximum 30 ppb) was produced within the laboratory with a TKA Lab HP 6UV/UF instrument. Reagents were pro-analysis grade (phosphoric acid) or ion-pair grade (alkylsulphonates) from Merck. The analytes are commercially available compounds, having purities higher

than 99% and were purchased from Sigma Aldrich. Their structures are shown in Fig. 1.

**Chromatographic conditions:** A Zorbax Eclipse XDB column, packed with extra-densely bonded doubled end-capped octadecyl silicagel, 150 mm length, 4.6 mm i.d. and 5  $\mu$ m particle size, was used under isocratic elution conditions. All experiments were performed at 25°C (unless specified), using a flow-rate of 1 mL/min. UV detection was used by monitoring the absorbance at 285 nm wavelength.

The aqueous component of the mobile phase contained 0.1% H<sub>3</sub>PO<sub>4</sub> (pH = 2) or 10 mM NaH<sub>2</sub>PO<sub>4</sub> / Na<sub>2</sub>HPO<sub>4</sub> brought to the indicated pH with 0.1% H<sub>3</sub>PO<sub>4</sub> (pH = 2.5 - 7.4) and different concentrations of the ion pairing agent. Sodium pentane- (PNT), hexane- (HXN), heptane- (HPT) and octane-sulphonates (OCT) were used as ion pairing agents. Methanol (MeOH) was used as organic modifier in mobile phase. The two components were mixed in different volume ratios by means of the binary HPLC pump, as further described in the text. Column re-equilibration with different mobile phase compositions was done (approx. 15 min).

The working solutions of each tested compound were prepared in water at 250  $\mu$ g/mL (NE, DA, S) or 500  $\mu$ g/mL (E, LD, D) concentration level each and injected as a volume of 1  $\mu$ L. Retention factors were computed according to the relationship  $k = (t_R - t_0)/t_0$ . Absolute retention time values ( $t_R$ ) are considered as mean resulting for experiments carried out in triplicate (the maximum relative standard deviation calculated for the experimental values of  $k$  was 2%). KNO<sub>3</sub> peak was used as the dead time ( $t_0$ ) indicator.

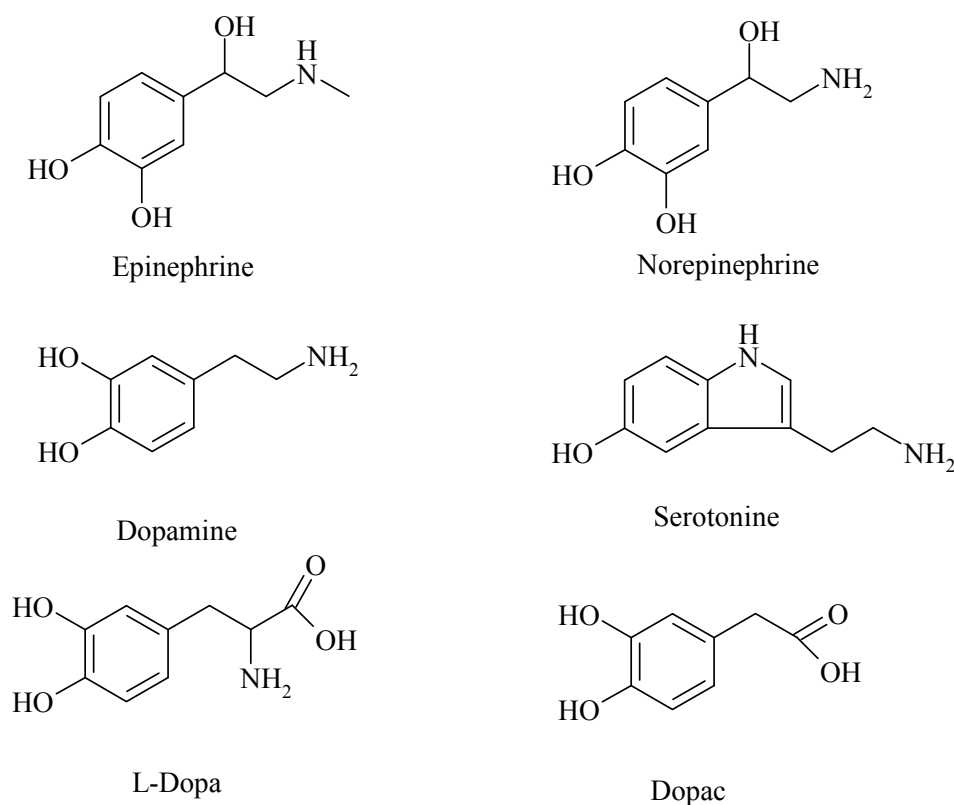


Fig. 1 – Chemical structures of studied compounds.

## RESULTS AND DISCUSSION

Initially, a separation study was performed in the aim of optimizing the parameters for further investigations. A satisfactory baseline separation between all the analytes was achieved for a mobile phase containing 75% aqueous phase in acidic conditions using 10 mM HPT as IPA (Fig. 2). For the rest of the experiments, performed in richer organic modifier compositions, coelutions of (epinephrine, norepinefrine) and (dopamine, L-Dopa), respectively, were observed and for further studies the separate injections were performed.

