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Investigation into the phenomena affecting the retention behavior of basic analytes in chaotropic chromatography: Joint effects of the most relevant chromatographic factors and analytes' molecular properties

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ABSTRACT

The aim of this study was to systematically investigate the phenomena affecting the retention behavior of structurally diverse basic drugs in ion-interaction chromatographic systems with chaotropic additives. To this end, the influence of three factors was studied: pH value of the aqueous phase, concentration of sodium hexafluorophosphate, and content of acetonitrile in the mobile phase. Mobile phase pH was found to affect the thermodynamic equilibria in the studied system beyond its effects on the analytes' ionization state. Specifically, increasing pH from 2 to 4 led to longer retention times, even with analytes which remain completely protonated. An explanation for this phenomenon was sought by studying the adsorption behavior of acetonitrile and chaotropic additive onto stationary phase. It was shown that the magnitude of the developed surface potential, which significantly affects retention – increases with pH, and that this can be attributed to the larger surface excess of acetonitrile. To study how analytes' structural properties influence their retention, quantitative structure–retention modeling was performed next. A support vector machine regression model was developed, relating mobile phase constituents and structural descriptors with retention data. While the ETA_EtaP_B_RC and XlogP can be considered as molecular descriptors which describe factors affecting retention in any RP-HPLC system, TDB9p and RDF45p are molecular descriptors which account for spatial arrangement of polarizable atoms and they can clearly relate to analytes' behavior on the stationary phase surface, where the electrostatic potential develops. Complementarity of analytes' structure with that of the electric double layer can be seen as a key factor influencing their retention behavior. Structural diversity of analytes and good predictive capabilities over a range of experimental conditions make the established model a useful tool in predicting retention behavior in the studied chromatographic system.

1. Introduction

Chaotropic salts have been used extensively for the analysis of protonated basic compounds in reverse-phase chromatographic systems (RP-HPLC). Significant effort has been invested into explaining the role of chaotropic salts in RP-HPLC systems and their influence on the chromatographic behavior of protonated basic analytes [1–8]. Different retention models have been developed with the aim to appropriately describe analytes' retention in chromatographic systems modified with classical ion-par agents [9–11] or chaotropic agents [12–15]. However, certain aspects of the retention phenomena in these systems have seldom been investigated. For example, the pH of the mobile phase is usually adjusted to the value which will assure complete protonation of the analyte without degrading the column package material. While this is often an appropriate choice, it is not entirely clear whether pH variance affects retention in the presence of chaotropic agents, beyond well-described effect on analytes' ionization state. Furthermore, very few attempts have been made to describe the relationship between analytes' structure and their retention in the presence of chaotropic salts [15–17]. It is commonly observed that analytes' sensitivity to chaotropes increases with their lipophilicity, but apart from this finding little is known on the specific structural determinants that govern analytes' retention in these systems.

The aim of the present work was to systematically investigate these outstanding questions regarding the retention behavior of basic drugs in ion-interaction chromatographic systems with chaotropic additives. To this end, pH value of the aqueous phase,

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concentration of the chaotropic salt (sodium hexafluorophosphate – $NaPF_6$), and the content of acetonitrile in the mobile phase were varied and the retention behavior of 34 structurally diverse basic drugs was monitored. The obtained retention data was first analyzed from a qualitative point of view revealing unexpected interaction between the studied factors. A quantitative model was subsequently developed, relating mobile phase composition and structural descriptors with retention data. In addition to its predictive capabilities over a range of experimental conditions, the analysis of the established model provided novel insights into the structural factors influencing analytes' retention behavior in the considered chromatographic system.

2. Material and methods

2.1. Chemicals

All used chemicals were of the analytical grade. Acetonitrile (Fluka, Steinheim, Germany), sodium hexafluorophosphate – $NaPF_6$ (Aldrich, St. Louis, MO, USA), hydrochloric acid (Zorka Pharma, Šabac, Serbia), sodium hydroxide (Zorka Pharma, Šabac, Serbia) and water (HPLC grade) filtered through Simplicity 185 (Millipore, Billerica, MA, USA) were used for the preparation of the mobile phases for the chromatographic profiling of the analytes and the determination of adsorption isotherms. For the determination of the column surface area *para*-toluenesulfonic acid (Acros Organics, Geel, Belgium) was used as a solute, while the mobile phase was prepared of methanol (Fluka, Steinheim, Germany) and the buffer solution made of potassium dihydrogen phosphate (Merck, Darmstadt, Germany) and *ortho*-phosphoric acid (J.T. Baker, Deventer, Holland). Reference standards of analyzed substances: moclobemide, clonazepam, maprotiline hydrochloride, sulpiride, thioridazine hydrochloride, perazine dimalonate, pheniramine maleate, chlorpheniramine maleate, duloxetine hydrochloride, amitriptyline hydrochloride, fluvoxamine maleate, clomipramine hydrochloride, desloratadine, loratadine, sertraline hydrochloride, sumatriptan succinate, eletriptan hydrobromide, rizatriptan benzoate, lorazepam, carbamazepine, prazepam, diazepam, bromazepam, fluoxetine hydrochloride, venlafaxine hydrochloride, selegiline hydrochloride, lamotrigine, risperidone, olanzapine, cinnarizine, cetirizine, clozapine, tramadol, mianserin hydrochloride were obtained from LGC GmbH (Luckenwalde, Germany).

