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Determination and importance of temperature dependence of retention coefficient (RPHPLC) in QSAR model of nitrazepams' partition coefficient in bile acid micelles

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ABSTRACT

Linear dependence between temperature (t) and retention coefficient (k, reversed phase HPLC) of bile acids is obtained. Parameters (a, intercept and b, slope) of the linear function $k=f(t)$ highly correlate with bile acids' structures. Investigated bile acids form linear congeneric groups on a principal component (calculated from $k=f(t)$) score plot that are in accordance with conformations of the hydroxyl and oxo groups in a bile acid steroid skeleton.

Partition coefficient (K_p) of nitrazepam in bile acids' micelles is investigated. Nitrazepam molecules incorporated in micelles show modified bioavailability (depo effect, higher permeability, etc.). Using multiple linear regression method QSAR models of nitrazepams' partition coefficient, K_p are derived on the temperatures of 25 °C and 37 °C. For deriving linear regression models on both temperatures experimentally obtained lipophilicity parameters are included (PC1 from data $k=f(t)$) and *in silico* descriptors of the shape of a molecule while on the higher temperature molecular polarisation is introduced. This indicates the fact that the incorporation mechanism of nitrazepam in BA micelles changes on the higher temperatures. QSAR models are derived using partial least squares method as well. Experimental parameters $k=f(t)$ are shown to be significant predictive variables. Both QSAR models are validated using cross validation and internal validation method. PLS models have slightly higher predictive capability than MLR models.

1. Introduction

The aim of Quantitative Structure Activity (Property) Relationship (QSA (P) R) research is to find functional dependence between molecule structure and its pharmaco-biochemical activities or physico-chemical properties. Important feature of derived mathematical model is its ability to predict activity (property) of molecules not directly included in experiment (molecules not yet synthesised or those with limited *in vivo* and *in vitro* experiments due to economic or ethical reasons). Mathematical models give additional information about activity of a molecule and through correlations with molecular descriptors explain receptors binding places (enzyme, ionic channel, etc.) [1–8].

Bile acids (BAs) are surface active molecules with steroid skeleton [9–11]. Besides their well known physiological role in lipid metabolism regulation, BAs are used as promoters in transport of some drugs through the cell membrane or other physiological barriers (blood–brain barrier, etc.) [12–15]. Above certain concentration – critical micellar concentration BAs form aggregates i.e. micelles that have the possibility to accept hydrophobic molecule – guest (drug) changing thus its bioavailability [16–18]. Interaction between BA micelle and its hydrophobic guest can be described with partition coefficient (K_p) [19]. If a BA is more hydrophobic it has a higher capacity to accept hydrophobic drug so its effect on BA bioavailability is higher.

Everything mentioned above indicates importance of bile acids' hydrophobicity (lipophilicity) in describing interactions between their micellar solutions and nitrazepam. Thus, it is expected that QSAR model for partition coefficient contains descriptor for lipophilicity of bile acids. Bile acids' lipophilicity is usually expressed as a logarithm of partition coefficient between 1-octanol and water ($\log P$). Traditional shake flask method for deriving $\log P$ is shown to be less precise and reproductive than different chromatographic parameters [20]. Because of that, goal of the first part of our work was to find a temperature dependence (t) of retention coefficient (k) obtained in reversed phase chromatography (RPHPLC) in order to gain experimental, predictive BA variables that describe the change of molecules' hydrophobicity [21], i.e. to derive a novel chromatographic parameter for describing bile acids' lipophilicity. According to that, hydrophobicity parameters should

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be extracted from chromatographic data ($k=f(t)$) that describe the best structural i.e. conformational characteristics of bile acids. The second part of the work deals with deriving QSAR model between nitrazepams' (probe molecule) partition coefficient (K_p) and BA structure using multiple linear regression (MLP) and partial least square (PLS) methods. Experimentally obtained lipophilicity parameters of temperature dependence of BA retention coefficient (k) and *in silico* molecular descriptors (topological and electronic) are used as predictive variables in deriving QSAR model. Parameters of lipophilicity are included as predictive variables because a lot of molecular descriptors calculated from the molecular graph have the same value if they belong to the same congeneric group (for instance the cholic acid and its keto derivatives have the same value of Wieners' index which is 2045). In this paper a novel molecular descriptor (ND) is introduced which describes bile acids' steroid skeleton i.e. spatial orientation of substituents and their mutual distance in the BA steroid skeleton. Descriptor ND has characteristics of both 2D and 3D topological descriptors. That is the reason why bile acids from the same congeneric group have different ND descriptor values [22]. The relationship between chromatographic descriptors (descriptors gained from $k=f(t)$ is observed as well and *in silico* descriptors where ND is included). BAs with one, two and three hydroxyl group and their glyco-, tauro and oxo derivatives are investigated (Fig. 1). Particular attention is paid to BA oxo derivatives that have growing pharmacological use due to lower membranolytic activity [12,23].

