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Review

Liquid Chromatography on the Different Methods for the Determination of Lipophilicity: An Essential Analytical Tool in Medicinal Chemistry

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Abstract: Lipophilicity is one of many parameters involved in the biological activity of drugs, as it affects their pharmacokinetic and pharmacodynamic behavior. Generally, lipophilicity is assessed by the partition coefficient of a compound between a nonpolar phase (*n*-octanol) and an aqueous phase (water), expressed as P (partition coefficient) or as its decimal logarithm (Log P). The gold standard method for the experimental determination of Log P is the shake-flask method. In this context, chromatographic methods enable the direct and simple quantification of the partitioned compound between the two phases. This review discusses the use of liquid chromatography (LC) for direct and indirect determination of lipophilicity. Beyond the classical isotropic log P determination, methods for assessing anisotropic lipophilicity are also reviewed. Several examples are discussed that highlight the versatility of LC technique and current trends. The last section of this review focuses on a case study describing an experience of our group and emphasizing the dual role of LC in determining Log P.

Keywords: lipophilicity; Log P; liquid chromatography; partition coefficient; reversed-phase high-performance liquid chromatography; immobilized artificial membrane chromatography; micellar liquid chromatography

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1. Lipophilicity and Its Importance in Drug Discovery and Design

Lipophilicity, as defined by the International Union of Pure and Applied Chemistry (IUPAC), is a "physicochemical property which describes a partitioning equilibrium of solute molecules between water and an immiscible organic solvent, favouring the latter" [1]. Commonly, lipophilicity is expressed as the logarithm to the base 10 of the partition coefficient (log P) of a solute [2]:

$$\log \mathrm{P} = \log \frac{[substance]_{\mathrm{nonaqueous}}}{[substance]_{\mathrm{aqueous}}} \quad (1)$$

where $[substance]_{\mathrm{nonaqueous}}$ is the solute equilibrium concentration in a nonaqueous (non-polar phase) and $[substance]_{\mathrm{aqueous}}$ is its equilibrium concentration in an aqueous phase (polar phase). Classically, the aqueous phase is water, but the partition coefficient can also

be determined using buffer solutions. In this case, the partitioning involving co-existing electrical species of a solute at a given pH is expressed as the distribution coefficient log D:

$$\log D^{pH} = \log \sum_{i}^{n} \left(f_i \times P^i \right) \quad (2)$$

where P^i is the partition coefficient of the electrical species i and f_i, its molar fraction [3].

Classically, the nonaqueous phase is an isotropic organic solvent, such as *n*-octanol. However, artificial and natural membranes, such as liposomes and micelles (Figure 1a), have been recently used as alternative nonaqueous phases [4]. These systems are anisotropic because they establish different topographical relations between the solute and the nonaqueous phase involving different interaction forces [3]. The choice of the nonaqueous phase impacts the type of intermolecular forces encoded in the partition coefficient value. Lipophilicity is the product of all intermolecular forces involved in the partition of a solute between the two phases, assembling not only the contributions of hydrophobicity, the tendency of apolar groups or molecules to associate in an aqueous environment, but also the contributions of polarity [5]. The different intermolecular forces encoded in lipophilicity are shown in Figure 1, and they are different depending on the nonaqueous phase. While isotropic lipophilicity results from the net sum of hydrophobicity minus polarity, anisotropic lipophilicity also considers the ionic bonds [3]. Therefore, isotropic and anisotropic partition coefficients express lipophilicity on different scales.

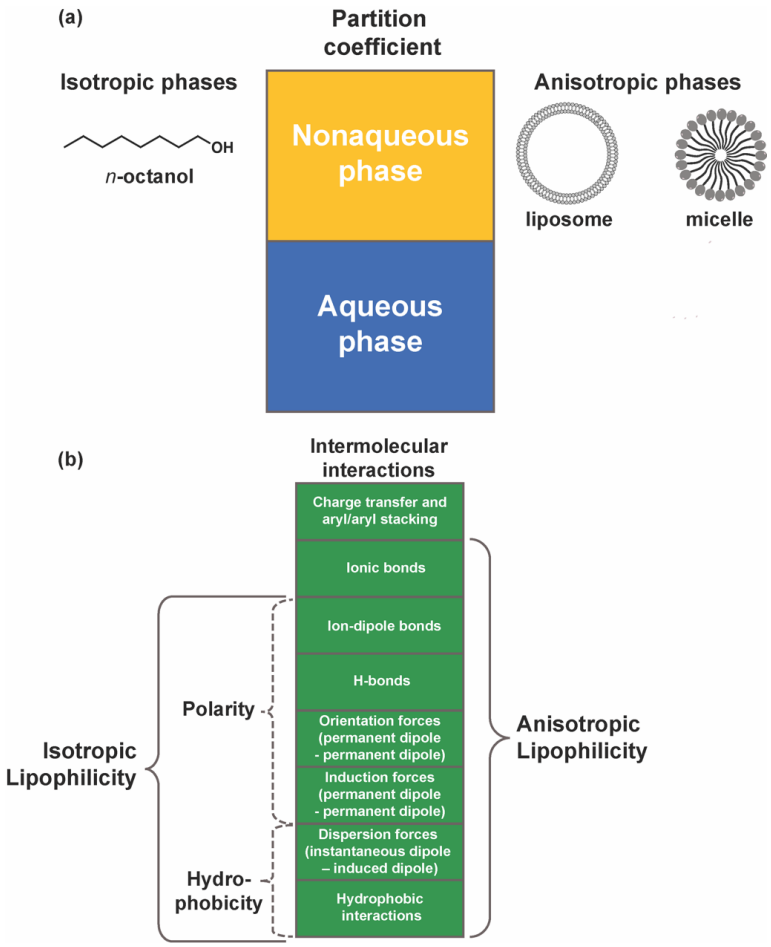

Figure 1. (**a**) Models used to determine isotropic and anisotropic lipophilicity; (**b**) forces governing intermolecular interactions between drugs and biological systems and their relationship with isotropic and anisotropic lipophilicity.

Lipophilicity is a key parameter on drug discovery and design. Every single pharmacokinetic component, namely the absorption, distribution, metabolism, excretion, and toxicity (ADMET), is modulated by lipophilicity. Absorption is related to the lipophilicity because drugs typically must cross biomembrane portals to reach the sites of action. Passive permeation into or across biomembranes is modulated by the partition of the drug into the lipid membrane and from the membrane into the aqueous intracellular environment [6]. The rate-limiting step for hydrophilic compounds is the partitioning into the hydrophobic membrane layer, and for hydrophobic compounds is partitioning into the hydrophilic intracellular medium [7]. Solubility is another parameter that is modulated by lipophilicity in such a way that solubility can be calculated by an empirical formula that correlates melting point and log P with solubility [2]. In general, compounds with lower log P tend to be water-soluble. In addition to solubility, other physical parameters are related to lipophilicity, such as the flexibility of a molecule based on the presence of rotatable bonds and on the ratio of sp^3 carbons, the presence of polar groups, and the presence of intramolecular hydrogen bonding [8].

Drug distribution is also affected by lipophilicity. Lipophilic compounds have a large volume of distribution due to affinity to lipophilic tissues such as adipose tissue [2]. Nevertheless, lipophilicity also indirectly influences the distribution of drugs through plasma protein binding, as lipophilic compounds tend to have high plasma protein binding [9,10]. The facility to cross the compact and protected blood–brain barrier (BBB) is also dependent on lipophilicity [6]. In general, small and highly lipophilic compounds can penetrate easier across the BBB, and increasing lipophilicity is likely to enhance BBB permeation [11]. However, increasing lipophilicity can also be associated with higher binding affinity to efflux pumps, such as P-glycoprotein [2]. Lipophilicity is also related to metabolic rate as in vivo metabolism often converts lipophilic drugs into more polar metabolites to facilitate their clearance. In addition, lipophilic compounds are more likely to be toxic [12]. One example is the interaction with the human ether-a-go-go related gene (hERG), especially for positively charged lipophilic compounds, which can cause increases in the cardiac QT interval [13].

Despite being mostly recognized for its role in pharmacokinetics, lipophilicity also plays a crucial role in the pharmacodynamic behaviour of a drug. Lipophilicity contributes to understanding ligand–target interactions, considering the different forces that govern the intermolecular interactions involved in biochemical and pharmacological processes. In fact, lipophilicity is a parameter considered in numerous quantitative structure–activity relationship studies [5,14]. The presence of lipophilic moiety offers lead compounds with a higher probability of having anchors on protein targets with large and hydrophobic binding sites [15]. However, increasing lipophilicity to the pursuit of potency through increasing lipophilicity across the drug development comes with costs. One is the trend, known as 'molecular obesity', for seeking huge and lipophilic molecules that, despite being highly potent, do not have a suitable pharmacokinetic profile [7]. Another risk is target promiscuity. Compounds that achieve their potency through the increase of lipophilicity are more likely to be less selective, increasing the risk of off-target interactions and undesirable toxicity [16].

In a nutshell, lipophilicity is probably the single most informative physicochemical parameter in modern drug discovery. Therefore, drug design seeks to control this property within a defined optimal range [15]. To fulfil this aim, suitable methods for the determination of lipophilicity are required mainly in the early steps of the drug discovery pipeline.

2. Methods for Determination of Lipophilicity

The determination of lipophilicity has become a routine process for medicinal chemists [17]. Lipophilicity can be measured or can be calculated [2]. The experimental methods to determine lipophilicity can be divided into two categories: direct and indirect methods [18,19]. In direct experimental methods, the partition coefficient is obtained directly from the concentration ratio in the equilibrium of a compound partitioned between the nonaqueous and aqueous phases.

In indirect experimental methods, the partition coefficient is estimated through a correlation, usually the correlation between a compound retention factor in a reverse-phase chromatographic system and its partition coefficient. Liquid chromatography (LC) plays a relevant role both in the direct and indirect determination of lipophilicity.

2.1. In Silico Determination of Lipophilicity

Since the pivotal work of Hansch et al., which found out that the partition coefficients have an additive constitutive character, a myriad of in silico methodologies have been developed to predict lipophilicity from the molecular structure of a molecule [20]. If some of these methods are very simple [21], others exist that are more sophisticated and based on deep neural networks [22]. Excellent reviews on the in silico estimation of isotropic [23] and anisotropic lipophilicity [24,25] are available in the literature.

Independent of the computational method, the calculation is always based on a set of experimentally obtained values [26]. Therefore, the calculated values should be regarded as approximations. Often, calculated log P values are inaccurate, and the reliability of calculation methods is low for highly complex compounds [27]. In addition, in silico methods can also be imprecise as computed values for a class of compounds could vary up to two log P units depending on the approach used [28,29]. For the above-mentioned reasons, calculated values should be considered when [30]:

- Choosing the experimental method;
- Selecting conditions for a LC analysis;
- Examining the plausibility of experimentally obtained values;
- Providing an estimate when experimental methods are not applicable.

In the early discovery process, in silico methods are very useful for filtering drug-like compounds [31]. However, as soon as possible, the predicted values should be substituted by more accurate measured values [28].

2.2. Direct Experimental Determination of Lipophilicity

Direct determination of lipophilicity requires the quantification of the compound concentration present in the nonaqueous and in the aqueous phase. The direct method known as the shake-flask method is the gold standard for log P determination. Direct methods are usually accurate when measuring log P values in the range −2 to 4, but they are labor-consuming and usually require relatively large amounts of pure compounds [32].

In direct methods, LC is the method typically used to quantify the amount of substance in each phase of the biphasic system. When compared to other analytical methods, LC provides a wider range of applicability than gas chromatography and provides a lower detection limit than UV/Vis spectroscopy. The detection limit is particularly relevant for highly lipophilic compounds because compounds with log P values larger than 4 are often limited by the analyte minimum detection limit in the aqueous phase [19]. The use of LC is similar in the different direct methods. Therefore, in the next subchapters the different direct methods that can be coupled with LC will be discussed, providing more focus on the biphasic system rather than on the LC conditions.

2.2.1. Shake-Flask Method

The shake-flask method uses *n*-octanol (hydrophobic) and water (hydrophilic) as the biphasic liquid system, and it is the standard method recommended by Organization for Economic Co-operation and Development (OECD) [32]. This classical procedure consists of dissolving the sample, shaking until equilibrium has been reached, and measuring the compound concentration in each phase of the biphasic system [32].

The shake-flask method is a direct measurement of the partition coefficient, and that is its main advantage [33]. However, this procedure has several drawbacks. It requires control over a large number of experimental parameters (different experimental conditions usually produce different values of log P for the same analyte) [34]. It is a highly time-consuming method as the partition needs to reach equilibrium. The time needed to reach

equilibrium concentrations varies hugely (from 1 to 24 h), and partitioning rates may be fast or slow depending on the log P of the solute and the degree of shaking [35]. It consumes large amounts of solvent per analyte [35], and it is not suitable for degradable compounds and surface-active materials [27]. Finally, it is not appropriate for poorly soluble compounds [35].

Modification of the shake-flask method has been proposed that enables the determination using different alternatives to the *n*-octanol/water system aimed to imitate different physiological cell barriers (e.g.: chloroform/water, alkane/water, or propylene glycol dipelargonate/water) [36].

2.2.2. Slow-Stirring Method

In the slow-stirring method, *n*-octanol and aqueous phases are mixed under slow stirring (instead of shaking) [37]. By proceeding this way, the formation of emulsions can be prevented. The obtained values with the shake-flask and slow-stirring method are very similar for compounds exhibiting log P < 4.5, but they differ for compounds with higher log P, which can be attributed to the formation of octanol emulsions in the shake-flask procedure [38]. As a drawback, this method requires a long period of stirring, up to 2–3 days, to reach equilibrium [37].

