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ORIGINAL PAPER

Fast log *P* determination by ultra-high-pressure liquid chromatography coupled with UV and mass spectrometry detections

Yveline Henchoz · Davy Guillarme · Sophie Martel · Serge Rudaz · Jean-Luc Veuthey · Pierre-Alain Carrupt

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Abstract Ultra-high-pressure liquid chromatography (UHPLC) systems able to work with columns packed with sub-2 μm particles offer very fast methods to determine the lipophilicity of new chemical entities. The careful development of the most suitable experimental conditions presented here will help medicinal chemists for high-throughput screening (HTS) log P_{oct} measurements. The approach was optimized using a well-balanced set of 38 model compounds and a series of 28 basic compounds such as β-blockers, local anesthetics, piperazines, clonidine, and derivatives. Different organic modifiers and hybrid stationary phases packed with 1.7-μm particles were evaluated in isocratic as well as gradient modes, and the advantages and limitations of tested conditions pointed out. The UHPLC approach offered a significant enhancement over the classical HPLC methods, by a factor 50 in the lipophilicity determination throughput. The hyphenation of UHPLC with MS detection allowed a further increase in the throughput. Data and results reported herein prove that the UHPLC-MS method can represent a progress in the HTS-measurement of lipophilicity due to its speed (at least a factor of 500 with respect to HPLC approaches) and to an extended field of application.

Keywords Lipophilicity · UHPLC · Reversed-phase liquid chromatography · Partition coefficients · Physicochemical profiling

Introduction

Precise knowledge of physicochemical properties of new chemical entities (NCEs) is essential in early steps of investigations of a new drug [1, 2]. Among these properties, lipophilicity is a key parameter to understand and somehow predict pharmacokinetic processes such as absorption, distribution, metabolism, excretion, and toxicity (ADMET), particularly in terms of membrane permeation. Lipophilicity also significantly contributes to understand and model ligand–target interactions underlying the pharmacodynamic phase [3]. Moreover, this parameter appears in many (quantitative) structure–activity relationships (Q)SAR of various classes of compounds [4], which further emphasize its importance.

In silico methods are widely used for lipophilicity estimation despite their lack of accuracy due to the neglect of the 3D structure effect or to unknown fragments [5]. It is therefore necessary to build suitable and extended chemical libraries of experimental lipophilicity data [6, 7].

Different in vitro approaches have been reported to measure lipophilicity. The classical shake-flask technique still remains the reference for lipophilicity measurements, although the procedure is time-consuming, sensitive to impurities and the measurable log P_{oct} (octanol–water partition coefficient sometimes called log $P_{o/w}$) range restricted to −3 to 3 [8–10]. Several studies using

Electronic supplementary material The online version of this article (doi:10.1007/s00216-009-2862-1) contains supplementary material, which is available to authorized users. Experimental section for capillary electrophoresis (CE) measurements, list of the 38 compounds of the calibration set and solvatochromic analyses of the extrapolated retention factors and partition coefficients.

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potentiometry have been reported but this method is only appropriate for ionizable solutes [8, 9, 11]. More recently, microemulsion electrokinetic chromatography (MEEKC) [12–14] has been proposed for log P_{oct} determination. This separation technique is characterized by many advantages, namely low sample consumption, insensitivity to impurities, potential for automation, high throughput, and low cost. However, there are several issues to overcome the lack of electroosomotic flow when working at low pH values. The LC approach has also been widely used and recognized as a good alternative to these methods because it presents some evident benefits (similar to those observed in MEEKC) [12, 14–19]. However, it is difficult to handle highly basic or highly lipophilic compounds, due to the instability of silica-based stationary phases in high pH conditions and to long analysis time, respectively [20]. Centrifugal partition chromatography (CPC) has also been scarcely reported for log P determination but cannot be considered as a high-throughput method [21–24].

LC lipophilicity measurements can be performed either in isocratic or gradient mode. In isocratic conditions, the log k_w is obtained by extrapolation to 100% water plotting isocratic log k values as a function of the mobile phase composition. The relationship between isocratic log k values and organic modifier concentration depends on the experimental conditions. With methanol, isocratic log k values are generally linearly correlated to the organic modifier percentage φ in the mobile phase:

$$\log k = \log k_w - S \times \varphi \tag{1}$$

where k is the retention factor and S is a constant for a given solute and organic modifier. Using acetonitrile, the correlation between log k and organic modifier percentage in the mobile phase is generally appropriately fitted with a quadratic model:

$$\log k = \log k_w + B \times \varphi + A \times \varphi^2 \tag{2}$$

where A and B are constant for a given solute and organic modifier.

Linear correlations between log P_{oct} and log k_w obtained in isocratic conditions have been reported in the literature with determination coefficients (r^2) included in the range 0.81 to 0.99. The best correlations are generally obtained with analogs or with a low number of analytes [12, 14–16, 19, 25, 26].

Gradient approaches have been developed to accelerate the procedure of lipophilicity determination. However, one of the main problems associated with gradient elution remains the complexity of the involved phenomenon and associated mathematical treatment. The Linear Solvent Strength (LSS) Theory elaborated by Snyder and Dolan [27] allows to express the analyte retention time t_R with a linear-gradient separation in a convenient way:

$$t_R = \frac{t_0}{b} \times \log(2.3 \times k_i \times b + 1) + t_0 + t_D \tag{3}$$

where k_i is the k value in the initial composition of the gradient, t_0 is the column dead time (min) and t_D is the system dwell time for gradient elution (min). The gradient steepness parameter b can be described by the following relationship:

$$b = \frac{t_0 \times \Delta\varphi \times S}{t_G} \tag{4}$$

where t_G is the gradient time from the beginning to the end (min) and $\Delta\varphi$ the change in composition during the gradient (ranging from 0 to 1). Eqs. 3 and 4 contain two unknown terms k_i and S, which can be determined by two chromatographic runs differing only in t_G. These two values then allow to estimate log k_w with the help of Eq. 1 when using methanol as an organic modifier or Eq. 2 with acetonitrile. Appropriate calculating procedures of the unknown coefficients are included in several commercially available software such as Drylab (Rheodyne, CA, USA) or Osiris (Datalys, Grenoble, France). As in isocratic mode, numerous linear correlations between log P_{oct} and a retention property (such as log k_w) have been reported in the literature [12, 14, 17, 19] with r^2 generally included in the range 0.88 to 0.99.

