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Limits of rapid log P determination methods for highly lipophilic and flexible compounds

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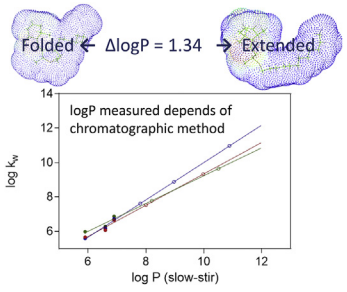
HIGHLIGHTS

- Different log P values were obtained for phenylalkanoic acids series including the pro-perfume Haloscent®D, depending on the chromatographic method used for log P estimation.
- Molecular modelling suggested that log P variations may be due to the chromatographic conditions applied, responsible of specific conformations of the molecule in solution.
- For flexible compounds, chromatographic methods have to be used with caution and considered as a good tool to estimate a log P range, that depends on the conformational state.

GRAPHICAL ABSTRACT



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ABSTRACT

Lipophilicity is of crucial importance in many fields including pharmaceutical, environmental, cosmetic and food industries. Whereas different experimental strategies have been developed for rapid lipophilicity determination of new chemical entities, log P determination of highly lipophilic compounds is always challenging. In this study, three published chromatographic methods have been compared on a series of phenylalkanoic acids including the pro-perfume Haloscent®D (HD-C12). Different log P values were obtained depending on the chromatographic method used for log P estimation. Molecular modelling suggested that log P variations may be due to the chromatographic conditions applied (isocratic or gradient mode, ratio methanol/water in the mobile phase), responsible of specific conformations of the molecule in solution. Thus, for flexible compounds, published methods have to be used with caution and considered as a good tool to estimate a log P range, depending on the molecular conformational state.

1. Introduction

Lipophilicity is a major parameter that controls many phenomena in life sciences such as absorption, distribution,

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metabolism, excretion and toxicity (ADMET) [1] or bioaccumulation [2]. The knowledge of log P as a quantification of the lipophilicity is therefore crucial for companies, not only for ADME predictions but also in environmental considerations as surrogate for bioaccumulation evaluation. Indeed, an evaluation of the ecotoxicity is important for the commercialization of a new chemical entity. Since the implementation of REACH in 2006 [3,4], chemicals toxicity and environmental impact have to be evaluated prior to compounds production or importation higher than one ton per year [5]. Environmental risk assessment may require intensive animal testing, as is the case for bioaccumulation potential measurement which requires sacrificing tens of fish [6]. This may appear contradictory with the strong will to limit or ban animal testing in REACH directive, for instance with the prohibition of animal testing for cosmetics since 2009 [7]. Therefore, strategies to evaluate the *in vitro* bioconcentration factor are required. The widely used model for the prediction of chemicals bioconcentration factor implies the n-octanol/water partition coefficient (log P_{oct}), as hydrophobicity is one of the main driving force of bioconcentration. However, other parameters influence bioconcentration such as metabolism or transport through membranes [2]. Nevertheless, a large number of models based on log P_{oct} values were reported to predict the bioconcentration factor (log BCF) measured in fish [8].

Therefore, log P determination is of crucial importance in many fields and explains the large number of methods developed for several decades. The golden standard method for log P determination remains the shake-flask method. Many different approaches have also been proposed so far including potentiometry, centrifugal partition chromatography, capillary electrophoresis [9–11], and in particular liquid chromatography [12–15]. Chromatographic approaches, based on correlation between partition coefficients (for measuring log P) and retention factors (for measuring log k), gained acceptance due to their rapidity, ease of automation, low sample consumption, and insensitivity to impurities [9,13,14,16–19]. Nevertheless, measuring log P values is always challenging for lipophilic compounds: all approaches have limited log P range estimation (ca. from −3 to +5) [20]. Recently, chromatographic methods specifically designed for the determination of log P up to 8 were published [21–23].