The slow-stirring method can also be applicable for the determination of anisotropic lipophilicity using a liposome/buffer system. The difference between the procedures is in the quantification step, as liposomes are separated from the buffer phase by inducing liposome aggregation followed by filtration [39].

2.2.3. Water-Plug Aspiration/Injection Method

The water-plug aspiration/injection method is another shake-flask modification particularly suitable for highly lipophilic compounds. In the shake-flask procedure, it is difficult to manually separate the water phase from the *n*-octanol phase without contaminating the water phase because of the high viscosity of *n*-octanol. Improper phase separation can impact the determination with a higher impact for highly lipophilicity compounds [40]. To avoid this problem, a small volume of water is aspirated into the needle before collecting the aqueous phase in order to decrease the potential contamination, as water in the needle should repel contaminating *n*-octanol phase [40]. The sampling of the water phase can be automated, but the obtained values must be manually inspected to identify instrument problems or solubility issues. The need for manual inspection can be suppressed by the use of an injection marker [41].

2.2.4. Flow-Based Method

Flow injection analysis can be exploited to set a variant of the shake-flask method that allows standardization of the measurement [42,43]. In this method, the test substance is dissolved in either *n*-octanol or water phase. The substance is then injected into the flow of a capillary tube with the corresponding phase, and the two immiscible phases are continuously pumped to a mixing point in the system. The substance partitions between the two phases, and after equilibration, a fraction of the aqueous flow is separated, and the concentration of the analyte is determined. The large surface area and short distance result in the fast transport of the analyte from one phase to the other, allowing it to rapidly reach equilibrium [42].

2.2.5. Miniaturization of Shake-Flask Method

To increase the throughput of log P measurements and to decrease the amounts of compound needed, the traditional shake-flask method was transposed to a 96-well format. In this technique, the partition coefficient of a solute is measured between a polymer phase and an aqueous phase [44]. The polymer phase is prepared in 96-well microplates. The tested compound is added to a polymer phase prepared in a 96-well microplate, and after incubation, in a shaker, the amount of compound is determined in the supernatant.

2.2.6. Vortex Liquid–Liquid Microextraction (VALLME) Method

VALLME is a microextraction method that, coupled with LC, aims to increase the throughput of log P measurements. In VALLME method, microvolumes of *n*-octanol are dispersed in the aqueous phase using vortex agitation [35]. The mechanical stress promoted by vortex agitation bursts the *n*-octanol phase into several smaller droplets forming an emulsion (Figure 2). The fine microdroplets formed to increase the interfacial contact area and reduce the thickness of the stagnant aqueous film, which is usually present at the *n*-octanol/water interface [35]. Consequently, the time required to reach equilibrium is dramatically shortened (equilibrium conditions are achieved in 2 min of vortex agitation) [35]. As the formed dispersion is unstable, the target analyte can be easily separated using centrifugation [45]. The microdroplet is then collected with a microsyringe, and the solute concentration is determined by LC [35].

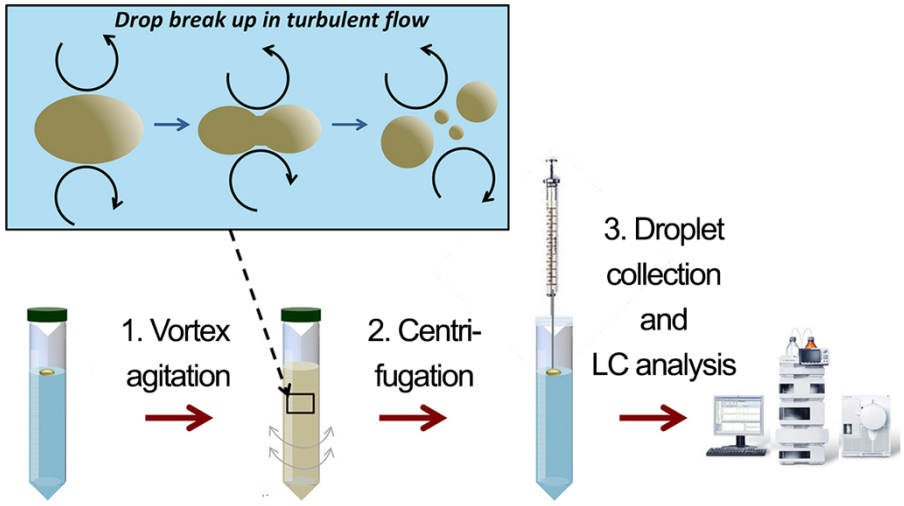

Figure 2. VALLME workflow (adapted from [45]). (Reprint with permission from [45], Copyright (2019) ELSEVIER).

The log P values are calculated as the ratio of equilibrium concentrations in the *n*-octanol and water phases. The equilibrium concentration of the analyte in the *n*-octanol phase is calculated each time using calibration curves obtained with the octanolic individual stock standard solutions. The log P values were calculated for each concentration level using the mass balance Equation (3) [46]:

$$\log P = \log \left(\frac{\text{vol.}_{\text{water}}}{\text{vol.}_{\text{water}}} \times \frac{[\text{octanol}_{\text{eq.}}] \times \text{vol.}_{\text{octanol}}}{[\text{water}_{\text{i}}] \times \text{vol.}_{\text{water}} - [\text{octanol}_{\text{eq.}}] \times \text{vol.}_{\text{octanol}}} \right) \quad (3)$$

where $\text{vol.}_{\text{octanol}}$ is the volume of *n*-octanol phase, $\text{vol.}_{\text{water}}$ is the volume of the water phase, $[\text{octanol}_{\text{eq.}}]$ is the concentration of the compound in the *n*-octanol phase calculated externally by the calibration curve, and $[\text{water}_{\text{i}}]$ is the initial concentration in the aqueous phase. The main advantages of VALLME are its reliability, simplicity, low cost, minimal solvent, and solute consumption [35]. The main disadvantage of VALLME is the requirement of a low-density organic solvent as a nonaqueous phase. From a practical point of view, only *n*-octanol is suitable to be used in VALLME, as it is the only one that can restore to its initial single-drop shape after centrifugation [47].

2.2.7. Nano-Absorbent Based Method

Nanoparticles with absorbent properties can be used for the determination of partition coefficient. One of these methods is based on the use of porous silica-encapsulated magnetic nanoparticles, which are preloaded with a known amount of *n*-octanol [48,49].

These nanoparticles are then dispersed into an aqueous phase containing the tested compound. The small size of the *n*-octanol droplets and the efficient mixing provided by the nanoparticles increase the interfacial contact area between the two phases, which significantly reduces the time required to achieve equilibrium. The magnetic properties of the nanoparticles allow the easy separation of phases. The partition coefficient is determined by measuring the concentration of the targeted analyte in the aqueous phase before and after partitioning.

Despite being used for isotropic lipophilicity determination, nano-absorbent-based methods acquire particular relevance for the determination of anisotropic lipophilicity. Nano-sized artificial membranes, like liposomes [50] or micelles [51], act as absorbents enabling the direct determination of the lipophilicity in these biomimetic nonaqueous phase. The partitioning occurs just by mixing the artificial membranes with an aqueous solution of the compound to be tested. The major technical difficulty is the phase separation after reaching equilibrium. While *n*-octanol and water are two immiscible solvents, liposomes and micelles form a colloidal suspension in water. The most used techniques for phase separation are ultrafiltration [50] and ultracentrifugation [51]. The partition coefficient is determined by quantifying the compound present in the filtrate or in the supernatant.

An alternative method for the determination of anisotropic lipophilicity is based on solid-supported lipid membranes (SSLMs). SSLMs are silica nanobeads coated with a liposomal membrane that was noncovalently bound to the bead [52]. SSLMs beads are added to an aqueous solution of the compound to be tested, and the mixture is incubated to allow the partition between the lipid and aqueous phase. Phase separation is performed by centrifugation with a regular bench centrifuge [52] or by a short filtration step [53]. SSLM silica beads are commercially available and offer a high throughput as the time-consuming ultracentrifugation or ultrafiltration steps are not required.

2.2.8. Dialysis-Based Method

Dialysis bags can be used for the determination of isotropic lipophilicity [54]. In this method, the dialysis bag is filled with a solution of the compound under investigation dissolved in *n*-octanol saturated with water or buffer. The dialysis bag is then immersed into the aqueous phase saturated with *n*-octanol, and sonication is applied to shorten the equilibration time. After sonication, the phases are easily separated and analyzed by LC.

Similar to the nano-absorbent-based methods, dialysis-based methods are more important for the determination of anisotropic lipophilicity than for the determination of isotropic lipophilicity. In fact, the "gold standard" to measure lipid-water partitioning is the equilibrium dialysis method [52]. The dialysis cell consisted of two glass chambers separated by a dialysis membrane [55]. For the experiment, two dialysis cells are required: a reference cell and a measurement cell. One chamber of each cell is filled with a solution of the compound under is added, but the other chamber is filled with buffer and with the membrane suspension in the reference and measurement cell, respectively. The dialysis membrane allows the diffusion across the two chambers of the free compound but impedes the diffusion of the compound bound to the liposomes. The partition coefficient is determined by LC quantification of the compound concentration in the chambers without membrane suspension in the reference and in the measurement cells [56].

2.3. Indirect Experimental Determination of Lipophilicity

Due to the disadvantages associated with direct experiments, there is an increasing demand for developing methods that are able to estimate lipophilicity without quantification requirements [34]. These methods provide a greener and high-throughput measurements than can direct methods while simultaneously can reach good accuracy and versatility [19].

Since the 1980s, chromatographic procedures have been used to obtain physicochemical parameters associated with structural characteristics, namely log P [57]. Exploiting the relationship between the compound retention in a hydrophobic stationary phase and the compound lipophilicity, LC methods really shine in the indirect measurement of lipophilic-

ity. In the following subchapters, the different LC methods used for indirect experimental determination of lipophilicity will be discussed.

2.3.1. Reversed-Phase Thin-Layer Chromatography

Reversed-phase thin-layer chromatography (RP-TLC) is the easiest LC method that can be used for the indirect determination of isotropic lipophilicity [58]. In RP-TLC [59] and reversed-phase-high performance thin layer chromatography (RP-HPTLC) [60], the stationary phase is a commercially available C8 or C18 silica gel plate (Figure 3). Alternatively, it is possible to prepare a non-polar stationary phase from the commonly available silica gel plates after its pulverization with *n*-octanol 5% (*v*/*v*) in diethyl ether. The mobile phases consist of binary mixtures of water (or buffer) and an organic solvent (modifier), usually methanol, which is the preferred solvent due to its compatibility with water, or alternatively tetrahydrofuran or acetonitrile [27].

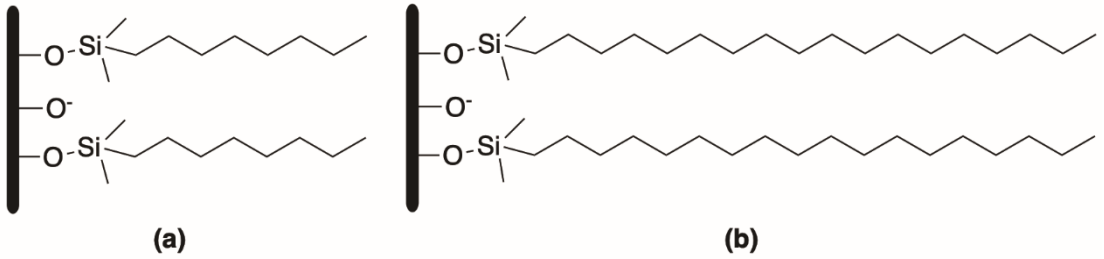

Figure 3. Octyl (**a**) and octadecyl (**b**) silica-based stationary phases.

The retention in reversed-phase systems is mainly governed by partitioning between the stationary and mobile phases [61,62]. Therefore, the lipophilicity index measured in RP-TLC can be derived from the RM value [61,62]:

$$\mathrm{RM} = \log\left[\left(\frac{1}{\mathrm{Rf}}\right) - 1\right] \quad (4)$$

where Rf is the retention factor which is obtained by dividing the distance covered by the sample by the distance covered by the mobile phase.

RM values are determined in the presence of different organic solvent concentrations in the mobile phase. The linear relationship between the RM values and different mobile phase proportions is established, and the partition coefficient is calculated by extrapolating to a pure water mobile phase [63].

2.3.2. Reversed-Phase High-Performance Liquid Chromatography

Reversed-phase high-performance liquid chromatography (RP-HPLC) is the most widely used indirect method to experimentally determine the lipophilicity [58,64] and the standard procedure for measuring log P is officially recommended by OECD [30]. The wide use of RP-HPLC is justified by the advances in the elucidation of solute interactions, the availability of well-characterized stationary phases, and the ubiquitous presence of automated HPLC systems [2]. Table 1 provides a list of reported RP-HPLC methods to illustrate the versatility and large applicability of this technique, including for each example the aim of the study, the compounds under analysis, and the chromatographic method and conditions used. In addition, Table 1 also describes other indirect methods coupled with LC, which will be discussed in the following subchapters. The bibliographic research of assembling Table 1 was conducted using Web of Science and Scopus without any temporal restriction. The keywords used were "lipophilicity AND chromatography", "lipophilicity AND chromatography", "partition AND reversed-phase high-pressure liquid chromatography, "micellar liquid chromatography", and "immobilized artificial membrane".

Table 1. Indirect methods for determining lipophilicity coupled with LC (full list of abbreviations is provided at the end as table footnote).