Solvatochromic analysis, an approach based on linear solvation–energy relationships (LSERs), enables a better characterization of the stationary phases used for lipophilicity determination and can thus lead to an improvement of correlations in isocratic and gradient modes. This method was applied to identify and evaluate the intermolecular forces involved in the partitioning mechanism of compounds in various organic/aqueous biphasic systems [28–31] and then extended to chromatography to determine the intermolecular forces responsible for the retention on LC stationary phases [32–35]. LSERs can be expressed by the following equation:

$$S_p = v \times V_w + p \times \pi^* + a \times \alpha + b \times \beta + c \tag{5}$$

where S_p is a given molecular property of a neutral organic compound, which can correspond to log P_{oct} or retention factors (log k_w) determined on LC stationary phases. The four structural terms are the Van der Waals volume V_w, the dipolarity/polarizability π^*, the hydrogen-bond-donor acidity α and the hydrogen-bond-acceptor basicity β. The regression coefficients v, p, a, and b reflect the absolute contribution of each solute parameter and c is a constant.

Several strategies were successfully applied these last years to speed up lipophilicity determination by LC such as the use of shorter columns, higher flow rates, monolithic

supports, or mass spectrometry detection [36–38]. Short columns as well as high flow rates decrease the analysis time and extend the range of measurable log P_{oct} [39–42]. However, these two strategies generate a significant reduction of the chromatographic performance and accuracy of the results. Additionally, some overloading problems and reduced column lifetime have also been reported [43]. With monolithic supports, a study showed promising results as analysis time was reduced by a factor ten with results almost equivalent to those obtained with silica-based stationary phases [44]. However, the field of application of these columns remains limited due to the poor resistance at high pH and the lack of available chemistry at the moment. Recently, the development of columns packed with small porous particles (sub-2 μm) has allowed a significant improvement of chromatographic performance in LC [45–48]. It is indeed possible to increase the efficiency by a factor 3 when changing particle size from 5 to 1.7 μm while maintaining an equivalent column length. The column length can also be drastically reduced compared to conventional LC to reach high-throughput separations. For instance, it is possible to decrease analysis time by a factor 9 when changing particle size from 5 to 1.7 μm while maintaining equivalent efficiencies. However, a system compatible with very high pressure conditions (up to 1,000 bar) and possessing a low dead volume is required to work in such conditions. This strategy is known as ultra-high-pressure liquid chromatography (UHPLC) and a suitable chromatographic system was commercialized in 2004 by Waters under the trademark ultra performance liquid chromatography (UPLC).

In this work, UHPLC coupled with UV or MS detection was used for log P_{oct} determination. Firstly, several new silica-based stationary phases packed with 1.7-μm particles were tested using a well-balanced set of 38 compounds. Lipophilicity determination was performed with methanol and acetonitrile as organic modifiers, in both isocratic and gradient modes to point out their respective advantages and limitations. This method was then applied to a set of 28 basic pharmaceutical compounds owing to the high stability of these stationary phases in basic pH conditions, expanding the application field of this approach to basic drugs. Finally, a general strategy is proposed for high-throughput measurements of NCEs.

Experimental section

Materials and reagents

The majority of compounds were obtained from Sigma-Aldrich (Steinheim, Germany), except 2-chloroaniline and *m*-dichlorobenzene from Riedel-de Haën (Seelze, Germany), ethylacetate from Merck (Dietikon, Switzerland), metipranolol from Boehringer (Mannheim, Germany), carazolol and carvedilol from Roche Diagnostics (Mannheim), penbutolol from Sanofi-Aventis (Hoechst Marion Roussel, Paris, France), bupivacaine and mepivacaine from Sintetica (Mendrisio, Switzerland), lidocaine from Hänseler (Herisau, Switzerland), and rilmenidine from Servier (Neuilly-sur-Seine, France). Clonidine, rilmenidine and 5-bromo-*N*-(4,5-dihydro-5-methyl-1*H*-imidazol-2-yl)-6-quinoxalinamine (Cmp 1) were provided by Professor Pascal Bousquet (Laboratoire de Neurobiologie et Pharmacologie Cardiovasculaire, unité INSERM 715, Faculté de Médecine, Université Louis Pasteur, Strasbourg, France) and piperazines by Professor Serge Labidalle (Laboratoire de Synthèse, Physico-Chimie et Radiobiologie, Département de Synthèse Organique, Faculté des Sciences Pharmaceutiques, Université Paul Sabatier, Toulouse, France). Samples were set at a concentration of 20–100 μg/mL for UV detection and 2 μg/mL for MS detection. Methanol (10% to 50%, *v*/*v*) was added to enhance compound solubility.

To ensure that the compounds were mostly on their neutral form, four different buffers with pH values equal to 2.0, 5.0, 9.0, and 10.5 were used depending on the investigated compound nature (acidic, basic or neutral). The aqueous pH 5.0, 9.0, and 10.5 buffers set at a 20-mM ionic strength were prepared from acetic acid, ammonium acetate, or ammonia obtained from Fluka (Buchs, Switzerland). Trifluoroacetic acid (Fluka) was used to obtain pH 2.0. The buffer compositions were calculated by the Phoebus software 1.0 (Analis, Namur, Belgium) and the pH value measured with a Mettler-Toledo Seven-Multi pH meter (Schwerzenbach, Switzerland). HPLC grade methanol and acetonitrile were purchased from VWR (Leuven, Belgium), 1-octanol from Romil (Cambridge, England). Ultra-pure water was supplied by a Milli-Q Waters Purification System from Millipore (Bedford, MA, USA).

