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The use of immobilized artificial membrane chromatography to predict bioconcentration of pharmaceutical compounds

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ABSTRACT

The potential of immobilized artificial membrane chromatography (IAM) to predict bioconcentration factors (BCF) of pharmaceutical compounds in aquatic organisms was studied. For this purpose, retention factors extrapolated to pure aqueous phase, $\log k_{w(IAM)}$, of 27 drugs were measured on an IAM stationary phase, IAM.PC.MG type. The data were combined with retention factors on two IAM columns, IAM.PC.MG and IAM.PC.DD2 types, reported previously by our research group and correlated with logBCF values predicted by Estimation Program Interface (EPI Suite) Software. Linear models were established upon exclusion of ionic or highly hydrophilic nonionic drugs, for which a constant value of logBCF equal to 0.50 was arbitrarily assigned by EPI Suite Software. As additional physicochemical parameter BioWin5 proved to be statistically significant, expressing the decrease of bioaccumulation potential as a result of biodegradation in the aquatic environment. The constructed IAM model was successfully validated by application to a set of pharmaceuticals, whose experimental BCF values are available. Better predictions compared to EPI Suite Software were achieved for the dataset under study. Since bioconcentration process involves electrostatic interactions, IAM retention may be a better measure for BCF values, especially for ionic species, compared to octanol-water partition coefficients widely implemented in environmental sciences. The developed approach can be considered as a novel tool for the prediction of bioconcentration of pharmaceutical compounds in aquatic organisms in order to minimize further experimental assays in the future.

1. Introduction

Pharmaceutical compounds and their metabolites constitute an important group of unregulated emerging pollutants. They continuously enter the environment via various routes, such as excretion as unmodified parent drugs or bioactive metabolites, disposal of leftover drugs or even applications to animal- feeding operations (Jagiello et al., 2015; Brillas et al., 2015). There are sufficient reports of their widespread distribution in environmental compartments, even at low concentrations, ranging from nanograms to micrograms per liter in the case of surface, ground and drinking water (Daughton, 2001; Huerta et al., 2012; Li, 2014; Brillas et al., 2015; Puckowski et al., 2016). However, even such low concentrations can favor the development of multi-resistant strains of microorganisms in the case of antibiotics, exert toxic effects on fishes, algae and invertebrates and disrupt the endocrine system of living organisms (Jagiello et al., 2015; Brillas et al., 2015). Moreover, pharmaceuticals are known as "pseudo-persistent" contaminants, since their high transformation/ removal rates in the environment are compensated by their continuous introduction into the aquatic systems, leading to a prolonged exposure of aquatic organisms (Daughton, 2001; Schultz et al., 2011; Puckowski et al., 2016).

According to the environmental sciences, bioaccumulation can be defined as the simple uptake of a xenobiotic from the environment or the retention of the chemical to aquatic organisms (Zenker et al., 2014). A special case of nondietary bioaccumulation of a substance dissolved in water is referred as bioconcentration (Meylan et al., 1999). It is expressed by the bioconcentration factor (BCF), which is the ratio of the equilibrium concentration of the chemical in the exposed organism and in the surrounding water environment (Meylan et al., 1999; Zenker et al., 2014). As humans are consumers of fish and shellfish, BCF constitutes a measure of human exposure to chemicals in the environment (Meylan et al., 1999).

BCF can be experimentally determined by standardized procedures,

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provided by authorities, such as the U.S. Environmental Protection Agency's (EPA) for fish (U.S. E.P.A., 1996b, Fish BCF) and oyster (U.S. E.P.A., 1996a, Oyster BCF). These tests are laborious, time consuming and expensive, while several experimental factors have to be controlled, contributing to a considerable inter-laboratory variability on the experimental reported values (Escuder-Gilabert et al., 2009). The measurement of BCF of the thousands of chemical substances of potential regulatory interest is not possible (Meylan et al., 1999). Furthermore, experimental measurements of BCFs undergo to ethical limitations, such as the legal requirement to avoid investigations in animals, associated with the principles of "3Rs" (Replacement, Reduction and Refining) of animal testing for scientific purposes, according to Directive 2010/63/ EU (Directive 2010/63/EU of 22 September 2010 on the protection of animals used for scientific purposes).