### The influence of the hydrophobicity of the IPA

Generally, IPA added to mobile phase can influence the retention of the analytes through his electric charge, hydrophobicity or its concentration in the mobile phase. The hydrophobicity of IPA influences both the monolayer capacity and the free energy of adsorbtion. Increasing the number of carbon atoms in the IPA molecule results in an incremental change in the value of the adsorbtion term ( $n_0K_{IPA}$ ) in the eq. 1. For a constant ionic

strength and both the analyte and IPA single charged, eq. 1 can be rewritten as:

$$\ln k_B^{IPA} = \alpha + \frac{1}{2} \cdot \ln(n_0K_{IPA}) + \frac{1}{2} \ln c_B \quad (4)$$

Keeping constant the ionic strength and IPA concentration, respectively, the equation becomes

$$\ln k_B^{IPA} = \alpha + \frac{1}{2} \cdot \ln(n_0K_{IPA}) \quad (5)$$

, where the term  $\alpha$  is a constant depending on the IPA concentration, organic modifier and ionic strength. The adsorbtion term depends on the hydrophobicity of the IPA and on the organic modifier concentration.

In this study, four alkylsulphonates were studied in order to find out the influence of the number of carbon atoms on the retention of studied compounds (C5–C8). A linear dependence was observed between  $\ln k$  and the number of carbon atoms, with correlations higher than 0.999. Examples of such dependences between  $\ln k$  and the number of carbon atoms are illustrated in Fig. 3 and the regressions parameters are given in Table 1, for 10 mmoles/L alkylsulphonates used as IPA.

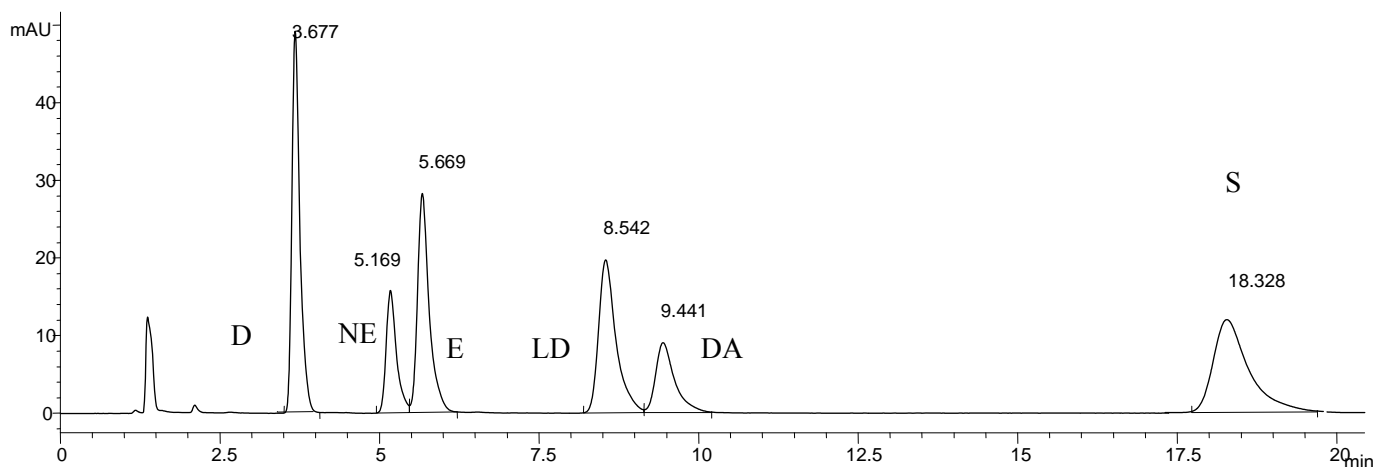


Fig. 2 – Separation of a mixture of studied compounds under following chromatographic conditions; stationary phase: Zorbax Eclipse XDB – C18 (4.6 mm/ 150 mm/ 5  $\mu$ m); mobile phase: 25% MeOH/ 75% aq (0.1%  $H_3PO_4$ + 10 mM sodium heptanesulphonate); isocratic elution; flow 1 mL/min.; detection 285 nm; temperature 25°C; injected amounts for E, LD, D – 500 ng each, NE, DA, S – 250 ng each.