Apparatus

All retention measurements were performed on a Waters Acquity UPLC system hyphenated with a UV–VIS programmable detector possessing a 500 nl flow cell or with a single quadrupole SQD mass spectrometer possessing an upper mass limit of *m*/*z* 2000 (Milford, MA, USA). This instrument includes a binary solvent manager with a delivery flow rate set at 0.5 mL/min, a sample manager with a 2 μl injection loop (full loop mode) and a column oven set at 30 °C. Data acquisition, data handling, and instrument control were performed by Empower 2.0 Software (Waters). UV–VIS wavelength was selected according to the compound structure to reach maximum absorbance level. Time constant was always set at 12.5 ms and data sampling rate at 80 Hz. The ESCi® dual ionization

source was used in positive ESI mode. All the experiments were performed in single-ion monitoring (SIM) mode using a dwell time of 20 ms. The capillary, cone, and source extractor voltages were set at 3 kV, 30 V, and 2 V, respectively. The source and desolvation gas temperatures were maintained at 120 °C and 250 °C, while the desolvation and cone gas flows were set at 500 and 20 l/hr, respectively. The four tested columns were Acquity BEH Shield RP18, Acquity BEH C18, Acquity BEH C8, and Acquity BEH Phenyl (30 mm×2.1 mm I.D., 1.7 μm) purchased from Waters.

System characterization: determination of column dead time

Precise knowledge of the column dead time t_0 is mandatory for accurate determination of log k values. Several approaches summarized in a review by Dorsey et al. were evaluated, each of them presenting some inherent advantages and limitations [49]. The most accurate methodologies are clearly the pyconometry or the use of homologous series but both are time-consuming. An unretained neutral marker was used to determine t_0 as it is a fast, simple, and non-destructive method. However, it has to be carefully selected to provide an adequate measure of the dead time. Uracil, acetone, $NaNO_3$, and D_2O were evaluated (data not shown) and uracil was found to be the most appropriate as acetone can be retained on a C18 support, $NaNO_3$ presents Donnan exclusion effects, and D_2O is not UV–visible. In this study, uracil was injected at a concentration of 50 ppm with a mobile phase composition of 40% acetonitrile/60% pH 5.0 aqueous buffer. These conditions were selected as a compromise between low acetonitrile percentages where uracil could be retained on the support and high percentages detrimental for peak shape.

System characterization: determination of extra-column volume and delay time

When working with short columns at high-mobile-phase linear velocities, it is important to apply some corrections to experimental t_R and t_0 values to discard the influence of the chromatographic system and thus obtain accurate log k values. To fully characterize the chromatographic system, the elution time of uracil was measured at different flow rates ranging from 50 to 1,000 μl/min without chromatographic column (replaced by a zero-dead volume union). The elution times t_E were then plotted as a function of 1/flow-rate (1/F) and a linear regression was performed. The y-intercept represents the incompressible delay time t_{delay} due to the UPLC system and the slope corresponds to the extra-column dead volume V_{ext}. These two parameters have to be deduced from t_0 and t_R measurements. In our UPLC system, t_{delay} was equal to 0.010 min (6% of t_0 value), V_{ext} to 16.2 μl in UV detection configuration, and 21.8 μl in mass-detection configuration (20% and 26% of t_0 value at 500 μl/min, respectively). Without considering these two corrections, t_0 values would have been overestimated by about 30% at 500 μl/min. The influence of t_{delay} and V_{ext} was less significant on t_R measurements, although still relevant. Therefore, the following equation was used to calculate accurate log k values in isocratic mode:

$$\log k = \log\left(\frac{t_R - t_{delay} - V_{ext}/F}{t_0 - t_{delay} - V_{ext}/F} - 1\right) \quad (6)$$

In gradient mode, corrected t_R and t_0 values were directly entered in the modelization software (Eq. 3 and 4).

System characterization: determination of gradient dwell volume

The system dwell volume or gradient delay volume V_D corresponds to the volume between the mixing point of solvents and the head of the analytical column. This parameter is of prime importance and has to be considered for any modelization in gradient mode because the sample is prone to an initial isocratic migration. This parameter was experimentally determined according to a procedure described by Dolan [50]. A 5-min gradient of 0.1% acetone in acetonitrile was performed in triplicate at a flow rate of 0.5 mL/min. The average V_D value determined with the 2-μl injection loop was 100 μl. The latter was considered in Eq. 3.

Procedures

Isocratic mode

Calculations of log k values were performed with t_R measurements at five different mobile phase compositions. The investigated eluent compositions were determined according to the compound lipophilicity and the nature of the organic modifier, as summarized in Table 1. Each compound was injected once with each mobile phase composition and t_R determined from the apex of the peak. The t_R measurements were not repeated because RSD values for triplicate analyses of model analytes were always lower than 0.2% (data not shown). Finally, log k_w values were obtained by extrapolation to 0% organic modifier using linear or quadratic relationships between log k values and methanol or acetonitrile percentages, respectively (Eqs. 1 and 2).

Gradient mode

A generic procedure including two gradients was applied to modelize the behavior of a compound in the whole organic

Table 1 Investigated eluent compositions according to the compound log P_{oct} and the nature of the organic modifier

	Methanol	Acetonitrile
log P_{oct}<1	20–60%	10–50%
1<log P_{oct}<2	30–70%	20–60%
2<log P_{oct}<3.5	40–80%	30–70%
3.5<log P_{oct}<4.5	50–80%	40–80%
log P_{oct}>4.5	60–80%	50–80%

Literature log P_{oct} values [64]

modifier composition range. Gradient profiles were selected to obtain k_e (retention factor of the solute in the eluted mobile phase composition) equal to 3 and 10. The gradient slopes were 7.5 and 22.5%/min according to the investigated column geometry and mobile phase flow rate. The initial and final eluent compositions were set at 2 and 95% organic modifier, respectively. With t_R data obtained from two gradient runs differing only in gradient time, log k_w values were calculated by solving a set of two equations equivalent to Eq. 3 and then using Eq. 1. For this purpose, an iteration procedure included in the HPLC modeling software Osiris v. 4.1.1.2 was used.