Analytes	Aim	Chromatographic Method	Chromatographic Conditions	Ref.
Series of corticosteroids	Estimate lipophilicity	RP-HPLC (254 nm)	Columns: Zorbax Eclipse XDB-C18 (150 × 4.6 mm, 3.5 µm) and Zorbax Phenyl (250 × 4.6 mm, 5 µm); MP: mixtures of ACN (5–70%) and water with HCOOH 0.1%; FR: 1.0 mL/min	[65]
17β-Carboxamide glucocorticoids	Estimate lipophilicity	RP-HPLC	Columns: Zorbax Eclipse XDB-C8 (150 × 4.6 mm, 5 µm), Zorbax Eclipse XDB-phenyl (150 × 4.6 mm, 5 µm), and Zorbax SB-CN (150 × 4.6 mm, 5 µm); MP: mixtures of MeOH or ACN (50–90%) and water; FR: not specified	[66]
Antibiofilm agents	Estimate lipophilicity	RP-HPLC	Columns: end-capped RP BDS Hypersil C18 (30 × 4.0 mm, 3 µm) and end-capped Kinetex C8 (150 × 4.6 mm, 5 µm); MP: mixtures of ACN (45–55%) and water with ammonium acetate; FR: 1.0 mL/min	[67]
Steroid derivatives	Estimate lipophilicity	RP-HPLC (210 nm)	Column: Zorbax SB-C18 (250 × 3.0 mm, 5 µm); MP: mixtures of MeOH or ACN (70–80%) and water; FR: 0.6 mL/min	[68]
Antioxidant compounds	Determine the effects of stationary phase on the retention in terms of hydrophobicity	RP-HPLC (254 nm)	Columns: Purosphere RP-18e (125 × 3.0 mm, 5 µm), Zorbax Eclipse XDBC8 (150 × 4.6 mm, 5 µm), Discovery RP-Amide C16 (150 × 4.6 mm, 5 µm), CN100 Lichrosphere (250 × 4.0 mm, 5 µm), and pentafluorophenyl Kinetex (150 × 2.1 mm, 2.6 µm); MP: mixtures of MeOH (55–55%) and water with 0.1% formic acid; FR: 0.7 or 0.2 mL/min	[69]
Tetrahydrothiophen-3-one based thiazoles	Estimate lipophilicity	RP-HPLC (254 nm)	Column: Superspher 100 RP-18; MP: mixtures of MeOH (60–95%) and water; FR: 0.7 mL/min	[70]
N-Hydroxyethylamides of aryloxyalkyllen and pyridine carboxylic acids	Study the role of the stationary and mobile phases on the determination of log P	RP-HPLC/ UV-Vis (254 nm)	Columns: LiChrosorb RP-18 (125 × 4.0 mm, 5.0 µm), LiChrospher 60 RP-Select B (125 × 4.0 mm, 5.0 µm), Zorbax (5 µm, L = 150 × 4.6 mm; 5.0 µm), Zorbax-Eclipse XDB-C18 (150 × 4.6 mm; 5.0 µm); MP: mixtures of MeOH (0–60%) or ACN (0–50%) and water or 20 mM phosphate buffer at pH 7.0 or 20 mM tricine buffer at pH 7.0; FR: 1.0 mL/min	[71]
Basic and neutral drugs	Evaluate the performance of [EMIM][BF_4] in producing extrapolated log k_w indices	RP-HPLC/ UV-Vis (220–254–268 nm)	Columns: Hypersil end-capped BDS C18 column (250 × 4.6 mm, 5.0 µm) and ABZ^+ (150 × 4.6 mm, 5.0 µm); MP: mixtures of MeOH (20–80%) and 20 mM MOPS at pH 7.4 with [EMIM][BF_4] (1%) and with octan-1-ol (0–0.2%); FR: not specified	[72]
Deuterated benzene, toluene, *p*-xylene, pyridine, and aniline, and their isotopomers,	Compare partition coefficients of deuterium atoms by shake-flask and HPLC methods	RP-HPLC/ UV-Vis (254 nm)	Column: LiChrosorb RP-18 (300 × 4 mm, 10 µm); MP: mixtures of MeOH and water; FR: not specified	[73]

Table 1. *Cont.*

Analytes	Aim	Chromatographic Method	Chromatographic Conditions	Ref.
β-Blockers and anti-arrhythmic drugs	Determine the effects of stationary phase on the retention in terms of hydrophobicity	RP-HPLC/DAD (220, 230, 254 and 280 nm)	Columns: SG-CHOL (250 × 4.6 mm) cholesterolic; SG-AP (250 × 4.6 mm); End-capped Supelcosil C18-DB (250 × 4.6 mm, 5.0 µm); Merck Chromolith Performance RP-18e (100 × 4.6 mm, 5.0 µm); MP: mixtures of ACN or MeOH (20–100%) (gradient mode); FR: 1.0 mL/min	[74]
Arylpiperazines and tetrahydroisoquinoline derivatives with imidazo [2,1-f]purine-2,4-dione moiety	Estimate lipophilicity	RP-HPLC/DAD (230 nm)	Column: LiChrospher 100 R C18 (100 × 4.6 mm, 5.0 µm); MP: mixtures of ACN (35–65%) and water with 0.01% TFA; FR: 1.0 mL/min	[75]
meta and *para* Substituted benzenesulfonamides	Estimate lipophilicity	RP-HPLC/ UV-Vis (254 nm)	Column: µ-bondapak C18 (150 × 3.9 mm,10 µm); MP: mixtures of MeOH or ACN and water; FR: 1.0 mL/min	[76]
Terpenoids	Determine lipophilicity by RP-HPLC and compare with shake-flask and predicted values	RP-HPLC/DAD (215 nm); RP-HPLC/RI	Column: Alltech Altima C18 (150 × 4.6 mm, 5 µm); MP: mixtures of MeOH (30–75%) and 0.02 M MOPS buffer at pH 7.2; FR: 1.0 mL/min	[77]
Anilides of pyrazine-2-carboxylic acid and 4-benzylsulfanylpyridine derivatives	Determine lipophilicity by RP-HPLC and compare with predicted values	RP-HPLC/ UV-Vis (254 nm)	Column: Chromolith RP18e; (100 × 4.6 mm); MP: mixture of MeOH (37–42%) and 0.05 M phosphate buffer at pH 7.4; FR: 4.0 mL/min	[78]
Esters of substituted 6-aminohexanoic acid	Determine lipophilicity by RP-HPLC and compare with predicted values	RP-HPLC/DAD (204 nm)	Column: Zorbax Eclipse XDB C18 (150 mm × 4.6 mm, 5 µm); MP: mixture of MeOH (85%) and water; FR: 0.4 mL/min	[79]
Set of compounds representing various functional groups such as ketones, acids, or alcohols	Determine lipophilicity by RP-HPLC and compare it with reported values	RP-HPLC/ UV-Vis (220 and 254 nm)	Columns: Discovery RP Amide C16 (150 × 4.0 mm, 5.0 µm) and Discovery RP Amide C16 (20 × 4.0 mm, 5.0 µm); MP: mixtures of MeOH and phosphate buffer at pH 3 with octanol (0.25%); FR: 1.0 or 2.0 mL/min	[80]
N-(Bemothiazol-2-yl)-α-amino alkyl phosphonic diesters	Compare lipophilicity determination between RP-HPLC and RP-HPTLC	RP-HPLC/ UV-Vis (230 nm)	Column: ODS (250 × 4.6 mm, 5 µm); MP: mixtures of MeOH (70–90%) and water, FR: 1.0 mL/min	[81]
Acyclovir esters	Determine lipophilicity by RP-HPLC and compare with predicted values	RP-HPLC/ UV-Vis (240 nm)	Column: LiChrospher RP-18 (250 × 4.0 mm, 5.0 µm); MP: mixtures ACN or MeOH and 20 mM phosphate buffer pH 6.7; FR: 1.5 mL/min	[82]

Table 1. *Cont.*

Analytes	Aim	Chromatographic Method	Chromatographic Conditions	Ref.
Diverse set of compounds	Compare the solvation equations obtained for different RP chromatographic retention parameters	RP-HPLC	Column: ODS2-IK5 Inertsil (150 × 4.6 mm); MP: mixtures of ACN and 0.1% phosphoric acid or ammonium acetate buffer at pH 9.5 (gradient mode); FR: 1.0 mL/min	[83]
[(5Z)-(5-Arylalkylidene-4-oxo-2-thioxo-1,3-thiazolidin-3-yl)]acetic acids derivatives	Determine lipophilicity by RP-HPLC and compare it with predicted values	RP-HPLC/DAD (210 nm)	Column: end-capped Symmetry C18 (250 × 4.6 mm, 5 µm); MP: mixture of MeOH (70%) and water; FR: 0.9 mL/min	[84]
Weakly ionizable basic compounds and neutral compounds	Determine lipophilicity by RP-HPLC	RP-HPLC/DAD	Column: Phenomenex Gemini C18 (150 × 4.6 mm, 5 µm); MP: mixtures of MeOH and 0.02 M ammonium chloride buffer at pH 7.4 and 9.0; FR: 1.0 mL/min	[64]
4-Alkyl or alkoxy-4′-cyanobiphenyl derivatives	Determine lipophilicity by RP-HPLC	RP-HPLC/ UV-VIS	Column:C18 Nucleosil 7 (15 × 4.6 mm); MP: mixtures of MeOH (75–100%) and water; FR: 1.0 mL/min	[85]
Structurally diverse acidic drugs both in the neutral and ionized form	Determine lipophilicity by RP-HPLC and compare three columns in the presence and absence of octan-1-ol as additive	RP-HPLC/ UV-Vis (220, 254, 268 nm)	Columns: Supelcosil ABZ+ Plus (150 × 4.6 mm, 5 µm) and Supelcosil Aquasil (150 × 4.6 mm, 5 µm); MP: mixtures of MeOH (10–85%) and 20 m M MOPS buffer at pH 2.5 and 7.4 in the presence or absence of octan-1-ol; FR: 1.0 mL/min	[86]
Quinoline derivatives	Determine lipophilicity by RP-HPLC and compare with predicted values	RP-HPLC/DAD (210 nm)	Column: Symmetry C18 (250 × 4.6 mm, 5 µm); MP: mixtures of MeOH and water; FR: 0.9 mL/min	[87]
Amidoximes or substituted heterocyclic coumarin derivatives	Study the relationship between log P and various chromatographic indices, including the extrapolated capacity factors derived by HPLC and reversed-phase TLC	RP-HPLC/ UV-Vis (254 nm)	Column: BDS C18 (250 × 4.6 mm, 5 µm); MP: mixtures of MeOH (40–90%) and water; FR: not specified	[88]
Aliphatic hydrazide derivatives	Determine lipophilicity	RP-HPLC/ UV-Vis (320 nm)	Column: Hypersil BDS C18 (150 × 4.6 mm, 5 µm); MP: mixtures of MeOH (45–95%) with acetate buffer at pH 4.0; FR: 0.5 mL/min	[89]

Table 1. *Cont.*

Analytes	Aim	Chromatographic Method	Chromatographic Conditions	Ref.
Diverse set of compounds	Determine lipophilicity	RP-HPLC/DAD	Columns: Phenomenex Gemini NX (150 × 4.6 mm, 5 µm), Waters XTerra RP-18 (150 × 4.6 mm, 5 µm), Waters XTerra MS C18 (150 × 4.6 mm, 5 µm); MP: mixtures of ACN (40–50%) and pyrrolidine and ammonium hydrogenocarbonate buffer at pH 11.0; FR: 1.0 mL/min	[90]
Selenazole derivatives	Determine lipophilicity by RP-HPLC and compare with predicted values	RP-HPLC/ UV-Vis (210 nm)	Column: Zorbax SB-C18 (100 × 3.0 mm, 3.5 µm); MP: mixtures of MeOH (45–90%) and water; FR: not specified	[91]
Spironolactone	Determine lipophilicity by RP-HPLC and compare with predicted values	RP-HPLC/ UV-Vis (238 nm)	Column: C18 Eurospher 100–5 (250 × 4.0 mm, 5 µm), MP: mixtures of MeOH or dioxane (55–95%) and water; FR: 1.0 mL/min	[92]
6-Aminohexanoates derivatives	Determine lipophilicity by RP-HPLC and compare with predicted values	RP-HPLC/DAD (204 nm)	Column: C18 Zorbax Eclipse XDB (150 × 4.0 mm, 5 µm); MP: mixture of MeOH (85%) and water; FR: 0.4 mL/min	[93]
Marine natural products	Determine lipophilicity by RP-HPLC and compare with predicted values	RP-HPLC/DAD	Column: Hamilton polystyrene-divinylbenzene PRP-1 column (150 × 4.6 mm, 5 µm); MP: mixtures of ACN and 25 mM $AcONH_4$ at pH 4.5, 7.2, 9.8 in the range 0–100%; FR: 1.0 mL/min	[94]
Wide range of commercially available compounds	High-throughput log P measurement using parallel liquid chromatography/ultraviolet/mass spectrometry and sample-pooling	RP-HPLC/UV/MS	Columns: LUNA C18 (100 × 3.0 mm, 5 µm) for use on single channel LC/MS and LUNA C18 (50 × 3.0 mm, 5 µm) for high-throughput analysis on parallel LC/MS; MP: mixtures of MeOH (45–100%) with 20 mM ammonium carbonate at pH 8.0 or 20 mM ammonium formate at pH 1.0; FR: 0.45 mL/min	[95]
Thiosemicarbazide and 1,2,4-triazole-3-thione derivatives	Determine lipophilicity by RP-HPLC and compare it with predicted values	RP-HPLC/ UV-Vis (254 nm)	Column: RP-18 Waters Symmetry (150 × 4.6 mm, 5 µm); MP: mixtures of MeOH (55–80%) or ACN (40–80%) and water; FR: 1.0 mL/min	[96]
α-Asarone derivatives	Determine lipophilicity by RP-HPLC	RP-HPLC/DAD (254 nm)	Columns: RP-18e Purospher STAR C18 (150 × 4.0 mm, 5 µm); RP-8e Purospher STAR C8 (150 × 4.6 mm, 5 µm); Zorbax Eclipse XDB-C (150 × 4.6 mm, 5 µm); Nucleosil Phenyl (250 × 4.6 mm, 7 µm); MP: mixtures of MeOH or ACN and water; FR: 1.0 mL/min	[97]
Mesoionic 1,3,4-thuadiazolium-2-aminide derivatives	Determine lipophilicity by RP-HPLC	RP-HPLC/ UV-Vis	Column: C18 ODS-Shim-Pack (18.0 × 6.0 mm); MP: a mixture of MeOH (25–85%) and 0.005 M of phosphoric and glacial acetic acid buffer at pH 4.6; FR: not specified	[98]