Table 1

Regression parameters for the dependences of  $\ln k$  on Number of C atoms of IPA for the six compounds studied by RP-IPC

Analyte	Intercept	Slope	$r^2$
Epinephrine	-3.604	0.646	0.9998
Norepinephrine	-3.779	0.658	0.9998
Dopamine	-3.592	0.712	0.9999
Serotonine	-3.143	0.732	0.9999
L-DOPA	-3.637	0.703	0.9997
Dopac	-4.121	0.641	0.9999

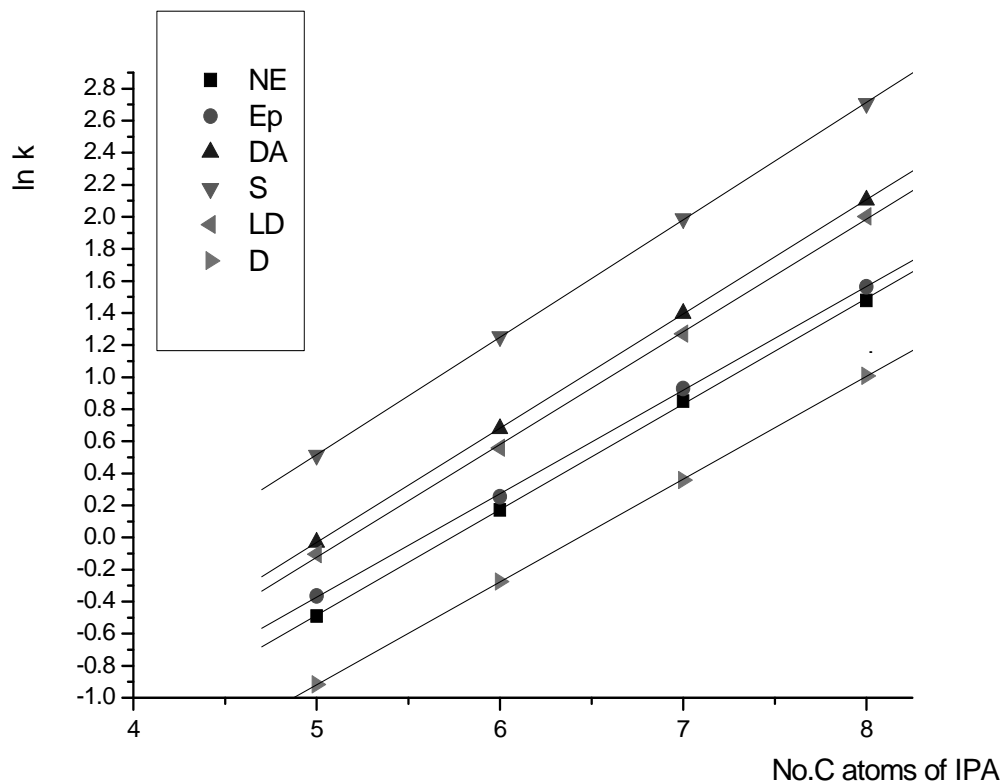


Fig. 3 – Dependence of the retention of studied compounds on the number of carbon atoms in ion pairing molecule (Experimental conditions: 10 mM IPA; pH 2; 30% MeOH/70% aq (10 mM IPA; 0.1% H<sub>3</sub>PO<sub>4</sub>)).

### The influence of ion pairing concentration in mobile phase

The dependence of the retention factor on this parameter must be a linear logarithmic dependence with a slope 0.5 for ionic charge for both analyte and IPA of 1. This equation can be written in the form:

$$\ln k_B^{\text{IPA}} = \beta - \frac{1}{2} \ln c_{\text{IPA}} \quad (6)$$

where the intercept  $\beta$  comprises the terms  $\ln k_B^0$  and  $0.5 \times (\ln \frac{n_0 K_{\text{IPA}} F^2}{\kappa R T \epsilon_0 \epsilon_m} + 1)$ . In this study were used concentration levels ranging from 5-20 mM HXN in the aqueous phase. The graphs representing this dependence gave good linear correlations between these two parameters and slopes around the value of 0.4, which is very close to the theoretical value (0.5). Similar values of the slope were reported for other dissociable compounds, for methanol or acetonitrile added to

the mobile phase compositions.<sup>21</sup> An example of the experimental dependence between these two studied parameters and the regression parameters for these dependences are given in Fig. 4. The retention obtained in Dopac case was almost without modification (data not shown).

### The influence of pH of aqueous component

The variation of the pH of the aqueous component of the mobile phase can lead to large changes in the protonation of amino groups of the analytes and hence in their ability to interact with the IPA. The influence of this parameter was studied on a broad pH range (2 – 7.4). Sigmoidal shapes were obtained for several compositions of the mobile phase, in accordance with the partition model and the eq. 3 describing this process. The retention behaviour might be explained by the protonation at  $-\text{NH}_2$  sites of the molecule, and leading to an ion pair,  $\text{BH}^+\text{IPA}^-$ , as illustrated in Figs. 5 and 6, for norepinephrine and dopamine.