1-octanol addition in isocratic mode

The aqueous buffers were saturated with 1-octanol and a 0.25% (*v*/*v*) amount of 1-octanol was added to methanol [39, 51]. t_R measurements as well as log k_w calculations were then performed following the same procedure as for isocratic measurements using methanol (cf. procedure: isocratic mode). However, from an experimental point of view it is recommended to perform t_R measurements after a sufficient equilibration time, beginning the experiments from the highest to the lowest methanol percentages according to a previously reported HPLC study (Girod L, Martel S, and Carrupt PA 2007, Unpublished results).

Results and discussion

Isocratic mode: choice of stationary phase and organic modifier

As described elsewhere [34], an optimal set of 80 calibration compounds was selected by cluster analysis from a larger set of 253 compounds according to their Van der Waals volume (V_w), polarity/polarizability (π*), H-bond donor acidity (α), H-bond acceptor basicity (β), and log P_{oct} values. Thirty-eight UV-active compounds of the optimal set were investigated. They were neutral, acidic (pK_a>3.6) or basic (pK_a<5.5) with log P_{oct} values ranging from 0 to 5 (Table S-1). In isocratic mode, linear relationships between log k_w and log P_{oct} values of the 38 calibration compounds were obtained with the four tested columns using either methanol or acetonitrile, as shown in Fig. 1. For each tested column and according to the obtained determination coefficients (r^2), better linear relationships were always achieved with methanol instead of acetonitrile as the organic modifier. In the literature, methanol was often regarded as the co-solvent of choice for measuring log P_{oct} by LC since quadratic extrapolation (Eq. 2) is less accurate than linear extrapolation (Eq. 1) [52]. Moreover, methanol is a protic solvent which can interact with free silanol groups. Therefore, secondary interactions between analytes and residual silanols can be reduced and a "pure" hydrophobic retention mechanism should be observed. During equilibration, methanol forms a monolayer on the stationary phase, providing a hydrogen bonding capacity, in better agreement with 1-octanol/water system [15, 20]. For these reasons, methanol was preferred to acetonitrile as the organic modifier in this study.

Concerning the stationary phase, the linear relationships with the highest r^2 were obtained with the Acquity BEH Shield RP18 column, containing an embedded carbamate group. Promising results [15, 53] had already been obtained in LC with polar-embedded stationary phases like Supelcosil LC-ABZ$^+$ or Discovery RP Amide C16 as silanophilic interactions are reduced due to a "shield" formed over the surface of the silica by the polar-embedded groups [54]. In this study, linear solvation free-energy relationships (LSERs) analyses were applied to the four sets of log k_w values and to the set of log P_{oct} values. These results are reported in Tables S-2 and S-3. The two main factors governing the retention for the four tested columns are the Van der Waals volume and H-bond acceptor basicity, whereas the polarity/polarizability and H-bond donor acidity are less significant. The Van der Waals volume and H-bond acceptor basicity are also the two principal parameters controlling the partitioning in octanol–water system, which is an additional confirmation that retention on these stationary phases and partitioning in biphasic liquid systems are governed by similar intermolecular forces. By taking into account the 95% confidence interval, the differences between the Acquity BEH C18 and Shield RP18 stationary phases are not significant. Therefore, the Acquity BEH Shield RP18 column was selected for further investigation as it exhibited the best linear correlations between log k_w and log P_{oct} values and it is characterized by a good discrimination power.

Using methanol as the organic modifier and the Acquity BEH Shield RP18 column, the following model between