Table 1. *Cont.*

Analytes	Aim	Chromatographic Method	Chromatographic Conditions	Ref.
Arylamino-2-ethane-1,1-diyl- and benzoxazole-2-methylenebisphosphonates	Determine lipophilicity by RP-HPLC and compare with predicted values	RP-HPLC/DAD (210 nm)	Column: Symmetry C18 (250 × 4.6 mm, 5µm); MP: mixture of MeOH (90%) and water; FR: 1.0 mL/min	[99]
Pyrimidinic nucleoside derivatives	Determine lipophilicity by shake-flask, RP-TLC, RP-HPLC, and in silico methods	RP-HPLC/ UV-Vis (265 nm)	Column: CLC-ODS(N)PN 228-17873-91(15 cm) QTY:2, packed with a C18 chemically bonded non-polar stationary phase; MP: mixtures of MeOH (30–80%) and water; FR: 1.0 mL/min	[100]
Nicotinates esters	Determine lipophilicity by RP-HPLC	RP-HPLC/ UV-Vis (262 nm)	Column: LiChrosorb RP-18 (250 × 4 mm, 10 µm); MP: mixtures of MeOH or ACN and 0.02 M MOPS buffer at pH 7.0 wit *N*-decylamine (0.2%); FR: 1.5 mL/min	[101]
Structurally diverse set of neutral, acidic, and basic compounds	Determine lipophilicity by RP-HPLC	RP-HPLC/variable wavelength detector (220–300 nm)	Column: PRP-C18 (50 × 4.6 mm, 5 µm) or (33 × 2.1 mm, 5 µm); MP: mixtures of ACN and water with 0.2% phosphoric acid or 30 mM of diethylamine; FR: 2.0 mL/min	[102]
Esters of alkoxyphenylcarbamic acid	Study the QSRR models for potential local anaesthetic drugs	RP-HPLC/DAD (210–290 nm)	Column: Separon SGX C18 (150 × 3.2 mm, 5 µm) or Separon SGX Phenyl (150 × 3.2 mm, 5 µm); MP: mixtures of MeOH or ACN (80%) and water; FR: 1.0 mL/min	[103]
Methylated naphthalene derivatives	Determine lipophilicity by RP-HPLC	RP-HPLC/DAD (254 nm)	Column: octadecylsilyl SUPELCOSIL LC-PAH (250 × 4.6 mm, 5 µm); MP: mixture of ACN (40–100%) and water; FR: 2.0 mL/min	[104]
(Hetero)arylamides of 2-amino 4,6-dimethyl pyridine	Determine lipophilicity by RP-HPLC	RP-HPLC/ UV-Vis (254 or 285 nm)	Column: porous polystyrene/divinylbenzene copolymer PLRP-S (250 × 4.6 mm, 8 µm); MP: mixture of ACN (60%) and 0.02 M Na_2HPO_4 buffer at pH 9.4; FR: 1.5 mL/min	[105]
Neutral compounds of varied structure	Evaluate a RP column under isocratic and gradient elution conditions for estimating lipophilicity	RP-HPLC/ UV-Vis	Column: Supelcosil LC-ABZ (150 × 4.6 mm, 5 µm); MP: mixtures of MeOH and water; FR: 1.5 mL min	[106]
Herbicides including triazines and phenylurea derivatives	Determine lipophilicity by shake-flask, RP-HPLC, and in silico methods	RP-HPLC/DAD (218, 230, 245 or 270 nm)	Column: Spherisorb ODS 2 (125 × 4 mm, 5 µm); MP: mixtures of ACN (10–45%) and 1 mM ammonium acetate buffer at pH 6.7–7.3; FR: 0.8 mL/min	[107]
Allyl thiosemicarbazide, N1-thiocarbamylamidrazone derivatives	Determine lipophilicity by RP-TLC, RP-HPLC and in silico method	RP-HPLC/ UV-Vis (254 nm)	Column: RP-18 Waters Symmetry (150 × 4.6 mm, 5 µm); MP: mixtures of MeOH (50–80%) and water; FR: 1.0 mL/min	[108]

Table 1. *Cont.*

Analytes	Aim	Chromatographic Method	Chromatographic Conditions	Ref.
Amphoteric compounds	Determine lipophilicity RP-HPLC	RP-HPLC/ UV-Vis	Column: C18 Hypersil 5 ODS (250 × 4.6 mm, 5 µm); MP: mixture of MeOH (50%) and phosphate buffer at pH range of 3.0 to 8.0; FR: 1.0 mL/min	[109]
Succinimide derivatives	Determine lipophilicity by RP-TLC, RP-HPLC and in silico method	RP-HPLC/DAD	Column: XTerra MS C-18 (150 × 4.6 mm, 5 µm); MP: mixtures of MeOH (5–100%) and water; FR: 1.0 mL/min	[110]
2-Substituted phenylnitronyl nitroxides	Determine lipophilicity RP-HPLC	RP-HPLC/ UV-Vis (275 nm)	Column: Rainbow C18 (150 × 4.6 mm); MP: mixture of MeOH (40%) and 0.001 M phosphate buffer; FR: 0.25 mL/min	[111]
Sorgoleone	Determine lipophilicity RP-HPLC	RP-HPLC/ UV-Vis (280 nm)	Column: C18 (250 × 4.6 mm); MP: mixture of ACN (70%) and water; FR: 1.8 mL/min	[112]
Solutes and drugs with well-defined solvatochromic parameters	Compare two Stationary Phases Based on Retention Mechanisms	RP-HPLC/ UV-Vis	Columns: Supelcosil Silica-based Discovery-RP-Amide-C16 (50 × 4.6 mm, 5 µm) and Asahipak Polymer-based ODP-50-4B (50 × 4.6 mm, 5 µm); MP: mixtures of MeOH (10–80%) and 0.02 M phosphate buffer at pH 3.0, 4.0, or 7.0 with octan-1-ol (0.25%); FR: 0.5 or 1 mL/min	[113]
[^{11}C]Me-Halo-CGS 27023A analogues	Determine lipophilicity RP-HPLC	RP-HPLC/UV (240 nm) and γ-ray (NaI)	Column: Prodigy C18 (250 × 4.6 mm, 5 µm); MP: mixture of ACN, MeOH, and 20 mM $KHPO_4$ buffer at pH 6.7; FR: 1.5 mL/min	[114]
Set of compounds covering a wide and regular range of structural parameters	Understand the structural properties governing retention mechanisms on RP-HPLC stationary phases	RP-HPLC/ UV-Vis and RI	Column: ODS Supelcosil LC-ABZ (150 × 4.6 mm, 5 µm) pre-treated with electrostatic coating; MP: mixtures of MeOH (10–50%) and 0.02 M MOPS buffer at pH 7.4; FR: 1.0 mL/min	[115]
Local anesthetics	Compare a fast and direct method of determining lipophilicity against an indirect HPLC determination	RP-HPLC/UV	Column: C18 LiChroCART Purospher (125 × 3.0 mm, 5 µm); MP: not specified; FR: 0.5 mL min	[54]
Large and diverse group of drugs	Determine lipophilicity and dissociation constant	RP HPLC–ESI-TOF–MS	Column: XBridge-C18 (50 × 3.0 mm, 2.5 µm); MP: series of pH and organic modifier gradients; FR: 0.5 mL/min	[116]
Estradiol derivatives	Evaluate the predictive power of the calculation procedure for molecular hydrophobicity	RP-HPLC/ UV-Vis (254 nm)	Columns: LiChrosorb® RP-18 (250 × 4.0 mm, 5 µm) and LiChrosphert RP-8 (150 × 4.6 mm, 5 µm); MP: mixtures of MeOH (60–95%) or ACN (70–90%) and water; FR: 1.0 mL/min	[117]

Table 1. *Cont.*

Analytes	Aim	Chromatographic Method	Chromatographic Conditions	Ref.
Natural compounds (mycotoxins and alkaloids, and amines)	Determine lipophilicity RP-HPLC	RP HPLC/DAD/MS	Columns: LiChroCART, Purospher RP-18e (125 × 3.0 mm; 5 µm) or LiChro-CART, LiChrospher RP-18e (250 × 4.0 mm; 5 µm) or Zorbax, Eclipse XDB-C8 (150 × 4.6 mm; 5 µm); MP: mixtures of MeOH or ACN and water at pH of 9.6 or 2.8; FR: 0.5 or 0.8 mL/min	[118]
Pyrrolyl-acetic acid derivatives	Determine lipophilicity RP-HPLC at low pH and at pH 7.4	RP-HPLC/ UV-Vis (254 nm)	Column: Supelcosil ABZ$^+$ (15 × 4.6 mm, 5 µm); MP: different mixtures of MeOH (10–70%) and MOPS buffer at pH 3.0 and 7.4 with octan-1-ol (0–0.25%); FR: not specified	[119]
Protonated basic compounds	Determine lipophilicity by RP-HPLC	RP-HPLC/ UV-Vis (254 nm)	Column: LiChrosorb RP-18 (250 × 4 mm, 5 µm); MP: mixtures of MeOH and 0.02 M MOPS buffer with *N*-decylamine (0.2%); FR: 1.5 mL/min	[120]
Drugs and flavonoids	Study the influence of 1-octanol in the determination of lipophilicity by RP-HPLC	RP-HPLC/ UV-Vis (254 nm)	Column: Supelcosil Discovery-RP-Amide-C16 (50 × 4.6 mm, 5 µm); MP: mixtures of MeOH (10–70%) an 0.02 M phosphate buffer at pH 3.0 with octan-1-ol (0–0.25%); FR: 1.0 mL/min	[121]
Parabens	Lipophilicity determination with mobile phases containing low and medium hydrophobic alcohols	RP-HPLC/MWD (254 nm)	Column: double end-capped Zorbax Eclipse XDB-C18 (150 × 4.6 mm, 5 µm); MP: mixtures of MeOH with 1% of different alcohols and water with 0.1% H_3PO_4; FR: 1.0 mL/min	[122]
DDT and related compounds	Determine lipophilicity by RP-HPLC	RP-HPLC/ UV-Vis	Column: Kromasil C18 (150 × 4.6 mm, 5 µm); MP: mixtures of MeOH and water; FR: 1.0 mL/min	[123]
2-Substituted alkyl-6-(2,5-dioxopyrrolidin-1-yl)hexanoates	Investigate the activity as skin penetration enhancers	RP-HPLC/DAD (204 nm)	Column: Zorbax Eclipse XDB (150 × 4.6 mm, 5 µm); MP: mixture of MeOH and water; FR: 0.4 mL/min	[124]
Penicillins and cephalosporins	Determine lipophilicity by RP-HPLC	RP-HPLC/ UV-Vis (254 nm)	Column: porous silica gel bonded chemically with octadecyl chains (250 mm); MP: mixture of MeOH and 0.035 M ammonium chloride buffer at pH 7.4; FR: not specified	[125]
Set of diverse compounds	Determine lipophilicity by RP-HPLC	RP-HPLC/ UV-Vis (210, 230 and 254 nm)	Column: Interaction ACT-l (150 × 4.6 mm, 10 µm), Nucleosil Cs (150 × 4.6 mm, 5 µm), Hamilton divinylbenzene-styrene copolymer (PRP-1) (150 × 4.1 mm, 10 µm); MP: mixtures of ACN (60–70%) and 0.1 M ammonium acetate buffer at pH 4.6; FR: 0.75 or 2.0 mL/min	[126]