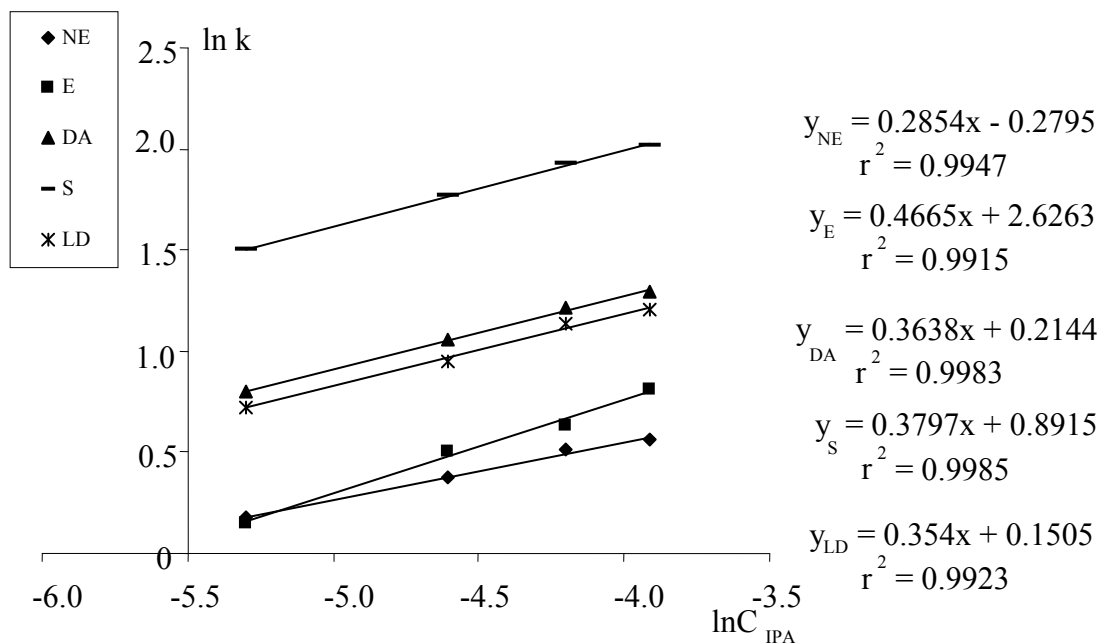


Fig. 4 – Dependence of the  $\ln k$  on the  $\ln C_{IPA}$  for studied compounds  
(Experimental conditions: 30% MeOH/ 70% Aq (x mM HXN and 0.1% H<sub>3</sub>PO<sub>4</sub>, pH = 2).

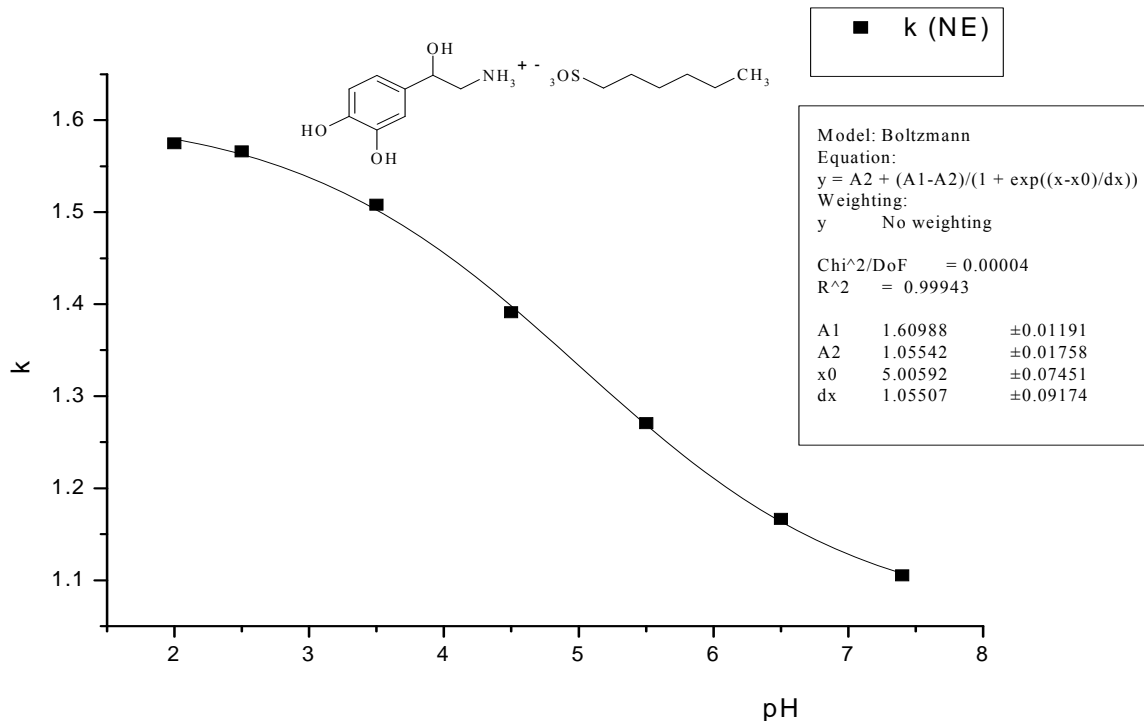


Fig. 5 – Dependence of the retention factor for norepinephrine on the pH of the aqueous mobile in ion-pairing mechanism (m.p.: 25% MeOH + 75% aqueous component consisting of 10 mmoles/L C<sub>6</sub>H<sub>13</sub>SO<sub>3</sub>Na + 10 mmoles/L Na<sub>2</sub>HPO<sub>4</sub> adjusted with H<sub>3</sub>PO<sub>4</sub> 85% solution).