Physicochemical modeling is an alternative to the experimental evaluation of ecotoxicity. The basis of such approaches is that the properties of a certain compound are determined by its structure, expressed by a range of descriptors. Thus, quantitative structure-activity relationships (QSAR) can be employed in order to estimate bioconcentration potential of xenobiotics and supply the relevant missing data. Most commonly, BCF is estimated on the basis of octanol-water partition coefficient, symbolized usually as log*P* in Medicinal Chemistry and as $\log K_{ow}$ in environmental sciences (EC, Joint Research Centre, European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM)). A very popular predictive tool in Environmental Sciences is the Estimation Program Interface (EPI Suite) Software (U.S. EPA, 2012), which predicts BCF from $\log K_{ow}$ values and correction factors. The determination of octanol-water distribution coefficients is however also laborious and time consuming, while they can not be reliably determined for highly lipophilic or compounds undergoing degradation. Alternatively, octanol-water partitioning can be predicted by a large arsenal of calculation systems (Meylan et al., 1999; Leo et al., 2000; Mannhold et al., 2009). Since such calculation algorithms are based on experimental data, their applicability domain largely depends on the degree of similarity of the compounds under investigation with the training set used (Mannhold et al., 2009). Thus, often different predictions are provided by the different algorithms, especially in the case of new chemotypes (Chrysanthakopoulos et al., 2009).

A popular alternative to octanol-water partitioning is Immobilized Artificial Membrane (IAM) chromatography, combining the simulation of amphiphilic microenvironment of biological membranes with rapid chromatographic measurements. IAM stationary phases consist by monolayers of phospholipids immobilized on propylamine- silica skeleton. The commercially available IAM.PC.DD2 and IAM.PC.MG columns differ in the end- capping of the free popylamino residues, but showed similar elution mechanism (Grumetto et al. (2013), (Grumetto et al., 2014; Tsopelas et al., 2015). Recent publications confirmed that IAM retention expresses both partitioning and electrostatic interactions, mainly in the case of basic compounds, due to the attraction of protonated species by phosphate anions, located close to the hydrophobic core of phospholipids (Vrakas et al., 2006, 2008; Tsopelas et al., 2015, 2016b). IAM retention proved to correlate with permeability through the main human physiological barriers, in particular the intestinal and the Blood- Brain Barrier (Tsopelas et al., 2016b). Despite its wide implementation to Pharmaceutical Sciences (Tsopelas et al., 2016b; Valko, 2016), application of IAM chromatography to environmental studies is rather rare. Recently, the contribution of IAM chromatography to the simulation of nonspecific toxicity to fishes (fathead minnow fish and tadpole) has been reported (Hidalgo-Rodriguez et al., 2012; Fernandez-Pumarega et al., 2015).

Thus, we considered it interesting to perform an investigation of the potential of IAM chromatography to model bioconcentration of pharmaceutical compounds in aquatic organisms, such as fish and mussels. For this purpose, IAM retention factors of 27 structurally diverse drugs were measured in the present study and combined with previous data of 98 pharmaceutical compounds obtained under analogous conditions. The combined 125 IAM retention factors were correlated with BCF values predicted by EPI Suite Software. Additional physicochemical parameters were also tested to improve the relationships. The validation of the developed model and comparison with EPI Suite Software was achieved using experimental data taken from the literature.

Table 1
Octanol- water partition (log*P*) and distribution coefficient at pH = 7.4, $\log D^{7.4}$ and the corresponding molecular fractions of positively (F^+), negatively (F^-) and zwitterionic species (F^z).

