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Usefulness of reversed-phase HPLC enriched with room temperature imidazolium based ionic liquids for lipophilicity determination of the newly synthesized analgesic active urea derivatives

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ABSTRACT

Lipophilicity of several novel analgesic active 1-(1-arylimidazolidyn-2-ylidyn)-3-arylalkyl urea derivatives has been estimated by the use of chromatographic method. The investigated compounds were analyzed by reversed-phase high performance liquid chromatography (RP-HPLC) using mixtures of methanol or acetonitrile and water with addition of imidazolium based room temperature ionic liquids varying in an anion chaotropicity as the mobile phases. The relationships between log k values vs. concentration of organic solvent was used for determination of the log k_w values by extrapolation technique. The partition coefficients (log P) values were calculated by means of the Pallas 3.1.1.2. and Spartan 10.0 softwares and further correlated with log k_w measured experimentally in classical organic–aqueous eluent system and systems modified with ionic liquids addition. It was found that log k_w values measured in eluent system modified with butyl-methyl imidazoilum chloride correlate the best with the logarithm of partition coefficient calculated by Pallas software (log $P_{calc.}$). Furthermore, it was found that the examined compounds form H-bonding with imidazoilum cation of modifiers improving the chromatographic peak parameters (the symmetry factor, the theoretical plates number) especially when ionic liquid's anion was more chaotropic. Amphiphilic ionic liquid possessing longer alkyl chain substituent (OMIM BF_4) can be considered as a new cationic surfactant. Micellar conditions improved separation selectivity of chloro- and methoxy substituted derivatives.

1. Introduction

Since the pioneering work of Overton and Meyer [1,2] at the beginning of the 20th century and later Hansch' [3] studies, lipophilicity has become fundamental descriptor in quantitative structure–activity relationships (QSAR) examining the potential pharmacological effect of newly synthesized drug candidates.

Lipophilicity can be either predicted computationally (fragmental methods, models based on molecular properties, atomic contribution, surface area etc.) or measured in liquid–liquid and solid–liquid systems based on the Nernst's law of distribution. One of the oldest methods of lipophilicity determination is the shake-flask method. The modern versions of liquid–liquid extraction offer flow injection analysis (FIA) and counter-current chromatography (CCC). In turn, such chromatographic techniques as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) or some of electrically driven separation methods use solid-liquid systems for lipophilicity determination [4–6].

Similarly to liquid–liquid extraction or crossing lipid barrier in the living organism, the main driving force in liquid chromatographic systems based on reversed-phase mode (RPLC) is partitioning of analytes between hydrophobic stationary phase and polar, usually organic, aqueous mobile phase. Relationship between retention factor (k) in chromatography and the partition coefficient ($P_{o/w}$) is expressed by Martin's equation [7]:

$$\log k = \log K + \log\left(\frac{V_s}{V_M}\right) \quad (1)$$

where log K is the chromatographic equilibrium constant, V_s, V_M are the volume of stationary and mobile phase. Assuming that the ratio $\log(V_s/V_m)$ is constant for a given chromatographic system and retention is governed by partition mechanism, we can expect that log k will be at least proportional to the distribution coefficient in its logarithmic form.

There are a few chromatographic lipophilicity parameters (log k_w, S φ_0). All of them are derived from Soczewinski–Wachtmeister

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equation describing relationship between retention factor (k) and the volume fraction (φ) of organic modifier in the mobile phase [8]:

$$\log k = \log k_w - S\varphi \quad (2)$$

According to the above equations, log k_w is retention extrapolated to pure water ($\varphi = 0$), φ_0 represents mobile phase composition permitting to obtain a retention factor of unity (log $k = 0$), S is the slope of above relationship.

Among chromatographic lipophilicity parameters, log k_w appears to be the most similar in the order of magnitude to octanol–water log $P_{o/w}$ [9,10]. It should be stressed however that the correlation between log $P_{o/w}$ or log P obtained by the use of popular computational methods and chromatographically derived lipophilicity parameters are statistically significant mainly for congeneric group of neutral solutes.

The lipophilicity measurements using RPLC appear to be imperfect mainly due to the activity of residual silanols, influences of the organic modifier on the extrapolated log k_w values, structural diversity of solutes and presence of ionizable groups in the solutes structure.

This work studies the influence of addition of ionic liquids to acetonitrile-aqueous mobile phase on lipophilicity determination of the newly synthesized derivatives of urea which are active on central nervous system (CNS) and exhibit, in vivo, significant analgesic activities with relatively low acute toxicities. An opioid-like mechanism was confirmed by reversion of the antinociception by naloxon in the Witkin test [11–13]. Their LD_{50} values were found to be in the range from 1100 to over 2000 mg kg^{-1}. The strength of their activity depends on the nature of substituents.