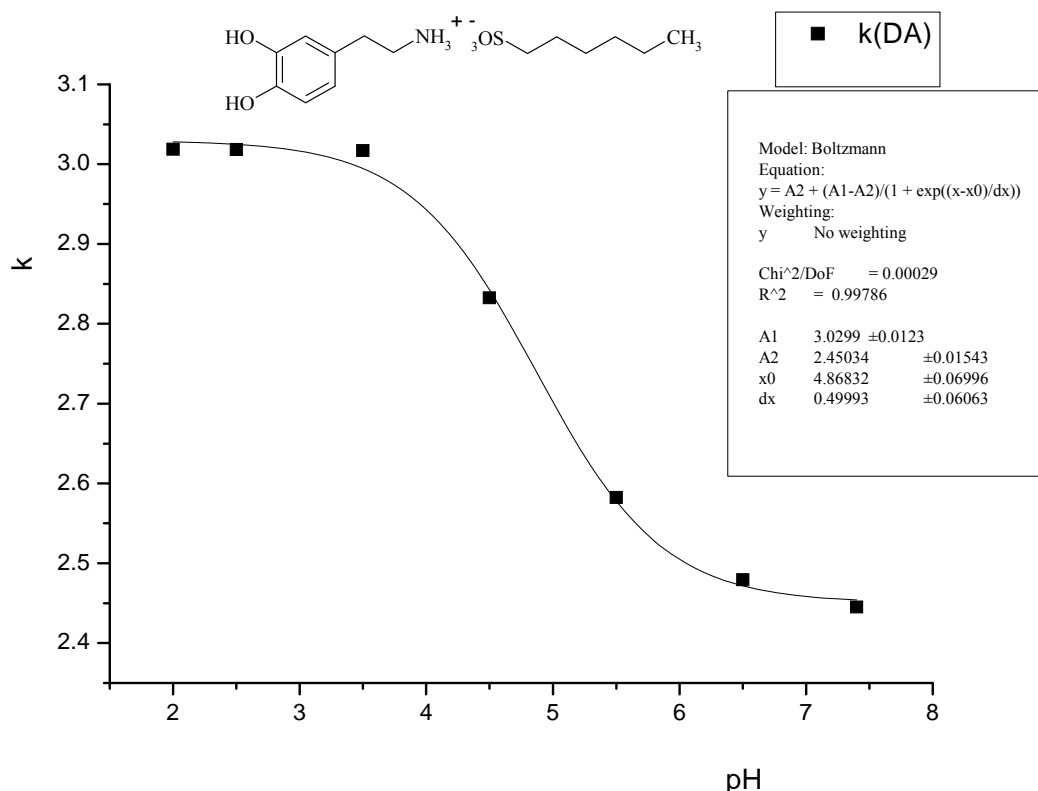


Fig. 6 – Dependence of the retention factor for dopamine on the pH of the aqueous mobile in ion-pairing mechanism (m.p.: 25% MeOH + 75% aqueous component consisting of 10 mmol/L  $C_6H_{13}SO_3Na$  + 10 mmol/L  $Na_2HPO_4$  adjusted with  $H_3PO_4$  85% solution).

### The influence of the organic modifier content

In ion-pairing liquid chromatography, the organic modifier content of the mobile phase influences the adsorption equilibrium of IPA at hydrophobic sites from stationary phase. Usually, the functional dependence between the ten base logarithms of the retention factors ( $\lg k$ ) and the concentration of the organic modifier in the mobile phase ( $C_m$  – volumetric percentage) observed on broader mobile phase composition intervals is given by a linear or polynomial regression up to a second order, according to the following equation:<sup>22</sup>

$$\lg k = \lg k_w + \alpha_1 C_{MeOH} + \alpha_2 C_{MeOH}^2 \quad (7)$$

where  $k_w$  represents the extrapolated value of the retention factor corresponding to a hypothetical mobile phase containing only the aqueous component (i.e.  $C_{MeOH} = 0$ ), and  $\alpha_i$  are regression parameters of the functional dependence. The intercept of this regression,  $k_w$  is proportional to the partition constant of formed ion pair between mobile phase and stationary phase, under the specified experimental conditions. Mathematically, this function has a minimum point resulted from

the condition  $\partial \lg k / \partial C_{MeOH} = 0$ , with coordinates

$C_{MeOH}^{min}$  and  $(\lg k)_{min}$ , as given below:

$$C_{MeOH}^{min} = -\frac{\alpha_1}{2\alpha_2} \quad (8)$$

$$(\lg k)_{min} = \lg k_w - \frac{\alpha_1^2}{4\alpha_2} \quad (9)$$

In this case, unsatisfactory linear correlations between  $\lg k$  and the methanol content in the mobile phase were obtained; the experimental data were fitted using an second order polynomial regression (eq. 7), resulting in a much higher correlation factor ( $r^2 > 0.999$ ), as can be seen in Table 2.