In a previous paper, the lipophilicity of Haloscent®D (HD-C12) and four homologues (HD-Cx with x = 4, 5, 6 and 8) has been studied by chromatography for risk assessment purposes [24]. Haloscent®D is a pro-perfume used for laundry applications, that, under certain conditions, releases an odorant molecule. This compound contains a twelve carbons alkyl chain and is a highly lipophilic substance. Haloscent®D homologues differ from HD-C12 by the number of carbons constituting the alkyl chain. Those compounds represent an interesting test set for published LC-based methods since they potentially undergo conformational changes that obviously changes apparent lipophilicity. Indeed, published methods for highly lipophilic compounds were generally benchmarked on a series of rigid compounds [23], while no highly flexible compounds were investigated. In this study, the log P of Haloscent®D and homologues including a non-previously published HD-C18 homologue were determined using the most relevant LC-based methods (in terms of best compromise between analysis time and accuracy) published by Guillot et al. [23]. Furthermore, because for half of HD-Cx no standard log P values were available, a series of phenylalkanoic acids differing by the number of carbons constituting the alkyl chain was chosen to complete the series. Indeed, log P values for smaller HD-Cx cannot be obtained due to a relatively lipophilic fixed scaffold. At the contrary, log P values of phenylalkanoic acids were easily measured by traditional methods (shake flask experiments) due to their hydrophilic head despite the presence of flexible chains similar to HD-Cx.

2. Material and methods

2.1. Chemicals

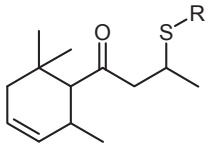
Haloscent®D (HD-C12) and Haloscent®D homologues (Fig. 1) were synthetized by Firmenich (Firmenich SA, Geneva, Switzerland). Synthesis protocols are described in Ref. [24]. Phenylalkanoic acids (Fig. 1) were obtained from commercial sources – phenylacetic acid from Fluka (Buchs, Switzerland, purity > 99%), phenylbutanoic, phenylpentanoic and phenylhexanoic acids from Aldrich (Steinheim, Germany, purity > 98%), and phenyloctanoic acid from Lancaster (Morecambe, United Kingdom, purity> 97%). Acetonitrile and methanol of HPLC quality were purchased from Biosolve (Valkenswaard, Netherland) and Fisher Chemicals (Loughborough, United Kingdom), respectively. Water was obtained with the Milli-Q Water Purification System from Millipore (Bedford, MA, USA), formic acid was provided by Fluka (Buch, Switzerland).

2.2. UHPLC measurements

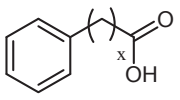
Formate buffer (pH 2.8; formic acid and sodium formate) was prepared at a fixed ionic strength of 20 mM, according to Phoebus software v1.0 (Analis, Namur, Belgium) to analyze all compounds in their neutral form (see pK_a of phenylalkanoic acids in Table 5). Buffer solutions were filtered through a 0.22 μm HA Millipore filter (Millipore, Bedford, MA, USA). 2000 μg/ml stock solutions in acetonitrile were prepared for all compounds and 20-fold diluted in methanol before injection to reach a final concentration of ca. 100 μg/ml.

UPLC measurements were performed on an Acquity UPLC system (Waters, Milford, USA) including a binary pumping system, an auto-sampler with an injection loop volume of 2 μL, a UV–VIS diode array programmable detector, a column manager with oven and a pre-column heater, for the UHPLC measurements. Systems were controlled by Empower Software v2.0 and the detection performed from 190 nm to 700 nm (6 nm resolution). Chromatograms were extracted at appropriate UV wavelengths for each compound.

Two stationary phases were used in this study: an Acquity BEH Shield RP18 column (30 × 2.1 mm ID, 1.7 μm) from Waters (Milford, MA, USA), and Hypersil™ GOLD Javelin HTS (10 × 2.1 mm ID, 1.9 μm, proprietary C18 phase, Thermo scientific, Waltham, USA).



R=C_4H_9, C_5H_{11}, C_6H_{13}, C_8H_{17}, $C_{12}H_{25}$ (Haloscent®D), $C_{18}H_{37}$



x=1, 3, 4, 5 and 7

Fig. 1. Structures of HaloscentD homologues and phenylalkanoic acids.