2. Materials and methods

2.1. Chemicals and solutions

Bile acids (1–14), 98% purity purchased from Sigma, New Zealand were used as starting compounds for the synthesis of its oxo derivatives (15–25). The syntheses of bile acids oxo derivatives and their transformation to sodium salts were carried out according to previously described procedures [9,16]. Methanol, HPLC grade was obtained from Carlo Erba Reagenti, Italy, KH_2PO_4 and Na_2PO_4 from Lachner, Czech Republic. Nitrazepam 99, 98% purity was purchased from Sigma, New Zealand and NaCl pro analysis from Merck, Germany.

2.2. Reverse phase HPLC method

The HPLC system Agilent 1100 Series, equipped with degasser, binary pump, automatic injector and DAD detector with software system for data processing AgilentChemStation was used and the analyses were performed on a reversed-phase C-18 column: Eclipse Plus C18 (250 mm × 3 mm, 5 μm, 250 Å) column (Zorbax SD). The mobile phase was 0.01 M phosphate buffer:methanol = 70:130 (v/v) maintained at pH 7 and the injection volume was 10 μL. Solutions of bile acids and their derivatives in mobile phase were prepared in concentration of 1 mg/ml. All separations were performed isocratically at a flow rate of 1 ml/min and a column temperature changing from 20 to 45 °C. The detection was performed at 210 nm [24].

The HPLC capacity factor (k) was calculated from the eluted peak retention time (t):

$$k = \frac{t_x - t_0}{t_0}$$

where t_x and t_0 are the retention times of the bile acids and the unretained solvent front respectively. Linear dependence between (k) and temperature (t) is calculated for the each BA:

$$k = a + bt \quad (1)$$

2.3. Spectrophotometric determination of nitrazepams' partition coefficient in bile salt micelles

Experiments were carried out according to De Castro et al. [19], using Agilent 8453 spectrophotometer equipped with the Peltier thermostated cell holder (25 and 37 °C). Critical micellar concentrations of bile salts (cholic acid and its keto derivatives) used for calculation of nitrazepams' partition coefficient were taken from [10,9].

2.4. Data treatment

For PCA, MLR and PLS analyses Statistica 7 was used [25].

3. Molecular descriptors

The molecular descriptors calculated with SciQSAR option of the molecular modeling computer program ALCHEMY 2000 [24] were the following: the first-order (^1X) and the third-order (^3X) connectivity index, the zero-order ($^0X^v$) and first-order ($^1X^v$) valence connectivity index, the third-order shape index for molecule ($^3K_\alpha$), the Wiener (W) index, volume (V), molar mass (M), dipole moment (DM), molecular polarizability, specific molar polarizability (SP), the largest positive charge over the atoms in molecule, in electrons (Q_+), the largest negative charge over the atoms in a molecule, in electrons (Q_-), the sum of absolute values of the charges on each atom of the molecule, in electrons (SQ), the sum of absolute values of the charges on the nitrogen and oxygen in the molecule, in electrons (SQ_{NO}) and the partition coefficient ($\log P$). Molecular ovality (Oval) and Connolly excluded volume (CSEV) are calculated with ChemBio3D Drew 10 software [26]. In all cases the structures of the compounds were pre-optimized with the Molecular Mechanics Force Field (MM+) procedure included in Hyperchem version 7.5 [27], and the resulting geometries were further submitted to the semi empirical method PM3 (Parametric Method-3) using the Fletcher–Reeves algorithm and a gradient norm limit of 0.009 kcal/Å.

The next formula, introduced in this paper, was used to calculate the novel descriptor (ND):

$$\mathrm{ND} = \frac{\frac{1}{n}\sum \angle_{O,a\mathrm{M}}}{\sum d_{O,O} + \sum d_{O,ph}}$$

where n represents the number of carbon atoms with hydroxyl and oxo groups in BAs steroid skeleton; $\angle_{O,a\mathrm{M}}$ represents the angle between β axial (a) methyl group and hydroxyl or oxo group in the proper Newmanns' projection formulas ($\angle_{O,a\mathrm{M}}$: $\alpha(a)$ OH = 180°; α(equatorial, e) OH or oxo = 120°; $\beta(e)$ OH or oxo = 60°), $d_{O,O}$ represents distance between carbon atoms with hydroxyl or oxo groups from steroid skeleton (number of single connection is taken as a unit), while $d_{O,ph}$ represents distance between carbon atoms with hydroxyl or oxo substituents and polar head of the side chain (as a unit the number of single connection as the shortest way in BA molecule graph is taken).

4. Results and discussions

4.1. Lipophilicity parameters: temperature dependence of retention coefficient

Linear equation (Eq. (1)) that connects BA retention coefficient (k) with temperature (t) fits very well with experimental data (Table 1). Linear model explains 96–99% of the whole variance (determination coefficient (R^2), Table 2). There is a good correlation (Eq. (2)) between the slope (b) and the intercept (a) in Eq. (1)