These dependences can be useful in estimating some different kinds of hydrophobicity descriptors assigned to the ion-pairs of these compounds with the mentioned agents. These descriptors can be useful in modeling the membrane crossing process or transport. From this point of view,  $\lg k_w$  is an very important parameter, related to the hydrophobic character assigned to the ion pair between the studied analyte and alkylsulphonate for a given pH value.

Table 2

Regression parameters and some extrapolated retention values obtained for dependences given by eq. 7 for studied compounds and HXN as IPA for pH = 5.5

Analyte	$\lg k_w$	$\alpha_1$	$\alpha_2$	$C_{\text{MeOH}}^{\text{min}}$	$(\lg k)_{\text{min}}$	$r^2$
NE	0.8723	-0.03510	$1.76 \cdot 10^{-4}$	99.99	-0.884	0.9999
E	1.0258	-0.0406	$2.29 \cdot 10^{-4}$	88.77	-0.776	0.9999
DA	1.6934	-0.0617	$4.18 \cdot 10^{-4}$	73.85	-0.585	0.9999
LD	-0.1643	-0.0194	$1.69 \cdot 10^{-4}$	57.33	-0.721	0.9955
S	2.4249	-0.0824	$5.86 \cdot 10^{-4}$	70.29	-0.471	0.9999

### The influence of the ionic strength

In accordance to the electrostatical pattern, the ionic strength influences the electrostatic surface potential. Theoretically, an increase of the concentration of the salt added in the aqueous phase leads to a decrease in the electrostatic surface potential. Not only the IPA ions are adsorbed on the stationary phase surface creating the surface potential, but the electrolytic counterions (e.g., bromide, acetate) can also be adsorbed on the surface layer reducing the net charge concentration on the surface. The ionic strength effect on the retention factor is embedded in  $\kappa$ , namely the inverse Debye length.<sup>18</sup> For constant organic modifier and IPA concentrations, eq. 2 can be simplified as:

$$\ln k_B^{\text{IPA}} = \chi - \frac{1}{4} \cdot \ln I \quad (10)$$

where  $I$  represents the ionic strength ( $I = \frac{1}{2} \sum C_i z_i^2$ ), and  $\chi$  is a constant depending on the

hydrophobicity and the charge of the analyte and pairing ion, organic modifier and the pairing ion concentration. In order to observe the effect of the ionic strength on the retention of the analytes, we kept the pairing-ion concentration constant, at 10 mmol/L, adding different amounts of salt (between 1 and 15 mmol/L) into the aqueous phase. As predicted by theory, for a given concentration of IPA in the mobile phase, increasing ionic strength led to a decrease in the retention of analytes ion pairs. The graphs representing the dependence  $\ln k$  vs.  $\ln I$  gave good linear correlations and slopes around the value of -0.4 (close to the theoretical value of -0.25). Examples of such dependences between these two studied parameters are given in Fig. 7. The regression parameters for these dependences using several amounts of KBr added in the aqueous phase are given in Table 3.

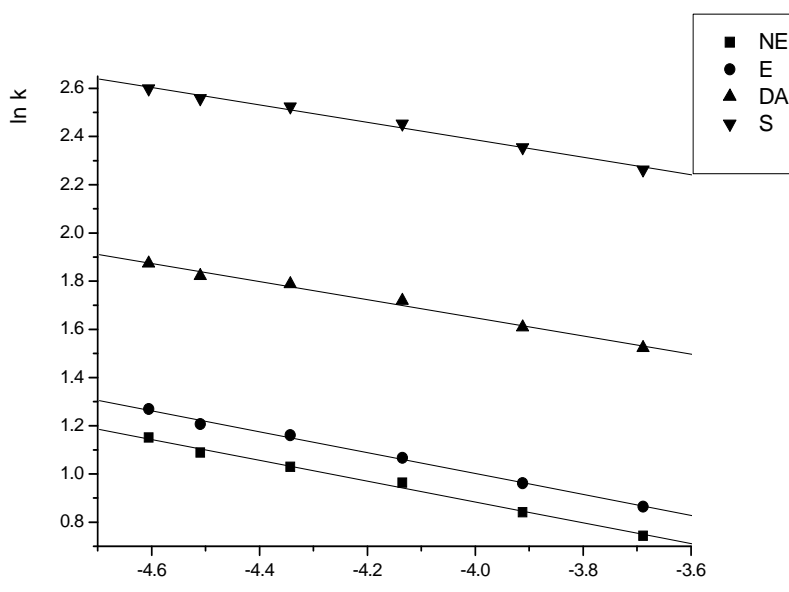


Fig. 7 – Dependence of the  $\ln k$  of studied compounds on the  $\ln I$  (Experimental conditions: 25% MeOH and 75% aqueous component consisting of 10 mmol/L  $C_7H_{15}SO_3Na$ , 0.1%  $H_3PO_4$  and KBr).