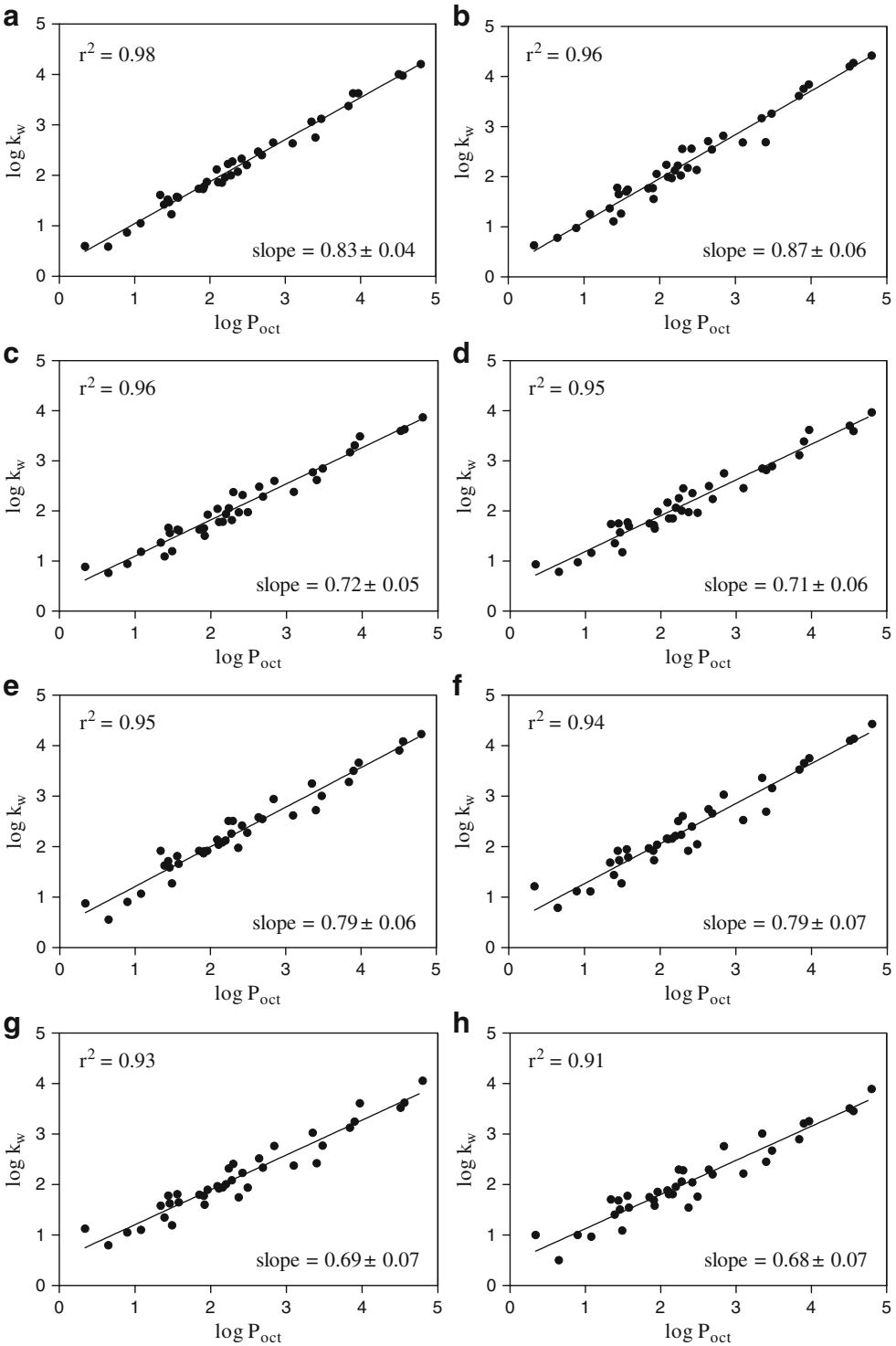

Fig. 1 Relationship between log k_w and log P_{oct} for the 38 calibration compounds in isocratic mode using methanol as the organic modifier and the Acquity BEH Shield (**a**), C18 (**b**), C8 (**c**) or Phenyl (**d**) columns, respectively. (**e**–**h**): idem, using acetonitrile as the organic modifier

log P_{oct} and log k_w values was derived from the 38 calibration compounds (Fig. 1a):

$$\log k_w = 0.83(\pm 0.04)\log P_{oct} + 0.21(\pm 0.10) \quad (7)$$
$$n = 38; q^2 = 0.98; r^2 = 0.98; s = 0.12; F = 2052$$

In this and following equations, n is the number of compounds, q^2 the cross-validated correlation coefficient, r^2 the determination coefficient, s the standard deviation, F the Fisher coefficient, and 95% confidence intervals are given in parentheses.

Gradient mode

In gradient mode, a good linear relationship between log k_w and log P_{oct} values of the 38 calibration compounds was obtained as previously demonstrated in a preliminary work involving UHPLC technology [55]:

$$\log k_w = 0.84(\pm 0.07)\log P_{oct} + 0.31(\pm 0.22)$$
$$n = 38; q^2 = 0.95; r^2 = 0.95; s = 0.21; F = 742 \quad (8)$$

To carefully compare the isocratic and gradient modes, many parameters have to be considered (Table 2). Firstly, the log k_w–log P_{oct} linear correlation is better in isocratic mode (Eq. 7) than in gradient mode (Eq. 8) in terms of r^2 (0.98 *versus* 0.95) and standard deviation (0.1 *versus* 0.2). Secondly, the average log P_{oct} determination time is similar in both modes (around 25 and 20 min/compound in isocratic and gradient mode, respectively). However, the approach is compound-dependent in isocratic mode as appropriate mobile phase compositions and run times have to be determined for each investigated compound (see procedure: isocratic mode) while it is generic in gradient mode. Finally, sensitivity might be an issue in isocratic mode as there is a serious decrease in peak height for compounds eluted with high retention factors, due to band broadening inside the column. In gradient mode, this problem is not observed since the peak width is almost constant whatever the retention time of the compound. In conclusion, the isocratic mode should be preferred to the gradient one in terms of trueness while the gradient approach is generic and thus far more suitable for high-throughput log P_{oct} measurements.

Assessment of the procedure with a set of basic pharmaceutical compounds

The chemical resistance of stationary phases such as the Acquity BEH Shield RP18 column in high pH conditions (up to pH 11) is a very interesting feature as classical silica-based stationary phases are only stable within a limited pH range (pH 2-8). These hybrid stationary phases with hydrophobic ethylene groups present throughout the particle backbone are believed to shield the silica units and prevent them from dissolution. Besides this hydrophobic shielding, the structural ethylene bridges increase the hydrolytic stability of the particles [56]. This stationary phase is thus well suited for log P_{oct} estimation of basic compounds in high pH conditions.

To take advantage of this chemical stability, 28 basic compounds with pK_a values ranging from 6.3 to 9.6 were selected and the quality of the relationship between log P_{oct} and log k_w evaluated in a similar way to the 38 calibration compounds. As shown in Fig. 2, basic drugs have a different behavior from the calibration compounds in both isocratic and gradient modes. Basic compounds are generally more retained on the stationary phase though the phenomenon seems family-dependent. For example, clonidine and derivatives have the same retention characteristics as calibration compounds while beta-blockers and local anesthetics behave in a much different way from all the other basic analytes. In the literature [20, 57–61] this increase in retention was usually attributed to silanophilic interactions due to the remaining free silanol sites, which can include hydrogen bonding, dipole interactions as well as electrostatic forces (ion exchange) [62].