The aim of the presented investigations was comparison of conventional reversed-phase high performance liquid chromatography (RP-HPLC) and RP-HPLC enriched with room temperature imidazolium based ionic liquids as for chromatographic behavior and agreement of obtained experimental lipophilicty parameters with theoretical values calculated by computer-assisted softwares. For the first time the existence of interaction between the cation of imidazolium based ionic liquid and unionized solutes, affecting the chromatographic behavior was demonstrated.

2. Experimental

2.1. Materials

The compounds: 1-(1-arylimidazolidyn-2-ylidyn)-3-arylalkyl urea derivatives investigated in the following study (Fig. 1) were synthesized in Department of Medical Chemistry, Medical University of Lublin. The synthesis procedure of this novel bioactive set of compounds was described in the Polish Patents [11–13]. The structures of all the investigated solutes were confirmed by ^{1}H NMR spectroscopy. Their purity was assessed by elemental analysis.

Ionic liquids: 1-butyl-3-methyl-imidazolium hexafluorophosphate (BMIM PF_6), 1-butyl-3-methyl-imidazolium chloride (BMIM Cl) and 1-butyl-3-methyl-imidazolium tetrafluoroborate (BMIM BF_4), 1-octyl-3-methyl imidazolium tetrafluoroborate (OMIM BF_4) were obtained from Fluka. The concentrations of the additives were in the range from 1 to 30 mmol l^{-1}, in the whole mobile phase. HPLC grade acetonitrile (ACN) and methanol (MeOH) were purchased from Merck (Darmstadt, Germany). HPLC water was obtained from Barnstead deionising system (Dubuque, IA, USA). All mobile phases were filtered with Nylon 66 membrane filter (0.45 μm) Whatman (Maidstone, England) by the use of a filtration apparatus.

2.2. HPLC apparatus

Experiments were performed using a LaChrom HPLC Merck Hitachi (E.Merck, Darmstadt, Germany) model equipped with diode array detector, column oven L-7350 and solvent degasser L-7612. The column (150 mm × 4.6 mm I.D.) was packed with 5-μm Zorbax Extend-C18 Agilent Technologies (Santa Clara, CA, USA); its void volume was determined to be 1.31 ml, by the injection of thiourea in acetonitrile–water (50:50) eluent system. Retention data were recorded at a flow-rate of 1 ml min^{-1}. The column was thermostated at 20 °C ± 0.1. Injected solutions were prepared at 0.1 mg ml^{-1} concentrations in aqueous methanol. The monitoring of the drugs was at 220 nm for organic aqueous eluent system and 265 nm for eluents modified with ionic liquids. Typical injection volumes were 2.5 μl. Duplicate injections were done.

2.3. RP-HPLC column

Extend-C18 column applied in this study offers a unique method of protection of the silica from dissolution at a wide range of eluent pH. Bidentate bonding technology combined with a double-endcapping process provides not only high pH stability but also prevents especially basic groups from any potential extremely disadvantageous silanophilic interactions.

Bonded phase	Pore size	Surface area	Temp. limits	pH range	End capped	Carbon load
ZORBAX Extend-C18	80 Å	180 m^2/g	60 °C	2.0–11.5	Double	12.5%

It should be noted that pH of the organic–aqueous eluent used in the present study independently of the modifier type can be generally referred to as pH 5–7. Even underivatized silanol groups will remain ionized taking into account their pK_a which is about 4.0. In turn at pH 5–7 the investigated compounds are neutral.

2.4. Calculation methods

Calculations devoted to theoretical plate number and asymmetry were performed using HSM program. The following equation was used to calculate the number of theoretical plates according to USP standards: $N = 16(R_T/W)^2$, where R_T stands for the actual full retention time of the appropriate peak, W equals the peak width obtained by drawing tangents to each side of the peak and by calculating the distance between the two points where the tangents meet the baseline. The HSM program uses the following equation to calculate asymmetry: A_s equals ½(1 + B/A), where A and B are evaluated at a 5% peak height of an appropriate peak. The capacity factor (k) was calculated as follows: $k = (t_r - t_0)/t_0$, where t_r stands for the actual retention time of the individual peak, t_0 is the elution of the unretained sample (thiourea).

Theoretical partition coefficients (log $P_{calc.}$) of the examined solutes were calculated using Pallas (version 3.1.1.2) software and Spartan (version 1.0.). Multivariate regressions were performed with Microsoft Office Excel 2007 and Statistica (version 5.1) respectively.

3. Results and discussion

3.1. Determination of chromatographic lipophilicity parameters on the basis of the relationships log k versus volume fraction of organic solvent in the mobile phase (φ)

Log k values were linearly extrapolated according to Eq. (2). In all cases very high correlation coefficients were obtained. Extrapolated log k_w values and the corresponding slopes (S) and φ_0 indices are presented in Table 1, along with their statistics.

Furthermore, existence of statistically significant correlations between log k_w and S either in acetonitrile or methanol containing mobile phase confirms structural similarity of investigated compounds and correctness of applied chromatographic method