Table 3

Regression parameters for the dependences of  $\ln k$  on  $\ln I$  for the four neurotransmitters studied by RP-IPC

Analyte	Intercept	Slope	$r^2$
Epinephrine	-0.7336	-0.4338	0.9980
Norepinephrine	-0.8461	-0.4324	0.9964
Dopamine	0.1431	-0.3761	0.9947
Serotonin	0.9383	-0.3619	0.9951

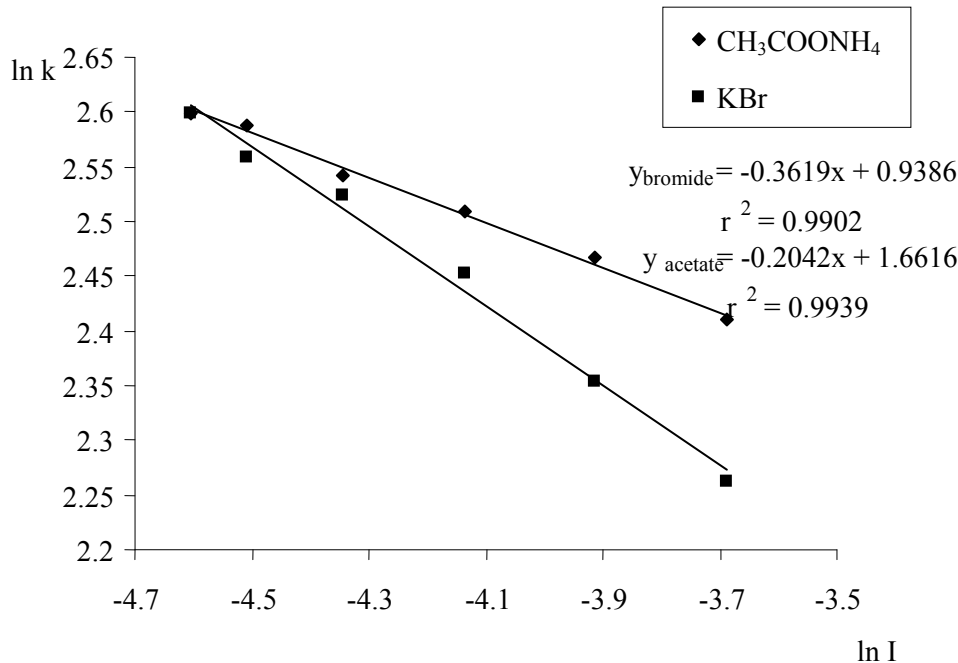


Fig. 8 – Dependence of the  $\ln k$  of serotonin on the  $\ln I$  (Experimental conditions: 25% MeOH and 75% aqueous component consisting of 10 mmol/L  $C_7H_{15}SO_3Na$ , 0.1%  $H_3PO_4$  and KBr /  $CH_3COONH_4$ ).

Two different electrolytes were added into the aqueous phase, potassium bromide and ammonium acetate, respectively, in order to observe their effect on the analytes retention. According to the Hofmeister series, the ammonium salt has a major ability to strengthen the hydrophobic interactions than potassium bromide. Experimentally, lower retention times were observed for both of the salts, but the magnitude of the decrease was larger when KBr was used (see Fig. 8).

### The influence of temperature

Temperature has a significant influence on all chromatographic separations. Generally, elevated temperatures decrease viscosity and increase solubility and diffusivity, thus the retention, peak shape, column efficiency, and total analysis time are affected. Particularly, in IP mechanism the main influence of increasing temperature is to decrease the

interaction between the ion pairs and the stationary phase, in accordance to the van't Hoff equation.<sup>23</sup> Linear  $\ln k$  vs  $1/T$  relationships have been obtained within the interval 20–55°C, as can be seen in Fig. 9 for the neurotransmitters investigated.

### CONCLUSIONS

Two theoretical models, i.e. electrostatic and partition models, can be used to explain the chromatographic behavior of the chosen analytes investigated under ion-pairing mechanism in liquid chromatography. Thus, the influence of the hydrophobicity of the ion pairing agent and its concentration in mobile phase as well as the ionic strength effect are fairly explained by the electrostatic model and good correlations were obtained for the dependences between experimental parameters and the chromatographic results as predicted by this theoretical model.