At this point, it is necessary to consider the effect of the organic modifier on pK_a values of the buffer, the acidic silanol groups, and the solutes [53, 63]. For example, the apparent mobile phase pH varied according to the organic modifier percentage. For experiments with basic compounds, the aqueous buffer pH was 10.54, whereas the ${}^{s}_{w}pH$ of the buffer in 40% and 70% methanol was 10.18 and 9.98, respectively. This might for instance influence the ionization state of the silanol groups on Acquity BEH

Table 2 Comparison between the four tested approaches

	log $P_{UHPLC\text{-}isocratic\text{-}UV}$ [a]	log $P_{UHPLC\text{-}gradient\text{-}UV}$ [b]	log $P_{UHPLC\text{-}isocratic\text{-}oct\text{-}UV}$ [c]	log $P_{UHPLC\text{-}50\%\text{-}UV}$ [d]
Standard deviation[e] (calibration compounds)	0.1	0.2	0.1	0.1
Standard deviation[e] (basic compounds)	*0.4*[f]	*0.4*[f]	0.2	0.2
Average log P_{oct} determination time (min)	25′	20′	25′	5′
Approach	Compound-dependent	Generic	Compound-dependent	Generic

[a] log P_{oct} determined by UHPLC-UV in isocratic mode by extrapolation to 100% water (Eq. 7)

[b] log P_{oct} determined by UHPLC-UV in gradient mode by extrapolation to 100% water (Eq. 8)

[c] log P_{oct} determined by UHPLC-UV in isocratic mode with 1-octanol addition by extrapolation to 100% water (Eq. 10)

[d] log P_{oct} determined by UHPLC-UV with a single measurement at 50% methanol (Eq. 9)

[e] Standard deviation calculated from the difference between literature log P_{oct} and log P_{UHPLC} with n=38 (calibration compounds) or n=28 (basic compounds)

[f] Aside from the high standard deviation, there is also a systematic bias for numerous basic compounds (Fig. 2)

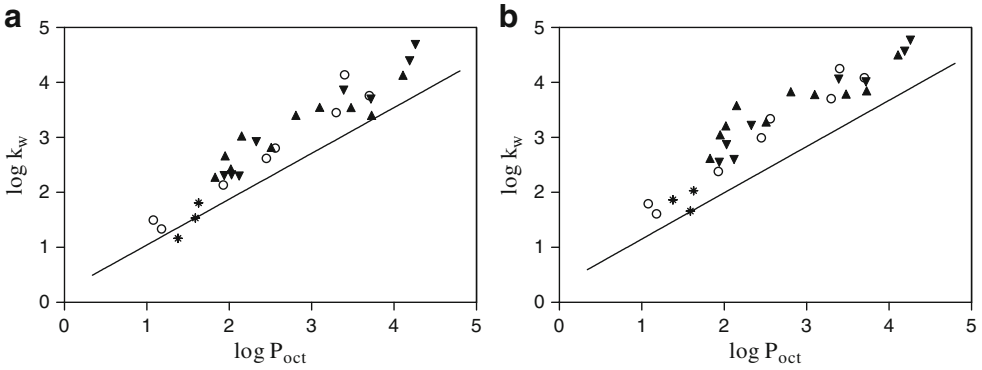

Fig. 2 Relationship between log k_w and log P_{oct} for the 28 basic compounds in isocratic (**a**) and gradient mode (**b**): beta-blockers (*filled upright triangles*), local anesthetics (*filled inverse triangles*), piperazines (*empty circles*) and clonidine + derivatives (*asterisks*). The *black straight line* is the linear regression for the 38 calibration compounds

columns as their acidity is low (pK_a>8) [56]. In addition, the ionization states of the most deviating basic compounds (beta-blockers and local anesthetics) were measured at different mobile phase compositions by capillary electrophoresis using a methodology detailed in the supporting information. Results are reported in Fig. S-1. From these measurements it can be concluded that beta-blockers were not completely in their neutral form (except carvedilol): there was about 10% of ionized form at each tested mobile phase composition, while local anesthetics were almost completely on their neutral form (except butacaine). Thus, the particular behavior of beta-blockers and local anesthetics seems not due to the percentage of ionized form and to electrostatic interactions with free silanol groups. However, it does not exclude hydrogen bonding, according to LSERs analyses (Tables S-2 and S-3). Indeed, silanophilic interactions can be present despite the carbamate-embedded group, which is usually considered to reduce such effects due to electrostatical shielding [20]. By comparing these results with those obtained on an Acquity BEH C18 column (data not shown), it can be concluded that the polar-embedded groups might prevent ionic interactions with free silanol groups but not hydrogen bonding with the stationary phase.

The effects of the organic modifier are significant and depend on its proportion in the mobile phase. When log k_w values are extrapolated from different organic modifier concentration ranges (depending on the lipophilicity of the