Table 1. *Cont.*

Analytes	Aim	Chromatographic Method	Chromatographic Conditions	Ref.
Acridinone derivatives	Determine the lipophilicity by RP-HPLC	RP-HPLC/DAD (254 nm)	Columns: Luna 5u C18(2) (150 × 4.6 mm, 5 µm), Candeza CD-C18 (150 × 4.6 mm, 3 µm), TSK-gel ODS-80TS (150 × 4.6 mm, 5 µm), Ascentis C18 (150 × 4.6 mm, 5 µm), Unison UK-C18 (150 × 4.6 mm, 3 µm), Zorbax SB-C8 column (75 × 4.6 mm, 3.5 µm); MP: mixtures of ACN or MeOH and water with 0.1% of formic acid; FR: 1.0 mL/min	[127]
Neutral drugs	Development of a method to determine the lipophilicity	RP-HPLC/DAD (235, 255, 265 and 275 nm)	Column: Supelcosil LC-ABZ (50 × 4.6 mm, 5 µm); MP: mixture of MeOH (15–70%) and 20 mM MOPS buffer at pH 7.4 with 0.25% of octanol; FR: 0.5, 1.0, or 2.0 mL/min	[28]
Structurally unrelated compounds	Determine the lipophilicity by RP-HPLC with a fast generic gradient	RP-HPLC	Column: ODS2-IK5 Inertsil (150 × 4.6 mm); MP: mixture of ACN (0–100%) and 50 mM ammonium acetate (pH ranging from 7.0 to 7.3); FR: 1.0 mL/min	[128]
Drugs with diverse chemical nature	Determine the lipophilicity by RP-HPLC	RP-HPLC/ UV-Vis	Column: XBridge^{TM} Shield RP_{18} (50 × 4.6 mm, 5 µm); MP: mixtures of MeOH (70 to 10%) and 0.02 M phosphate buffer at pH 7.0 with 1-octanol/buffer partitioning a 0.25%; FR: 1.0 or 0.5 mL/min	[129]
1*H*-Pyrazolo[3,4-*b*]pyridine derivatives	Determine the lipophilicity by RP-TLC and RP-HPLC	RP-HPLC/ UV-Vis (254 nm)	Column: LC8 SUPELCO (250 × 4.6 mm × 5µm); MP: mixtures of ACN (40–65%) and 0.1 M phosphate buffer at pH 7.4; FR: 1.5 mL/min	[59]
Isochromanone derivatives	Determine the lipophilicity by RP-HPLC	RP-HPLC/DAD	Column: Hypersil 5 MOS (250 × 4.6 mm, 5 µm); MP: mixture of ACN (40%) and triethyl-ammonium phosphate buffer; FR: 1.0 mL/min	[130]
Amphoteric compounds, amino acids, and small peptides	Determine the lipophilicity by RP-HPLC in a broad pH range	RP-HPLC/ UV-Vis	Column: Kromasil C18 (150 × 4.6 mm, 5 µm); MP: mixtures of MeOH (10–90%) and ammonium acetate buffer at different pH; FR: 1.0 mL/min	[131]
α-(4-Phenylpiperazine) derivatives of *N*-benzylamides	Determine lipophilicity by RP-HPLC and in silico methods	RP-HPLC/ UV-Vis (215 nm)	Column: Lichrospher RP18, (125 × 4.0 mm, 5 µm); MP: mixtures of ACN (45–65%) and water with addition of 0.1%TFA; FR: 1.0 mL/min	[132]
N-Alkylbenzenes and some OECD reference substances	Correlate log P shake-flask values and capacity factors derived from RP-HPLC	RP-HPLC/ UV-Vis	Column: C18-SIL-X-5 (250 × 4.0 mm, 5 µm); MP: mixtures of MeOH (60–95%) and water; FR: 1.0 mL/min	[133]
Natural secondary plant metabolites	Determine lipophilicity by RP-HPLC and in silico methods	RP-HPLC/DAD	Column: GraceSmart RP18 (150 × 4.6 mm, 5 µm); MP: mixtures of MeOH (90–30%) and 0.01 M phosphate buffer at pH 2.0; FR: 1.0 mL/min	[134]
Derivatives of *N*-substituted amides of 3-(3-ethylthio-1,2,4-triazol-5-yl)propenoic acid)	Determine the lipophilicity by RP-HPLC	RP-HPLC/ UV-Vis (254 nm)	Column: RP-18 Waters Symmetry (150 × 4.6 mm 5 µm); MP: mixtures of MeOH (50–75%) and water with or without ammonium buffer; FR: 1.0 mL/min	[135]

Table 1. *Cont.*

Analytes	Aim	Chromatographic Method	Chromatographic Conditions	Ref.
Cucurbitacins	Correlate the hydrophobicity index and the basal cytotoxicity on HepG2 cells	RP-HPLC/DAD	Column: Alltima C18 (250 × 4.6 mm, 5 µm) or Econosil C18 (150 × 4.6 mm, 5 µm); MP: mixtures of ACN and ammonium acetate buffer; FR: 1.0 mL/min	[136]
Carotenoids	Determine the lipophilicity by RP-HPLC	RP-HPLC/DAD	Column: Zorbax SB-C18 (250 × 3.0 mm, 5µm); MP: mixture of acetone (0–75%) and water (gradient mode); FR: 0.5 mL/min	[137]
Highly lipophilic benzoxazole-2ylphosphonates	Determine the lipophilicity by RP-HPLC	RP-HPLC/DAD (210 nm)	Column: Symmetry C18 (250 × 4.6 mm, 5 µm); MP: mixture of ethanol (90%) and water; FR: 1.0 mL/min	[138]
Bispyridinium derivatives	Determine lipophilicity by RP-HPLC	RP-HPLC/DAD (210 nm)	Column: Symmetry C18 (250 × 4.6 mm, 5µm); MP: mixture of MeOH (15%) and water; FR: 0.9 mL/min	[139]
1-(1-Arylimidazolidyn-2-ylidyn)-3-arylalkyl urea derivatives	Compare the conventional RP-HPLC and RP-HPLC enriched with room temperature imidazolium-based ionic liquids	RP-HPLC/DAD	Column: Zorbax Extend-C18 (150 × 4.6 mm, 5 µm); MP: mixtures of MeOH or ACN and water with the addition of butyl-methyl imidazolium-based ionic liquids; FR: 1.0 mL/min	[140]
Statin drugs	Determine lipophilicity by RP-HPLC	RP-HPLC/DAD	Columns: Zorbax SB-C18 (250 × 4.6 mm, 5 µm), LiChrospher RP-C8 (250 × 4.0 mm, 5 µm); MP: mixtures of ACN (20–80%) and water; FR: 1.0 mL/min	[141]
Basic and acidic analytes	Determine simultaneously pKa and lipophilicity by RP-HPLC	RP-HPLC/DAD	Column: XTerra MS C-18 (150 × 4.6 mm, 5 µm); MP: mixtures of MeOH with buffers at different pH (gradient mode); FR: 1.0 mL/min	[142]
Di-substituted 2-hydroxyacetamides	Correlate different lipophilicity parameters and antimycobacterial activities	RP-HPLC/ UV-Vis (205 nm)	Column: RP-C18 (250 × 4.0 mm, 5 µm); MP: mixtures of MeOH (10–50%) and water; FR: 1.0 mL/min	[143]
Fused 1,2,4-triazinones derivatives	Determine lipophilicity by RP-HPLC and in silico methods	RP-HPLC/ UV-Vis (245 and 366 nm)	Column: Supelcosil LC-18 (150 × 4.6 mm, 5 µm); MP: mixtures of ACN (10–70%) or dioxane (5–80%) or MeOH (10–80%) and water; FR: 1.0 mL/min	[144]
Polyprotic analytes	Determine pKa and lipophilicity by RP-HPLC	RP-HPLC/DAD	Column: XTerra MSC-18 (150 × 4.6 mm, 5 µm); MP: mixtures of MeOH and different buffers; FR: 1.0 mL/min	[145]
4,5-Dihydro-1*H*-1,2,4-triazol-5-one derivatives	Determine lipophilicity by RP-HPLC	RP-HPLC/ UV-Vis (254 nm)	Column: Luna C18 (250 × 4.6 mm, 5 µm); MP: mixtures of MeOH (25–100%) and water; FR: 1.0 mL/min	[146]

Table 1. *Cont.*

Analytes	Aim	Chromatographic Method	Chromatographic Conditions	Ref.
2-(4-Substituted phenyl)-3(2*H*)-isothiazolones derivatives	Determine lipophilicity by RP-HPLC and correlate it with biological activity	RP-HPLC/ UV-Vis (260 and 285 nm)	Column: octadecyl–poly(vinyl alcohol) (20 × 4.0 mm, 5 µm); MP: mixtures of MeOH and buffers at pH 2.0, 7.0 and 10.0 (gradient elution); FR: 1.5 mL/min	[147]
Nitrazepam in bile acid micelles	Evaluate the importance of temperature dependence of retention coefficient in QSAR model	RP-HPLC/DAD (210 nm)	Column: Eclipse Plus C18 (250 × 3.0 mm, 5 µm); MP: mixture of MeOH and 0.01 M phosphate buffer at pH 7.0; FR: 1.0 mL/min	[148]
Diazido Platinum(IV) complexes	Study the cellular accumulation, lipophilicity, and photocytotoxicity	RP-HPLC/UV	Column: Agilent Zorbax eclipse plus C18 (250 × 4.6 mm, 5 µm); MP: mixtures of MeOH and water with 0.1% TFA; FR: 1.0 mL/min	[149]
Organic compounds	Determine lipophilicity by RP-HPLC	RP-HPLC/DAD	Columns: Luna C18 (150 × 4.6 mm, 5 µm), Chromolith Performance RP-18e monolithic (100 × 4.6 mm); MP: mixtures of ACN or MeOH and buffers at different pH; FR: 1.0 or 2.0 mL/min	[150]
β-Blockers	Apply perfluorinated acids as ion-pairing reagents for RP chromatography and retention-hydrophobicity relationships	RP-HPLC/DAD (220 nm)	Column: Zorbax Extend-C18 (150 × 4.6 mm, 5 µm); MP: mixtures of MeOH and water with perfluorinated acids; FR: 1.0 mL/min	[151]
Cyclen derivatives	Determine lipophilicity by RP-HPLC	RP-HPLC/DAD (250 and 280 nm)	Column: Waters X-Bridge C-18 (150 × 4.6 mm, 5 µm); MP: mixture of ACN, MeOH and water (gradient mode); FR: 1.0 mL/min	[152]
Naturally occurring bile acids	Evaluate the bile acid lipophilicity	RP-HPLC/ UV-Vis (200 nm)	Column: C-18 (100 mm, 5 µm); MP: mixture of MeOH (35–65%) and 0.01 M phosphate buffer at pH 7.0; FR: 0.2 mL/min	[153]
Thiosemicarbazides and 1,2,4-triazole derivatives	Determine lipophilicity by RP-HPLC	RP-HPLC/DAD	Column: Zorbax 100 Extend-C18 (150 × 4.6 mm, 5 µm); MP: mixtures of can or MeOH and water; FR: 1.0 mL/min	[154]
Simple model compounds	Assess the lipophilicity by different techniques	RP-HPLC/DAD	Columns: C18 (125 × 4.0 mm); CN (125 × 4.0 mm); C18e (125 × 4.0 mm); DIOL (125 × 4.0 mm); MP: mixtures of ACN or MeOH and water (gradient mode); FR: 1.0 mL/min	[155]
Benzodiazepine-receptor ligands	Measure the lipophilicity by RP-HPLC and RP-TLC	RP-HPLC/ UV-Vis (254 nm)	Column: µBondapak Cl8 (30 × 3.9 mm); MP: mixtures of ACN (30–70%) and phosphate buffer at pH 7.0; FR: not specified	[156]
Halogenated organic pollutants	Determine lipophilicity by RP-HPLC	RP-HPLC/DAD	Columns: Venusil XBP C8 (150 × 2.1 mm, 5 µm); Venusil XBP C18 (150 × 2.1 mm, 5 µm); MP: mixtures of MeOH and water; FR: not specified	[157]

Table 1. *Cont.*

Analytes	Aim	Chromatographic Method	Chromatographic Conditions	Ref.
Thiosemicarbazides and analogues	Determine lipophilicity by chromatographic and in silico methods	RP-HPLC/ UV-Vis (254 nm)	Column: RP-18 Waters Symmetry (150 × 4.6 mm, 5 µm); MP: mixtures of MeOH (40–65%) and water; FR: 1.0 mL/min	[158]
Nicotine, cytisine and lobeline	Correlate the activity of cytisine and lobeline in ligand-binding and behavioural experiments and determine their lipophilicity	RP-HPLC/ UV-Vis (254 nm)	Column: Lichrosorb RP-18 (250 × 4.0 mm, 10 µm); MP: mixtures of MeOH and 0.02 M MOPS buffer at pH 7.4 with *N*-decylamine (0.2%); FR: 1.5 mL/min	[159]
Preservatives	Determine lipophilicity by chromatographic and in silico methods	RP-HPLC/DAD/MS (230, 254 and 366 nm)	Columns: endcapped C18 LiChroCART, Purosphere RP-18e (125 × 3.0 mm, 5 µm), double endcapped C8 Zorbax, Eclipse XDBC8, (150 × 4.6 mm, 5 µm), CN100 Lichrosphere, (250 × 4.0 mm, 5 µm), endcapped Supelcosil LC-NH2 (150 × 3.0 mm, 3 µm); MP: mixture of MeOH and water with 0.1% formic acid; FR: 1.0 and 0.6 mL/min	[160]
8-Aryl-2,6,7,8-tetrahydroimidazo[2,1-c][1,2,4]triazine-3,4-dione derivatives	Determine lipophilicity by chromatographic and in silico methods	RP-HPLC/ UV-Vis (254 nm)	Column: Zorbax Eclipse XDB C-18 (150 × 4.6 mm, 7 µm); MP: mixtures of MeOH and water; FR: 1.0 mL/min	[161]
2-Methyl-4-oxo-3*H*-quinazoline-3-alkyl-carboxylic acid derivatives	Determine the lipophilicity	RP-HPLC/ UV-Vis (270 nm)	Column: Ultrasphere C8 (250 × 4.0 mm, 5 µm); MP: mixtures of MeOH and water; FR: 1.0 mL/min	[162]
Neutral and basic drugs	Determine the lipophilicity	RP-HPLC/ UV-Vis	Column: Hypersil BDS C-18 (250 × 4.6 mm, 5 µm); MP: mixtures of MeOH (85%) and 20 mM MOPS at pH 7.4 with *N*-decylamine (0.15–20%) or octan-1-ol (0.25%); FR: not specified	[163]
Acidic and basic drugs	Evaluate the effect of octan-1-ol in the mobile phase	RP-HPLC/DAD	Column: Gemini C18 (150 × 4.6 mm, 5 µm), Gemini C18 (50 × 4.6 mm, 5 µm); MP: mixtures of MeOH with 0.25% of octan-1-ol (40–55%) and buffer saturated with octan-1-ol; FR: 1.0 mL/min	[164]
p-Toluenesulfonyl-hydrazinothiazole and hydrazine-bis-thiazole derivatives	Compare between RP-HPLC and RP-HPTLC	RP-HPLC/ UV-Vis (210 nm)	Column: Zorbax SB-C18 (100 × 3.0 mm, 3.5 µm); MP: mixtures of MeOH (45–90%) and water; FR: not specified	[60]
1-Aryl-3-ethyl-3-methylsuccinimides	Compare between RP-HPLC and RP-HPTLC	RP-HPLC (254 nm)	Column: Luna C18 (250 × 4.6 mm, 5 µm); MP: mixtures of MeOH (55–55%) and water; FR: 1.0 mL/min	[165]
Basic compounds such as β-blockers, local anesthetics, and piperazines	Determine lipophilicity	RP-UPLC/ UV-Vis or RP-UPLC/MS	Columns: Acquity BEH Shield RP18, Acquity BEH C18, Acquity BEH C8, Acquity BEH Phenyl (30 mm × 2.1 mm, 1.7 µm); MP: mixtures of MeOH or ACN and aqueous buffer saturated in 1-octanol; FR: 0.5 mL/min	[166]