No	Drug molecule	log*P*[a]	$\log D^{7.4}$[a]	F^{+}[b]	F^{-}[b]	F^{z}[b]
1.	Alprazolam	2.12	2.12[c]	0.000	0.000	0.000
2.	Aripiprazole	4.38	3.96[d]	0.638	0.000	0.000
3.	Azithromycin	4.02	1.72[d]	0.996	0.000	0.000
4.	Benzocaine	1.86	1.86[c]	0.000	0.000	0.000
5.	Cefazolin	−0.58	−2.52[d]	0.000	1.000	0.000
6.	Chloramphenicol	1.14	1.14[c]	0.000	0.000	0.000
7.	Cimetidine	0.40	0.35	0.218	0.000	0.000
8.	Clarithromycin	3.16	1.94[d]	0.946	0.000	0.000
9.	Diclofenac	4.40	1.22	0.000	0.999	0.000
10.	Epinephrine	−1.20	−2.42[d]	0.944	0.000	0.002
11.	Esomeprazole	1.61[e]	1.06[e]	0.737	0.000	0.000
12.	Furosemide	2.03	−1.83[d]	0.000	1.000	0.000
13.	Hydrocortisone	1.60	1.60[c]	0.000	0.000	0.000
14.	Itraconazole	5.66	5.66[d]	0.011	0.000	0.000
15.	Levofloxacin	−0.25	−0.25	0.047	0.096	0.851
16.	Meloxicam	3.02	0.09	0.000	0.999	0.000
17.	Methylprednisolone	1.95	1.95[c]	0.000	0.000	0.000
18.	Metoclopramide	2.64	0.64	0.989	0.000	0.000
19.	Metronidazole	−0.02	−0.02[c]	0.000	0.000	0.000
20.	Nadolol	0.81	−1.16[d]	0.991	0.000	0.000
21.	Niflumic acid	4.81	0.80[d]	0.000	0.997	0.003
22.	Pantoprazole	1.80[e]	1.78[e]	0.000	0.054	0.000
23.	Progesterone	3.87	3.87[c]	0.000	0.000	0.000
24.	Sildenafil	2.73[e]	2.47[e]	0.371	0.060	0.042
25.	Testosterone	3.32	3.32[c]	0.000	0.000	0.000
26.	Venlafaxine	2.86	1.17[d]	0.982	0.000	0.000
27.	Warfarin	2.60	0.01	0.000	0.997	0.000

[a] The log*P* and log*D* data were taken from refs (Tsopelas et al., 2016a, Tsopelas et al., 2015, Klosinska- Szmurlo et al., 2014, Lombardo et al., 2001, Lombardo et al., 2000, Tsantili- Kakoulidou et al., 1997, Tsai et al., 1993) and therein cited references as well as the database (only experimental values) of ADME Boxes v 3.0 software (PharmaAlgorithms).

[b] Molecular fractions of positively (F^+), negatively (F^-) and zwitterionic (F^z) species were calculated according to ADME Boxes v. 3.0 software (PharmaAlgorithms).

[c] $\log D^{7.4}$ is equal to log*P* because drug molecule is unionized at pH = 7.4.

[d] Predicted by ADME Boxes v. 3.0 software (PharmaAlgorithms) and corrected by the difference between predicted and experimental log*P* values.

[e] Calculated by ADME Boxes v. 3.0 software (PharmaAlgorithms).

2. Experimental

2.1. IAM chromatography

The 27 drugs investigated in this work were donated by local pharmaceutical companies. They are presented in Tables 1, 2. Working solutions in a concentration of 10 mg L^{-1} of each compound were daily prepared by dissolving the necessary amount of the pharmaceutical compound in acetonitrile (≥99.9%, LC gradient grade, Merck). All reagents were of analytical grade unless otherwise mentioned. For the preparation of mobile phases KCl (≥99.5%, Riedel-de Haen), KH_2PO_4 (≥99.5%, Merck), Na_2HPO_4 (≥99.0%, Sigma- Aldrich), NaCl (≥99.5%, Sigma- Aldrich), CH_3CN (≥99.9%, LC gradient grade, Merck) and High Purity Water (HPW) were used. The HPW was obtained by means of an EASYpure II (Barnstead International, USA) water purification system. As void volume markers, sodium citrate·2x H_2O (≥99.0%, Sigma- Aldrich), $(COONH_4)_2{\cdot}H_2O$ (≥99.5%, Riedel-de-Haen) and L-Cystine (≥99%, Fluka Biochemika) were used.