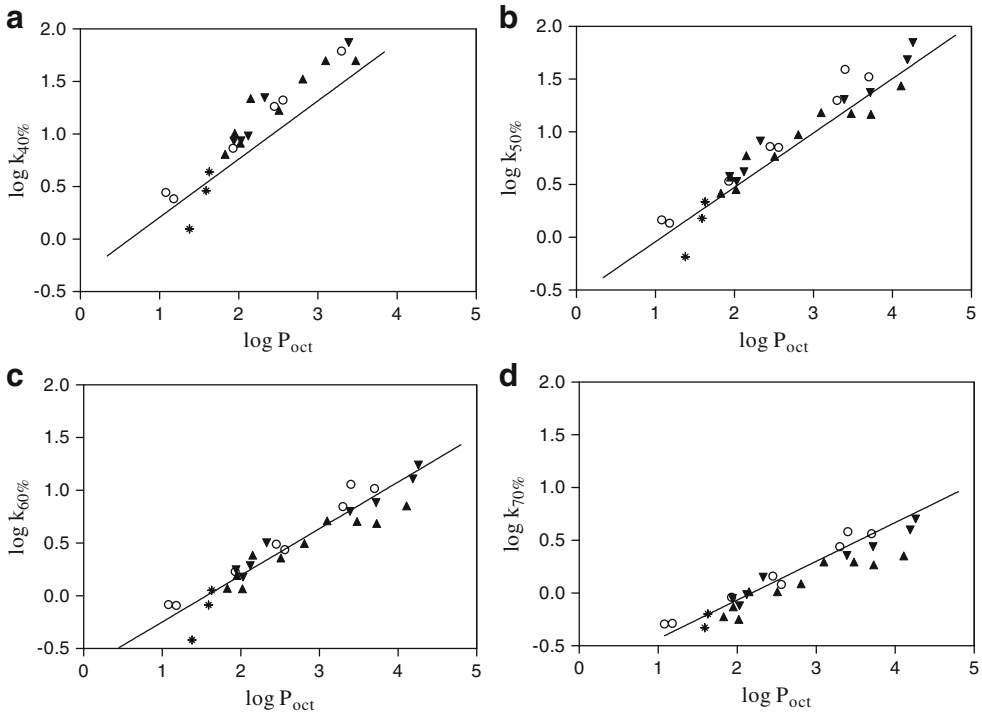

Fig. 3 Relationship between isocratic log k values and log P_{oct} for the 28 basic compounds: beta-blockers (*filled upright triangles*), local anesthetics (*filled inverse triangles*), piperazines (*empty circles*) and clonidine + derivatives (*asterisks*). Methanol percentage: 40% (**a**), 50% (**b**), 60% (**c**), and 70% (**d**). The *black straight line* is the linear regression for the 38 calibration compounds

investigated compounds), such effects are not taken into account. Therefore, correlations between log P_{oct} and isocratic log k values were explored, as depicted in Fig. 3. Indeed, the 28 basic compounds seem to have a similar behavior to the calibration set at 50% of methanol, as the linear correlation between log $k_{50\%}$ and log P_{oct} presents the lowest dispersion (r^2 0.95 including both the calibration and basic compounds sets). Moreover, the retention factors of compounds with $0<\log P_{oct}<5$ were acceptable at 50% methanol. Concerning the slope (i.e., discrimination power), the lowest percentage of methanol provided the highest slope. However, log $k_{50\%}$ presents the best compromise between low dispersion, suitable analysis time, and high discrimination power.

The following model between log P_{oct} and log $k_{50\%}$ values was derived from the 38 calibration compounds:

$$\log k_{50\%} = 0.52(\pm 0.02)\log P_{oct} - 0.56(\pm 0.05)$$
$$n = 38; q^2 = 0.98; r^2 = 0.98; s = 0.08; F = 2048 \quad (9)$$

This equation enabled to calculate log P_{oct} values for the 28 investigated basic compounds. The obtained results are reported in Table 3 and demonstrate that a single analysis at 50% methanol allows an acceptable log P_{oct} estimation for basic compounds with a low standard deviation (0.2;

Table 3 Data obtained for the 28 basic compounds

Compound	pK_a	log P_{oct}	log $P_{UHPLC\text{-}50\%\text{-}UV}$ [a]	log $P_{UHPLC\text{-}50\%\text{-}MS}$ [b]	log $P_{UHPLC\text{-}isocratic\text{-}oct\text{-}UV}$ [c]
Pindolol	9.54[d]	1.8[d]	1.9	1.9	1.9
Metoprolol	9.63[d]	2.0[d]	2.2	2.2	2.2
Acebutolol	9.52[d]	2.0[d]	2.0	1.9	1.9
Bisoprolol	9.57[d]	2.2[d]	2.6	2.6	2.6
Oxprenolol	9.57[d]	2.5[d]	2.6	2.5	2.6
Metipranolol	9.54[d]	2.8[d]	3.0	2.9	2.9
Alprenolol	9.59[d]	3.1[d]	3.4	3.3	3.4
Propranolol	9.53[d]	3.5[d]	3.4	3.3	3.4
Carazolol	9.52[d]	3.7[d]	3.3	3.3	3.3
Carvedilol	7.97[d]	4.1[d]	3.9	3.8	3.9
Mepivacaine	7.72[e]	1.9[f]	2.2	2.2	2.2
Procaine	8.95[e]	2.0[f]	2.1	2.1	2.1
Prilocaine	7.84[e]	2.1[f]	2.3	2.3	2.3
Lidocaine	7.82[e]	2.3[f]	2.9	2.8	2.8
Tetracaine	8.35[e]	3.4[f]	3.6	3.6	3.7
Bupivacaine	8.06[e]	3.7[f]	3.7	3.7	3.8
Dibucaine	8.61[e]	4.2[f]	4.3	4.3	4.4
Butacaine	9.51[e]	4.3[f]	4.7	4.6	4.7
Cmp 1	7.78[f]	1.4[f]	0.7	0.8	0.8
Clonidine	8.11[f]	1.6[f]	1.4	1.4	1.5
Rilmenidine	9.23[f]	1.6[f]	1.7	1.8	1.8
SPLV 1058-2	9.05[f]	1.1[f]	1.4	1.4	1.4
SPLV 1058-1	8.73[f]	1.2[f]	1.3	1.4	1.3
SPLV 1058-9	8.33[f]	1.9[f]	2.1	2.1	2.1
SPNL 2075-1	8.36[f]	2.5[f]	2.8	2.7	2.7
SPLV 1058-5	6.26[f]	2.6[f]	2.7	2.7	2.7
SPLV 1077	7.60[f]	3.4[f]	4.2	4.1	4.2
SPLV 1058-7	8.41[f]	3.7[f]	4.0	4.0	4.0

[a] log P_{oct} determined by UHPLC-UV with a single measurement at 50% methanol (Eq. 9); standard deviation 0.2 (Table 2)

[b] log P_{oct} determined by UHPLC-MS with a single measurement at 50% methanol (Eq. 9); standard deviation 0.2

[c] log P_{oct} determined by UHPLC-UV in isocratic mode with 1-octanol addition by extrapolation to 100% water (Eq. 10); standard deviation 0.2 (Table 2)

[d] Determined by potentiometry [65]

[e] Measured by capillary electrophoresis according to a method described elsewhere [66]

[f] Determined by potentiometry

Table 2). The precision of this determination is comparable to the precision of other experimental methods used for log P_{oct} measurement. Furthermore, this strategy is rapid as the average log P_{oct} estimation time is only 5 minutes per compound. Such approaches were previously reported in the literature and similar conclusions were drawn in conventional LC [37, 53].