Table 1. *Cont.*

Analytes	Aim	Chromatographic Method	Chromatographic Conditions	Ref.
Neutral, acidic, basic, and amphoteric drugs	Determine lipophilicity	RP-UPLC/DAD and RP-HPLC/UV-Vis	Columns: Gemini NX, (150 × 4.6 mm, 5 µm), Kinetex C18 (100 × 4.6 mm, 2.6 µm), Waters Acquity BEH C18 (50 × 2.1 mm, 1.7 µm); MP: mixtures of ACN (50%) and buffers at different pH values; FR: 0.5 and 1.0 mL/min	[167]
Diverse set of compounds	Determine the lipophilicity	RP-UPLC/ UV-Vis (215 or 220 nm)	Columns: Acquity BEH Shield RP18, Acquity BEH C18, Acquity BEH C8, AcquityBEH phenyl (30 × 2.1 mm, 1.7 µm); MP: mixtures of MeOH or ACN and aqueous buffer; FR: 0.5 mL/min	[168]
Androstane derivatives	Determine the lipophilicity	RP-UPLC/ UV-Vis (210 and 230 nm)	Column: ZORBAX Eclipse C18 (2.1 × 50 mm, 1.8 µm); MP: mixtures of MeOH (30–60%) or ACN (30–60%) and water); FR: 0.3 mL/min	[169]
N-[(4-Arylpiperazin-1-yl)alkyl]pyrrolidine-2,5-dione derivatives	Determine the lipophilicity	RP-UPLC–MS	Column: Acquity UPLC BEH C18 (2.1 × 100 mm, 1.7 µm); MP: mixtures of methanol (30–90%) and 0.01 ammonium acetate at pH 7.4); FR: 0.3 mL/min	[170]
Highly lipophilic compounds	Determine lipophilicity	RP-HPLC/ UV-Vis and UHPLC/UV-Vis	Columns: Discovery RPAmide C16 (20 × 4.0 mm, 5 µm) or Hypersil GOLD Javelin HTS (10 × 2.1 mm, 1.9 µm) or Acquity BEH Shield RP18 (30 × 2.1 mm, 1.7 µm); MP: mixtures of MeOH or ACN and water; FR: 0.5 or 1.0 mL/min	[171]
Huperzine A derivatives	Determine the lipophilicity	RP-HPLC/DAD and IAM-HPLC/DAD	Columns: ODS µBondapak C18 (300 × 3.9 mm) and IAM.PC.MG (150 × 4.6 mm); MP: mixtures of water and ACN; FR: 1.0 mL/min	[172]
Thioquinoline derivatives	Determine lipophilicity by RP-HPLC and IAM	RP-HPLC/UV-Vis and IAM-HPLC/UV-Vis (238, 215, 230, and 234 nm)	Column: LiChrospher RP18 (125 × 4.0 mm, 5 µm), IAM. PC DD2 Regis (100 × 4.6 mm, 12 µm); MP: mixtures of ACN (55–90%) and water or mixtures of ACN (35–60%) and phosphate buffer at pH 5.4; FR: 1.0 mL/min	[173]
Thiazolidinediones	Study the lipophilic behaviour and the relationships with PPAR-γ activity	RP-HPLC/UV-Vis and IAM-HPLC/UV-Vis (228, 267, or 254 nm)	Columns: Hypersil BDS C-18 (250 × 4.6 mm, 5 µm), Supelcosil ABZ+ Plus (150 × 4.6 mm, 5 µm), Supelcosil Aquasil (150 × 4.6 mm, 5 µm), IAM.PC.DD2; MP: mixtures of MeOH (35–80%) and 20 mM MOPS buffer at 7.4 or mixtures of ACN (10 to 35%) and 0.01 M PBS buffer in the pH range 2.5–7.4; FR: 1.0, 2.0, or 3.0 mL/min	[174]
2-(2,4-Dihydroxyphenyl) thieno-1,3-thiazin-4-one derivatives	Determine lipophilicity by RP-HPLC and IAM	IAM-HPLC/ UV-Vis (320 nm) and RP-HPLC/UV-Vis (380 nm)	Column: HypersilGold C18 (100 × 3 mm, 3 µm) and IAM.PC.DD2 (100 × 4.6 mm, 12 µm); MP: mixtures of MeOH (30–95%) and 20 mM phosphate buffer at pH 4.0 or mixtures of ACN (10–50%) and 20 mM phosphate buffer at pH 7.4; FR: 0.5 or 1.0 mL/min	[175]
Sα-(4-Phenylpiperazin-1-yl)-γ-phthalimido-butyramides derivatives	Determine lipophilicity by chromatographic and in silico methods	IAM-HPLC/UV-Vis and RP-HPLC/UV-Vis (210 nm)	Column: LiChirospher 100 RP18 (100 × 4.6 mm, 5 µm) and IAM.PC.DD2 (100 × 4.6 mm); MP: mixtures of ACN (35–55%), water with 0.01% TFA; FR: 1.0 mL/min	[176]

Table 1. *Cont.*

Analytes	Aim	Chromatographic Method	Chromatographic Conditions	Ref.
Solutes with sufficiently varied properties	Characterize an IAM column and study its similarity with common C18 chromatographic and biological systems	IAM-HPLC and RP-HPLC/ UV-Vis (254 or 214 nm)	Columns: IAM.PC.DD2 (100 × 4.6 mm; 12 µm), XTerra MS C18 (150 × 4.6 mm, 5 µm), XTerra RP18 (150 × 4.6 mm, 5 µm); MP: mixtures of ACN and 0.01 M phosphate aqueous buffer adjusted to pH 7.0; FR: 1.0 mL/min	[177]
Porphyrins	Study the lipophilicity of and retention in IAM, C8-C18, and HILIC chromatographic systems	IAM-HPLC and RP-HPLC/DAD (254 and 417 nm)	Columns: Gravity C18 (125 × 4.0 mm, 5 µm); Gravity C8 (125 × 4.0 mm, 5 µm); PolarTec (125 × 4.0 mm, 5 µm); HILIC (125 × 4.0 mm, 5 µm); IAM.PC.DD2 (30 × 4.6 mm, 10 µm); MP: mixtures of ACN (5–100%) and aqueous 20 mM ammonium acetate at pH 7.0 (gradient mode); FR: 1.0 mL/min	[178]
4,5-Disubstituted-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones derivatives	Study the chromatographic behaviour on different reversed-phase materials	IAM-HPLC and RP-HPLC/DAD	Columns: Zorbax Extend-C18 (150 × 4.6 mm, 5 µm); Cogent UDC Cholesterol (150 mm × 4.6 mm, 4 µm); Regis IAM.PC. DD2 (150 × 4.6 mm, 10 µm); MP: mixtures of MeOH (75–90%) or ACN (55–70%) and water; FR: 1.0 mL/min	[179]
Alkyl benzene derivatives and PAHs, flavonoids, nucleosides, and nucleic bases	Compare retention properties of stationary phases imitated cell membrane in RP HPLC	IAM-HPLC and RP-HPLC/DAD	Column: IAM.PC.DD2 (150 × 4.6 mm, 12 µm); Amino-P-C18 (125 × 4.6 mm, 5 µm); MP: mixtures of MeOH or ACN and water; FR: 1.0 mL/min	[180]
Macrolides	Investigate the relationships between retention factors and cellular permeation	RP-HPLC/MS IAM-HPLC/MS	Columns: Luna C18 (50 × 3 mm), IAM PC DD; MP: mixture of ACN (0–100%) and 0.05 M ammonium acetate at pH 7.4; FR: 1.0 mL/min	
Cephalosporins	Estimate lipophilicity	IAM-HPLC	Columns: IAM.PC.DD 2 (100 × 4.6 mm, 12 µm); MP: phosphate buffer at pH 6.9; FR: 1.0 mL/min	[181]
Structurally diverse basic and neutral drugs	Compare different IAM and reversed-phase retention factors and their relationships with lipophilicity data	IAM-HPLC	Column: IAM.PC.DD2, BDS C-18 (250 × 4.6 mm, 5 µm); MP: mixtures of ACN and 0.01 M PBS buffer or 0.02 M MOPS buffer at pH 7; FR: 1.0, 2.0, or 3.0 mL/min	[182]
Structurally non-related orally administered drugs	Investigate possible relationships between pharmacokinetic parameters and intestinal permeation data.	IAM-HPLC	Columns: IAM.PC.MG (150 × 4.6 mm; 12 µm), IAM.PC.DD2 (100 × 4.6 mm; 10 µm); MP: mixtures of ACN and 0.1 M phosphate buffer at pH 7.0; FR: not specified	[183]

Table 1. *Cont.*

Analytes	Aim	Chromatographic Method	Chromatographic Conditions	Ref.
Structurally diverse drugs	Investigate the relationships, retention factors, and volume of distribution	IAM-HPLC	Column: IAM PC.DD2 (150 × 3.0 mm); MP: Mobile phase A: mixtures of ACN (0–70%) and 0.05 M ammonium acetate at pH 7.4; FR: 1.0 mL/min	[184]
Quinolone drugs	Investigate possible relationships between IAM retention data and various lipophilicity scales	IAM-HPLC	Column: IAM.PC.MG (150 × 4.6 mm) and IAM.PC.DD2 (100 × 4.6 mm); MP: mixtures of ACN and 0.1 M phosphate buffer at pH 7.0; FR: 0.9 or 1.0 mL/min	[185]
Structurally diverse drugs	Characterize column and study the relationships between IAM retention data and DIPL	IAM-nano-HPLC	Monolith column prepared via copolymerization of MDPC and MDPA; MP: 0.05 M ammonium acetate at pH 7.4 or 4.5; FR: 0.6 µL/min	[186]
BBB permeant drugs	Investigate the relationships between BBB permeation data and lipophilicity	IAM-HPLC	Columns: IAM.PC.MG (150 × 4.6 mm; 12 µm); IAM.PC.DD2 (100 × 4.6 mm; 10 µm); MP: mixtures of ACN and 0.1 M phosphate buffer at pH 7.0; FR: 1.0, 2.0 or 3.0 mL/min	[187]
Kv11.1 inhibitors	Determine the lipophilicity by RP-HPLC and IAM	IAM-HPLC	Column: Supelcosil LC-ABZ (50 × 4.6 mm, 5 µm), IAM.PC-DD2 (100 × 4.6 mm, 10 µm); MP: mixtures of water and MeOH or ACN; FR: not specified	[188]
Set of basic, neutral, acidic and ampholytic drugs	Explore the effect of electrostatic interactions of anionic species	IAM-HPLC	Column: IAM.PC.DD2; MP: mixture of ACN (0–35%) and MOPS buffer pH: 7.4, 0.01 M PBS buffer at pH: 7.4, 0.01 M PBS buffer at pH: 5.0; FR: 1.2 or 3.0 mL/min	[189]
Structurally diverse drugs	Compare between IAM and equilibrium dialysis	IAM-HPLC (220 or 254 nm)	Column: IAM.PC.DD2 (100 × 4.6 m, 12 µm); MP: mixtures of methanol and 0.02 M phosphate buffer at pH 7.0; FR: 1.5 or 2.0 mL/min	[190]
Neutral, acidic, and basic drugs, including steroid hormones, local anesthetics, β-blockers, and NSAIDs	Characterize a reversed-phase amide column coated with phosphatidylcholine-based liposomes	ILC/DAD (230 or 254 nm)	Column: Ascentis® RP-amide HPLC column (50 × 4.6 mm, 5.0 µm) previously coated by immobilizing the PC liposomes on the column; MP: mixture of MeOH (20–80%) and phosphate buffer; FR: 1.0 and 2.0 mL/min	[34]
β-blockers, imidazoline derivatives, and p-alkylphenols	Characterize column coated with phosphatidylethanolamine-based liposomes	ILC/UV-Vis (220 nm)	Column: 5 mm glass column containing PE liposomes bounded to Sephacryl S-1000 gel by avidin–biotin binding; MP: 0.01 M HEPES with NaCl (0.15 M,) at pH 7.4; FR: 0.3 mL/min	[191]