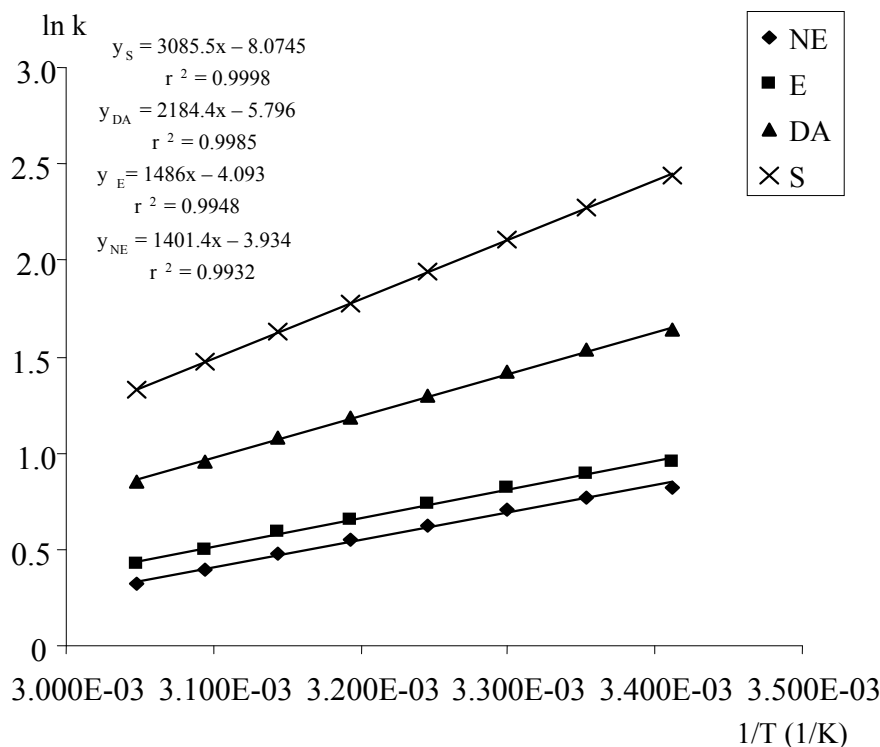


Fig. 9 – Dependence of the  $\ln k$  of the neurotransmitters on  $1/T$  (experimental conditions: 25% MeOH and 75% aqueous component consisting of 10 mmol/L  $C_7H_{15}SO_3Na$ , pH = 2).

The influence of the mobile phase composition and pH can be explained by the partition model, and the functional dependences between experimental parameters and the chromatographic outcome were very well correlated. Furthermore, these correlations can be used in estimating of some extrapolated values useful in characterizing the ion pair between analyte and ion pairing agent by means of the hydrophobicity descriptor.

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## REFERENCES

- G.J. Soleas, M. Carey and D.M. Goldberg, *Food Chem.*, **1999**, *64*, 49-58.
- A. Onal, *Food Chem.*, **2007**, *103*, 1475-1486.
- J. Lange, K. Thomas and C. Wittmann, *J. Chromatogr. B*, **2002**, *779*, 229-239.
- H. Siren and U. Karjalainen, *J. Chromatogr. A*, **1999**, *853*, 527-533.
- A. Kovacs, L. Simon-Sarkadi and K. Gazle, *J. Chromatogr. A*, **1999**, *836*, 305-313.
- F. Xu, M. Gao, G. Shi, L. Wang, W. Zhang, J. Xue, L. Jin and J. Jin, *Anal. Chim. Acta*, **2001**, *439*, 239-246.
- E.C.Y. Chan, P.Y. Wee, P.Y. Ho and P.C. Ho, *J. Chromatogr. B*, **2000**, *749*, 179-189.
- E.C.Y. Chan and P.C. Ho, *Rapid. Commun. Mass Spectrom.*, **2000**, *14*, 1959-1964.
- M.-Y. Zhang, C. E. Beyer, *J. Pharm. Biomed. Anal.*, **2006**, *40*, 492-499.
- E. Dadaova, M. Krizek and T. Pelikaova, *Food Chem.*, **2009**, *16*, 365-370.
- S. Zhang, Q. Xub, W. Zhang, L. Jin and J.Y. Jin, *Anal. Chim. Acta*, **2001**, *427*, 45-53.
- A. Kumar, J.P. Hart and D.V. McCalley, *J. Chromatogr. A*, **2011**, *1218*, 3854-3861.
- M.-Y. Zhang and C. E. Beyer, *J. Pharm. Biomed. Anal.*, **2006**, *40*, 492-499.
- M. C. Vidal-Carou, F. Lahoz-Portoles, S. Bover-Cid and A. Marine-Font, *J. Chromatogr. A*, **2003**, *998*, 235-241.
- N. Şanlı, S. Şanlı and G. Ozkan, *Hacettepe J. Biol. & Chem.*, **2007**, *35*, 129-134.
- C.S. Okereke, *J. Pharm. Pharmaceut. Sci.*, **2002**, *5*, 146-161.
- Human Metabolome Database – <http://www.hmdb.ca/metabolites/HMDB01336>
- A. Bartha and J. Stahlberg, *J. Chromatogr. A*, **1994**, *668*, 255-284.
- T. Cecchi, *J. Chromatogr. A*, **2005**, *1072*, 201-206.
- V. David, F. Albu and A. Medvedovici, *J. Liq. Chromatogr. Rel. Technol.*, **2005**, *28*, 81-95.
- M. Radulescu and V. David, *L. Liq. Chromatogr. Rel. Technol.*, **2012**, *35*, 2042-2053.
- V. David and A. Medvedovici, *J. Liq. Chromatogr. Rel. Technol.*, **2007**, *30*, 761-789.
- T. Cecchi, *Crit. Rev. Anal. Chem.*, **2008**, *38*, 161-213.