Alternatively, the well-known approach described by Lombardo et al. [39, 51] was tested for log P_{oct} determination of the 38 calibration compounds and 28 basic drugs. In this method, 0.25% (V/V) 1-octanol was added to methanol and buffers were saturated with 1-octanol. Under these conditions, the 28 basic drugs have a similar behavior to calibration compounds, as depicted in Fig. 4. Indeed, a good linear relationship is obtained between extrapolated log k_w and log P_{oct} values for calibration and basic compounds (r^2 0.96), thus allowing the derivation of the following equation for the 38 calibration compounds:

$$\begin{aligned} &\log k_w = 0.95(\pm 0.04)\log P_{oct} - 0.24(\pm 0.11) \\ &n = 38; q^2 = 0.98; r^2 = 0.98; s = 0.13; F = 2216 \end{aligned} \quad (10)$$

The log P_{oct} values of the 28 investigated basic compounds could be calculated with Eq. 10. The results are reported in Table 3 and demonstrate that extrapolated log k_w values measured in isocratic mode using 1-octanol as a mobile phase additive also allow a good log P_{oct} estimation for basic compounds with a low standard deviation (0.2) (Table 2). Moreover, the slope in Eq. 10 is higher than in Eq. 7–9, indicating a high discrimination power. It is also very close to unity, meaning that the retention process is energetically similar to the partitioning process in a 1-octanol/water system. In addition, LSERs analyses show that retention on the Acquity BEH Shield RP18 column with 1-octanol addition in the mobile phase and partitioning in biphasic liquid systems are similar phenomena (Tables S-2 and S-3). In fact, 1-octanol seems to form a coating on the silica support of the column during equilibration, conferring octanol-like properties to the stationary phase. These results prove that this method is an alternative to the log $k_{50\%}$ approach to determine log P_{oct} of basic compounds. However, the log $k_{50\%}$ approach is more generic and rapid with a log P_{oct} determination time of 5 minutes. It should thus be preferred to the method proposed by Lombardo for high-throughput log P_{oct} measurements.

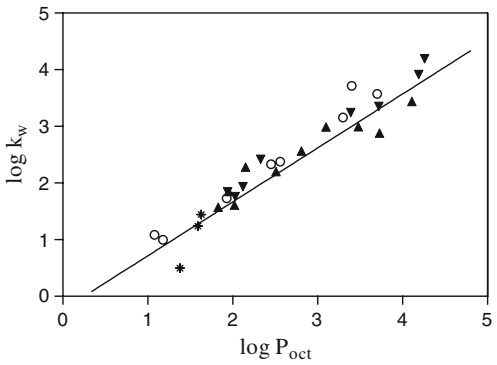

Fig. 4 Relationship between log k_w values obtained with 1-octanol as a mobile phase additive and log P_{oct} for the 28 basic compounds: beta-blockers (*filled upright triangles*), local anesthetics (*filled inverse triangles*), piperazines (*empty circles*) and clonidine + derivatives (*asterisks*). The black straight line is the linear regression for the 38 calibration compounds

Increased throughput with MS detection

The potential of UHPLC hyphenated to MS detection was investigated to further increase the throughput of log P_{oct} estimation. Indeed, due to the additional selectivity of MS instrument, several compounds can be analyzed simultaneously.

Generic MS conditions were found for log P_{oct} estimation of the 28 basic compounds at 50% methanol. 8-10 compounds were simultaneously analyzed to maintain an acceptable acquisition rate and allow a straightforward MS discrimination with the single quadrupole analyzer. If the molecular mass information is not available, this approach is perfectly compatible with SCAN mode determination. The log P_{oct} calculated with Eq. 10 are consistent with those obtained with UV detection, as reported in Table 3. The slight differences observed are probably due to small uncertainties of extra-column volume determination in UV and MS configurations. Another advantage of the UHPLC-MS strategy is the number of log P_{oct} determination that could be performed on a given column at pH 10.5. Finally, the UHPLC-MS approach allows high-throughput measurements since log P_{oct} of 8-10 compounds can be estimated in only 5 minutes.

Conclusion

Four approaches were tested for log P_{oct} determination by UHPLC, as summarized in Table 2. Firstly, suitable conditions in terms of stationary phase (Acquity BEH Shield RP18) and organic modifier (methanol) were found by correlation with log k_w values in the isocratic mode. This method is accurate for neutral, acidic (pK_a>3.6) and weak basic (pK_a<5.5) compounds but a systematic bias was observed when dealing with relatively basic (pK_a>6.3) compounds. Moreover, this strategy is not generic enough for high-throughput measurements.

Lipophilicity was also determined by correlation with log k_w values in gradient mode, which is a generic approach compatible with high-throughput measurements. However, this method is not suitable to relatively basic compounds

and not as good as the isocratic mode in terms of trueness. Moreover, data treatment remains complex.

The determination of log P_{oct} values by correlation with log k_w values in isocratic mode with 1-octanol addition allows an accurate log P_{oct} measurement for all tested compounds, including relatively basic compounds. Nevertheless, this strategy remains tedious and time-consuming (cf. 1-octanol saturation of aqueous phases and equilibration of UHPLC system).

The determination of log P_{oct} with a single analysis at 50% methanol turns out to be the method of choice as it is a generic, rapid (*ca* 5 minutes per compound) and accurate for all investigated compounds. Finally, the MS coupling allows more rapid lipophilicity determination (*ca* 5 minutes per 10 compounds).

In conclusion, the two most important features of the UHPLC approach for lipophilicity measurements are its high throughput (one compound per 5 min with UV detection and ten compounds per 5 min with MS detection) and its applicability to basic compounds due to the stability of the hybrid stationary phase at high pH values.

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