Table 1. *Cont.*

Analytes	Aim	Chromatographic Method	Chromatographic Conditions	Ref.
Structurally diverse drugs	Estimate lipophilicity and correlate it with absorption	ILC/UV-Vis (220 nm)	Column: 5–5.5 cm×5 mm glass column containing EPC, PS–chol, and EPC–PS–PE liposomes CNBr-Sepharose gel by avidin–biotin binding; MP: 0.01 M HEPES with NaCl (0.15 M,) at pH 7.4; FR: 0.3 mL/min	[192]
Fused azaisocytosine analogues	Compare between RP-HPLC, IAM, and MLC	IAM-HPLC MLC/UV-Vis	Columns: Spherisorb ODS-2 (125 × 4 mm, 5 µm), IAM.PC.DD2 (100 × 4.6 mm, 10 µm), Purosphere RP-18e column (125 × 4 mm, 5 µm); MP: 0.01 M phosphate citrate buffer at pH 7.4 with Brij 35 (0.15–0.075 M) and with isopropanol (7%); mixtures of ACN (20–50%) and 0.01 M phosphate citrate buffer at pH 7.4; FR: 0.1, 1 and 1.3 mL/min	[193]
Series of pharmaceutically related compounds	Study the effects of the variations in pH in MLC determination	MLC/UV-Vis (221 or 285 nm)	Column: RP cyanopropyl Spherisorb (150 × 4.6 mm, 5µm); MP: 0.1 M phosphate buffer with SDS at pH 3.0, 4.0, 5.0, 5.8, 6.4 and 7.0; FR: 1.35 mL/min	[194]
Benzene derivatives	Study the influence of mixtures of Triton X-100 and Brij 35	MLC/DAD	Column: Nucleodur C18 Gravity (2 × 125 mm, 5 µm); MP: HCl solution at pH 2 with different Brij 35/Triton X-100–ratios (0.05–0.30%); FR: 0.3 mL/min	[195]
Structurally diverse drugs	Estimate lipophilicity and correlate it with intestinal absorption and BBB	MLC/UV-Vis (210 or 300 nm)	Column: Grace GraceSmart C18 (150 × 2.1 mm, 3 µm); MP: 0.05 M phosphate buffer at pH 7.4 with 0.01 M miltefosine; FR: 0.2 mL/min	[196]
Structurally diverse drugs	Estimate lipophilicity and correlate it with absorption	MLC/UV-Vis	Column: Kromasil octadecyl-silane C18 (150 × 4.6 mm, 5 µm); MP: 0.05 M phosphate buffer at pH 6.5 and 7.4 with 0.04 M Brij35; FR: 0.5 mL/min	[197]
Structurally diverse drugs	Estimate lipophilicity and correlate it with absorption	MLC/UV-Vis (221 or 360 nm)	Column: Spherisorb (15 cm × 4.6 mm, 5 µm); MP: water with sodium deoxycholate (5–20 mM); FR: 1.34 mL/min	[198]
Structurally diverse drugs	Estimate lipophilicity and correlate it with absorption	MLC/UV-Vis (240 or 254 nm)	Columns: Spherisorb octadecyl-silane ODS-2 C18 (120 × 4 mm, 5 µm), Kromasil octadecyl-silane ODS-2 C18 (50×4.6 mm, 5 µm), Kromasil octadecyl-silane C18 (150 × 4.6 mm, 5 µm); MP: 0.05 M phosphate buffer at pH 7.4 with 0.02 M Brij35; FR: 1 and 1.5 mL/min	[199]
Anticonvulsant drugs	Estimate lipophilicity and correlate it with pharmacokinetic parameters	MLC/UV-Vis (220 or 240 nm)	Column: Kromasil C18 column (150 × 4.6 mm, 5 µm); MP: 0.05 M phosphate buffer at pH 7.4 with 0.04 M Brij35; FR: 1 mL/min	[200]

Table 1. *Cont.*

Analytes	Aim	Chromatographic Method	Chromatographic Conditions	Ref.
Quinolone drugs	Estimate lipophilicity and correlate it with pharmacokinetic parameters	MLC/UV-Vis (270 nm)	Column: Kromasil C18 column (150 × 4.6 mm, 5 µm); MP: 0.05 M phosphate buffer at pH 7.4 with Brij35 and with NaCl (9.20 g/L); FR: 1 mL/min	[201]
β-Hydroxy-β-arylalkanoic acids analogues	Estimate lipophilicity and correlate it with absorption	MLC/DAD	Column: Zorbax Extend-C18 (150 mm × 4.6 mm, 5 µm); MP: 40 mM solution of Brij35 in a mixture of ACN (20%) and 0.07 M phosphate buffer at pH 5.50 with0.04 M Brij35; FR: 1 mL/min	[202]
Structurally diverse drugs	Study the influence of pH, temperature, and ionic strength in MLC and compare it with IAM	MLC/UV-Vis (220 nm)	Column: High Stability C18 Supelco (150 × 4.6 mm, 5 µm); MP: 0.05 M phosphate buffer at pH 5.5 and 7.4 with NaCl (0–9.2 g/L) with 0.04 M Brij35; FR: 1 mL/min	[203]

ACN—acetonitrile; BEH—bridged ethyl hybrid; Chol—cholesterol; DAD—diode-array detection; DIPL—drug-induced phospholipidosis; [EMIM][BF4]—1-ethyl-3 methylimidazolium tetrafluoroborate; EPC—egg phosphatidylcholine; TFA—trifluoroacetic acid; FR—flow rate; MeOH—methanol; MDPC—12-methacryloyl n-dodecylphosphocholine; MDPA—12-methacryloyl n-dodecylphosphoric acid; MLC—micellar liquid chromatography; MOPS—morpholinepropanesulphonic acid; MP—mobile phase; NSAIDs—non-steroidal anti-inflammatory drugs; PAHs—polycyclic aromatic hydrocarbons; PBS—phosphate buffer saline; PE—phosphatidylethanolamine; PS—phosphatidylserine; RI—refractive index; RP-HPLC—reversed-phase high-pressure liquid chromatography; RP-HPTLC—reversed-phase high-pressure thin layer chromatography; SDS—sodium dodecyl sulphate.

As an indirect measurement of lipophilicity, RP-HPLC does not directly provide a partition coefficient but rather a lipophilicity index which is correlated with the partition coefficient. The lipophilicity index is inferred by the assumption that compounds are retained in proportion to their nonaqueous-aqueous partition coefficient, with hydrophilic compounds eluted first and lipophilic compounds last, and it is expressed by the retention factor k [30]. The retention factor k is easily calculated using the retention time (t_r), according to Equation (5) [27,30]:

$$\log k = \log\left(\frac{t_r - t_0}{t_0}\right) \quad (5)$$

where t_0 is the dead retention time (the retention time of an unretained solute) and t_r is the retention time of the solute. The major factors that influence the retention time are the nature, length, and diameter of the stationary phase and the composition and flow rate of the mobile phase [57]. Compounds that are either unretained or too retained may have to be assessed using different column lengths or eluent compositions [77].

Under suitable isocratic conditions, the log P of the compound under study is obtained by measuring its retention factor and then inputting the k values into the Collander equation:

$$\log P = a \times \log k + b \quad (6)$$

where a and b are regression constants for the slope and intercept obtained by linear regression of the log P of reference substances against the log k of the reference substances. In these conditions, the k-value of a given solute and stationary phase depends on the composition of the mobile phase [77]. To address this problem, pure aqueous eluent (log k_w) should be used as it is not affected by the organic modifier and reflects the partitioning behavior that occurred in a shake-flask measurement [77,85]. As under most conditions pure water cannot be used as mobile phase, $\log k_w$ is obtained by extrapolation to 100%

water [19,78,114]. The Snyder–Soczewinski equation (Equation (7)) is the most common approach to estimate log k_w [19]:

$$\log k = \log k_w - S\varphi \quad (7)$$

where φ is the volume fraction of the organic modifier in the mobile phase and S is a constant derived by linear regression analysis. The extrapolation for log k_w is performed by determining the log k values using different percentages of organic solvent in the mobile phases (at least four isocratic log k values) and then extrapolating to 100% of pure water eluent [19,81,204]. Triplicates should be made [30] and they should fall within a range of ±0.1 log units [30].

The classical method for the determination of log P by RP-HPLC employs isocratic conditions. For compounds with high retention factors, the determination may be challenging due to band broadening. Although it requires a more complex mathematical treatment, elution in gradient mode can be used for the determination of log P and offers faster analysis time and resolution with constant peak width [166]. In gradient methods, the lipophilicity index is expressed by a different chromatographic parameter—the chromatographic hydrophobicity index (CHI). CHI corresponds to the percentage of organic modifier required to achieve equal distribution of the analyte between the stationary and the mobile phase. CHI values are obtained by measuring the retention times in gradient mode (t_g) and then inputting the t_g into the equation [19]:

$$CHI = a \times t_g + b \quad (8)$$

where a and b are regression constants for the slope and intercept obtained by linear regression of t_g of reference substances against the CHI of the reference substances.

For measuring log P with RP-HPLC, the following features need to be considered:

- Precision: triplicates should be performed, and the obtained values of the log P values should be within a range of ±0.1 log units [26];
- Sensitivity: HPLC enables log P to be determined over a range of about 0–6 [30];
- Specificity: the presence of impurities may difficult the peak assignment and the interpretation of the obtained values [26];
- Accuracy: the obtained log P values can be within ±1 log unit of the shake-flask value [26].

To control the above-mentioned features, the choice of suitable stationary and mobile phases plays a pivotal role.

Various stationary phases have been applied to evaluate indirectly isotropic lipophilicity [34]. Most of the chromatographic methods for measuring lipophilicity use RP silica-based stationary phases containing long-chain hydrocarbon, like octyl (C8) and octadecyl (C18), bound onto silica (Figure 3) [19,26,30,81].

Silica is the most preferred packing material for RP columns, but it has the inconvenience of possible silanophilic interactions between the analyte and the remaining free silanol sites of the stationary phase. These interactions, more prominent in positively charged compounds, can produce distortions in peak shape and a considerable increase in the compound retention, which affects their log P determination [19,27]. In addition, these stationary phases are not stable at pH above 7.5 and can even break down [115]. OECD recommends that the stationary phases should have a minimal percentage of polar groups as they may impair the performance of the RP-HPLC column [30]. However, to overcome this problem, the best recommendation is to use highly end-capped columns for the RP-HPLC method, especially for basic compounds under high pH conditions [77,166]. One of the strategies for end-capping is to use base-deactivated silica stationary phases where the end-capping of the silanol residues is performed by the treatment with trimethylchlorosilane [86]. Another strategy to suppress silanophilic effects is the use of polar embedded stationary phases. Here, electrostatic shielding is achieved by the incorporation of functional groups, such as amide or carbamate, at the alky chains [86]. LC-ABZ$^+$ and Discovery-RP-

Amide-C16 are two examples of polar embedded stationary phases which have been used for lipophilicity determination [19,86].

However, other secondary interactions between these polar groups and certain analytes may occur. Many of these end-capped columns are still limited to pH below 8 because of their instability in an alkaline medium. Masking the silica units with hydrophobic ethylene groups prevents them from dissolution, and the structural ethylene bridges increase the column stability at a wide pH range (1–12) [19,166]. Acquity BEH Shield RP18 and XBridge Shield RP18 column are two examples of ethyl bridged columns that can be used to measure the lipophilicity not only for neutral and acidic compounds but also for basic ones [19,129].

Polymer-based stationary phases, composed of a rigid and porous cross-linked polymer, are another type of phases aimed at suppressing silanophilic effects. Polystyrene-divinylbenzene (PS-DVB) and octadecyl-poly(vinyl alcohol) (ODP) are two examples of these types of stationary phases that have been successfully used for log P determination. Silanophilic effects are suppressed because they do not have any polar site, and they can be used at a wide range of pH values (pH 1–13) [19,95,205]. However, these columns often have a longer retention time when compared to the silica-based ones [115] and the retention mechanism may be controlled by different structural properties of the polymer [19].

Regarding the mobile phase, the most extensively used are binary mixtures of water or buffer with an organic modifier [27]. HPLC-grade modifier and distilled water should be used to prepare the eluting solvent, which is degassed prior to use [30]. The use of binary mixtures is a requirement as the use of only water in RP silica-based columns is not feasible [61]. With high percentages of water, more than 98%, C18 and C8 columns exhibit poorly reproducible retention due to ligand collapse and the decreased contact between the mobile phase and the stationary phase [206]. For the lipophilicity assessment, methanol is considered the most suitable organic modifier. Being a protic solvent, methanol can interact with free silanol groups suppressing this way the silanophilic effects [166]. In addition, methanol forms a monolayer that mimics the *n*-octanol/water interface of the shake-flask method [27]. For lipophilicity determination, the volume fraction of methanol should be above 25–30% [30]. At a high-volume fraction of methanol, the eluent mixture is so unlike water; the phase aimed to be mimicked by the mobile phase, that the correlation between retention times and lipophilicity will be poor [77]. Acetonitrile is another organic modifier used for lipophilicity determination. When acetonitrile is used as an organic modifier instead of methanol, the correlation between retention times and organic modifier percentage in the mobile phase is generally better described by a quadratic model instead of a linear model [71,166]. In addition, the correlation between log P and log k_W was better when methanol was used instead of acetonitrile [71].

Additives to the organic modifier–water mixture can be used, particularly when dealing with dissociable compounds [19]. Hydrophobic amines, such as triethylamine, *n*-decylamine, and *N*,*N*-dimethyloctylamine, are the traditional examples of additives added to the mobile phase. These masking agents can reduce silanophilic interactions and are very useful when basic compounds are analyzed [19]. Ionic liquids, such as 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]), are greener alternatives to the traditional additives [19,72]. These additives are usually composed of relatively large asymmetric organic cations and inorganic/organic anions, meaning that both anion and cation can participate in the retention mechanism [19]. Therefore, the inclusion of a masking agent can selectively affect the compound retention times, and it should only be used on justifiable occasions [77].