2.2. Solutions

Stock solutions were prepared by dissolving the appropriate amount of standard substances in the mixture acetonitrile–water (50:50, v/v), except cinnarizine that was dissolved in pure acetonitrile, in order to obtain the concentrations of 1 mg/mL. Stock solutions were further diluted in mixture acetonitrile–water (50:50, v/v) to obtain working solutions in concentrations of 100 μg/mL.

2.3. Chromatographic conditions

The isocratic experiments, needed for the analysis of analytes' retention behavior, the experiment for the determination of the stationary phase total surface area, as well as the experiments for the determination of adsorption isotherms of acetonitrile from acidified water were performed on Finnigan Surveyor Thermo Scientific chromatographic system, which consisted of HPLC Pump, Autosampler Plus and UV/VIS Plus Detector. ChromQuest was used for data collection and analysis. Luna C18 column, 150 mm × 4.6 mm, 5 μm (Phenomenex, CA, USA) was used in all the experiments. Column temperature was set to 30 °C and flow rate was 1 mL/min.

Nine adsorption isotherms of $NaPF_6$, under different chromatographic conditions, were recorded on Acella Thermo Scientific chromatographic system, which consisted of HPLC Pump, Autosampler and PDA Detector. ChromQuest was used for data collection and analysis. Flow rate was 1 mL/min, while column temperature was set to 30 °C.

2.4. Chromatographic conditions for the analytes' profiling

For this investigation 36 different mobile phases consisted of acetonitrile and aqueous phase (1 mM, 4 mM, 7 mM or 10 mM of $NaPF_6$ with pH adjusted to 2, 3 or 4 with concentrated hydrochloric acid or 10 M sodium hydroxide) were prepared. Ratio of acetonitrile and aqueous phase content was 35:65, 37.5:62.5 or 40:60 (v/v). Detection was carried out at 220 nm for all analytes.

2.5. Determination of the column surface area

Frontal analysis was performed using a gradient delivery system with two pumps in seven steps (0–0.95 mM *p*-toluenesulphonate) at 235 nm. Mobile phase A consisted of 5% methanol and 95% 1 mM *p*-toluenesulphonate, while mobile phase B consisted of 5% methanol and 95% phosphate buffer. Ionic strength of both mobile phases was 100 mM and pH of both mobile phases was adjusted with *ortho*-phosphoric acid to pH 3. The amount of *p*-toluene sulphonate adsorbed onto the stationary phase LH (μmol) was calculated from the breakthrough times. Retention times of the breakthrough curve were corrected for the system delay time (retention time of breakthrough curve when the column is replaced with the zero-volume unit):

$$\mathrm{LH} = \mathrm{flowrate} \cdot (t_r - t_0) \cdot [H] \tag{1}$$

where t_r is a retention time of breakthrough curve corrected for the system delay time, t_0 is a void volume time (retention time of water injected in HPLC) and $[H]$ is the concentration of *p*-toluenesulphonate in the eluent (mM). The obtained data was fitted to the following equation [18]:

$$[H] = \frac{\mathrm{LH}}{K_{\mathrm{LH}} \cdot (L_T - LH)} \left(\frac{\mathrm{LH} \cdot f}{A} + \left(\left(\frac{\mathrm{LH} \cdot f}{A} \right)^2 + 1 \right)^{0.5} \right)^{2|Z_H|} \tag{2}$$

where $[H]$ is the concentration of *p*-toluenesulphonate in the eluent, LH represents its total amount adsorbed onto the stationary phase, A is the chromatographically accessible surface area of column, L_T indicates the total ligand sites (monolayer capacity of column, μmol) and K_{LH} corresponds to the constant of thermodynamic equilibrium for the adsorption of H onto the stationary phase free ligand site (L). The constant f (m^2/mol) was calculated from the experimental conditions [18]:

$$f = \frac{|Z_H| F}{\left(8 \varepsilon_0 \varepsilon_r R T \sum_i c_{oi} \right)^{0.5}} \tag{3}$$

where R is the universal gas constant, F is Faraday constant, T absolute temperature, ε_0 the vacuum permittivity, ε_r the dielectric constant of the mobile phase and Σc_i is the mobile phase concentration (mM) of singly charged electrolyte ions.

Fittings for the determination of total column surface area were processed in MATLAB® 7.10.0. (The MathWorks, Inc., USA). The best estimates of the adjustable parameters with 95% confidence interval were 241.5 m^2 for A, 0.1086 mM^{-1} for K_{LH}, 158.1 μmol for L_T. The correlation coefficient was 1.000, sum of squared errors (SSE) was 3.425e−06, and root mean square error (RMSE) was 9.254e−04.