In a similar way, ion suppressors can be added to the mobile phase to suppress the dissociation of ionizable compounds. Ion suppression is achieved by using buffer solutions based on phosphate, phosphate-buffered saline (PBS), or morphilinepropanesulfonic acid (MOPS) [19]. *n*-Octanol itself can be used as an additive to provide a better correlation with lipophilicity. The idea behind it is to render octanol-like characteristics to the column by saturating the column with *n*-octanol and then use of *n*-octanol saturated water as

eluent. However, the inclusion of *n*-octanol as an additive should be tested. Some reports show better correlations between log P and log k_W if better if *n*-octanol is supplementarily added [72,77] while others show the opposite trend [174].

Regarding the detection, most of the reported LC methods use UV-Vis or diode-array detectors (DAD), while mass spectrometry (MS) detection is only scarcely used (Table 1). This is attributed to the easier use and ubiquitous presence of UV-Vis detection and to the fact the majority of the compounds under study (drugs or drug candidates) tend to absorb in this range.

Strictly related to RP-HPLC, solvatochromic analysis is an approach based on linear solvation–energy relationships (LSER) that allow for the understanding of the contribution of each intermolecular interaction involved in the partition [166]. The model considers both solute properties, such as Van der Waals volume or dipolarity/polarizability or hydrogen-bond–donor acidity, as well as the properties of the chromatographic system properties, which are calculated by multiple linear regression analysis for a diverse group of compounds [107,167,205]. The establishment of a robust correlation with the nonaqueous/aqueous partition coefficients involves not only a correlation between the RP-HPLC retention factor and the log P values but also LSER equations that are in agreement with the structural parameters [115].

When compared to the "gold standard" shake-flask method, RP-HPLC offers several advantages: it requires a smaller amount of sample and solvent, it has higher reproducibility, and it is faster as only the determination of retention times is required, and retention times are independent of the injected compound concentration/amount [19,30,81]. When compared to the RP-HPTLC, RP-HPLC tends to provide similar results [60,165].

2.3.3. Ultra-Performance Liquid Chromatography

Ultra-performance liquid chromatography (UPLC) works with columns packed with sub-2 μm porous particles. Compared to the conventional LC, the drastic reduction in the column length allows a faster determination of lipophilicity with a significant improvement in chromatographic performance and efficiency [166]. Few reports of lipophilicity determination using UPLC have been reported [166–170] because this technique requires specific equipment.

The different stationary phases will yield different partition coefficient values. If the RP-phases are similar, the analyte chromatographic behavior might be different, but the relationship between log P and retention factors tends to be similar [71]. However, if the distinct RP phases are used, the results are not comparable. To demonstrate this, Giaginis et al. [174] evaluated the lipophilicity of a set of thiazolidinediones derivatives with different RP phases, including one that is endcapped, polar-embedded, and polar-endcapped. For all compounds under study, the one polar endcapped phase provided the lowest retention factors, and the endcapped provided the highest retention factors. When compared with the partition coefficient values obtained by the shake-flask method, the partition coefficient values obtained with the endcapped phase provided the best correlation, namely in the pH range 5–8.

2.3.4. Counter-Current Chromatography

Counter-current chromatography (CCC) uses two immiscible solvents: one will act as a mobile phase while the other will act as a liquid stationary phase [204]. The liquid stationary phase is retained by applying a gravitational or a centrifugal field [43]. The partition coefficient is determined by taking into account the retention volume of the substance and the volume of the stationary and mobile phases [205]. The advantages of this technique are the use of small sample sizes and insensitivity to impurities; however, it is only applicable for log D < 4.3, and it is time- and labor-consuming [43].

2.3.5. Immobilized Artificial Membrane Chromatography

Immobilized artificial membrane (IAM) chromatography is the most widely used method for the determination of anisotropic lipophilicity [207]. Table 1 describes numerous examples of IAM chromatography applied to lipophilicity determination. The motivation behind IAM stationary phase is to better mimic specific interactions that solutes or drugs can establish with the membrane phospholipidic bilayer. To fulfil this goal, IAM stationary phases are prepared by binding covalently phospholipids to a propylamino functionalized silica particles (Figure 4a) [27,208]. The incorporated phospholipid can have a single or a double chain, but a double chain can better simulate biomembranes [27]. The remaining free propylamine residues on the surface of the silica backbone should be treated to suppress the undesired silanophilic interactions discussed above [27]. The most recent generation of IAM columns contains double-chain phosphatidylcholine, they are end-capped with decanoic, and propionic anhydride and are commercially available (IAM.PC.DD2 from Regis Technologies) [209]. A simple and economical alternative is the preparation of the stationary phase by coating a conventional RP column with phospholipids [34].

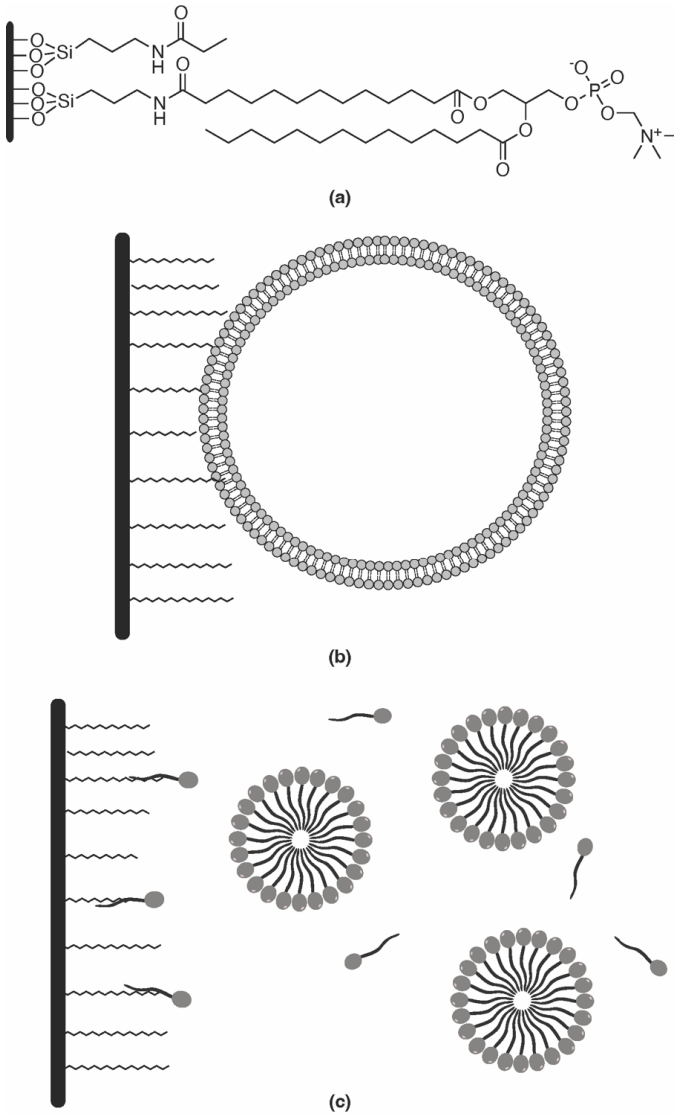

Figure 4. (**a**) IAM HPLC column; (**b**) immobilized liposome chromatography (ILC) HPLC column; (**c**) schematic representation of micellar liquid chromatography (MLC).

From a practical point of view, one of the main advantages of IAM columns is that they do not need the use of an organic modifier [27]. In fact, methanol as an organic modifier should be avoided as it disturbs the stability of the column by causing methanolysis of the phospholipids.

By simulating the phospholipid bilayer of biological membranes, the retention factor obtained with IAM chromatography correlates better with biological partition as the retention mechanism considers not only hydrophobic but also polar/ionic interactions [27,208]. For neutral solutes, the correlation between log P and the IAM partition coefficient (log $k_{W\text{-}IAM}$) is strong. For ionized compounds, the correlation is poor due to the contribution of electrostatic interactions, which are not recognized in the octanol–water system [189]. When compared with partitioning with liposomes, log k_{IAM} are well correlated for hydrophobic analytes [190]. Due to their resemblance to biological barriers, IAM chromatography has been successfully applied to calculate complex pharmacokinetic properties, such as cell permeation [210], intestinal absorption [183], unbound volume of distribution [184], and drug-induced phospholipidosis [186].

2.3.6. Immobilized Liposome Chromatography

Immobilized liposome chromatography (ILC) is another tool that has been used in anisotropic lipophilicity measurement. ILC stationary phases are prepared by immobilizing liposomes into the support silica particles by either covalent, hydrophobic, or electrostatic interactions (Figure 4b) [34,211].

When compared to IAM chromatography, ILC are better surrogates of the biological membranes because their liposomes have a bilayer structure and present a curvature effect [212]. In addition, the lipidic composition can be tailored to mimic specific biological barriers. Liu et al. studied different ILC column compositions aimed to mimic the small intestine membrane [192]. The authors suggested that liposomes composed of egg PC–PS–PE–cholesterol (5:1:2:2) were a suitable model for the prediction of the fraction absorption in humans. Despite its potential, the applications of the ILC method are very limited (Table 1) because of the low stability of liposomes [211].

2.3.7. Micellar Liquid Chromatography (MLC)

Micellar liquid chromatography (MLC) is a variant of RP-LC in which a surfactant at a concentration above the critical micellar concentration is added to the mobile phase, leading to the formation of micelles within the aqueous mobile phase (Figure 4c) [194,213]. MLC can be coupled to TLC, HPLC, and over-pressured-layer chromatography using micellar mobile phases [213].

In MLC, the micellar aggregates act as partition sites in the mobile phase, while the surfactant monomers present in the mobile phase modify the stationary phase either by both hydrophobic and/or silanophilic adsorption [203]. Due to the amphiphilic character of surfactants, the micellar mobile phase mimics the phospholipidic bilayer structure of biological membranes, while the modified stationary phase mimics not only the ordered array of the hydrophobic chains of phospholipids, mainly through the C18 carbon chains but also the polar heads of phospholipids, mainly through the adsorbed surfactant monomers [214]. During the elution, the solute can establish interactions with the micellar core, the micellar surface, the polar head of the surfactant bound to the stationary phase, and the alkyl chains bound to the stationary [194]. Therefore, solute retention involves the establishment of two different equilibriums: one between the bulk aqueous phase and the surfactant-coated stationary phase and another between the aqueous phase and the micelles [203]. The outcome index of MLC (log k_{MLC}) is a metric of anisotropic lipophilicity as it depends on hydrophobic, electronic, and steric interactions [215]. Due to its similarity with biological membranes and extracellular fluids, MLC is also known as biopartitioning micellar chromatography [203].

Since the first reports of MLCs in the early 1980s, modifications of MLC have been reported [194]. These modifications are aimed to solve the decreased column efficiency

in MLC due to slow mass transfer. The slow mass transfer is attributed to the poor wettability of a purely aqueous mobile phase and modification of the stationary phases by the adsorption of surfactant monomers [216]. To improve column efficiency, the main approaches are the addition of organic modifiers, the increase of column temperature, and decreased flow rate.

Exploiting the ability to mimic the phospholipidic barriers, log k_{MLC} values have been used to predict several pharmacokinetic parameters, such as human oral absorption (HOA) [196–199], blood–brain barrier (BBB) permeability [196], plasma protein binding (PPB) [200,201], the volume of distribution [200,201], and therapeutic parameters [200,201]. When compared to other chromatographic techniques, MLC appears to be in the mid-ground between IAM and RP-HPLC. As an example, the lipophilicity of a set of fused azaisocytosine analogues was evaluated using RP-HPLC, IAM, and MLC [193]. For all compounds under study, the log k_{MLC} values were higher than log $k_{W\text{-}IAM}$ but lower than log k_W. In another example, the lipophilicity of a set of 88 compounds was evaluated using IAM and MLC [203]. Here, the obtained log k_{MLC} values covered a narrower range than the obtained log $k_{W\text{-}IAM}$ values (log $k_{W\text{-}IAM}$ range: from −1.15 to 3.98, log k_{MLC} range: from 1.84 to 3.98). These differences are attributed to the strong electrostatic interactions between positively charged drugs and the IAM phase and to the improved distribution of lipophilic compounds in the micellar MLC mobile phase. The chromatographic retention factors were used to predict cell permeability, HOA, and PPB. MLC retention factors demonstrated a better predictor capacity than IAM for cell permeability, while similar results were found for the remaining parameters. The advantage of the MLC is that it uses a conventional and easily available RP column, which is usually available in any analytical laboratory, while IAM requires a specific and expensive column.

3. Case Study: LC in Lipophilicity Assessment of Xanthone Derivatives

To emphasize the dual role of LC in the determination of lipophilicity and its importance as a medicinal chemistry tool, we provide a case study describing the experience of our group with xanthone derivatives. Xanthone derivatives comprise an important class of oxygenated heterocyclic compounds in medicinal chemistry concerning not only its large range of biological and/or pharmacological activities [217–219], but also other applications, including as fluorescence probes [220,221], and chiral stationary phases for LC [222,223]. For the last several years, the search for new xanthone derivatives with potential bioactivity has remained one of the main areas of interest for our group [224].

Recently, the determination of lipophilicity of a small library of bioactive xanthone derivatives was performed in our group [225]. The chosen xanthone derivatives were previously synthesized and exhibited interesting biological activities, namely growth inhibitory effects on different tumour cell lines [226], nerve conduction blockade acting in a similar manner to local anaesthetics [227], and the inhibition of cyclooxygenases, the enzymes involved in inflammatory processes [228].

Among the different methods used for lipophilicity assessment, LC was applied for both indirect and direct determination of log P, specifically with RP-HPLC and with VALLME coupled with HPLC [225].

Regarding the RP-HPLC method, two hydrophobic RP silica-based columns with a C8 and a C18 containing hydrocarbon long-chain (same length, diameter, and particle size) were used to evaluate their influence on the experimental lipophilicity. Different percentages of methanol in the aqueous mobile phase were chosen with increments of 5% or 10%, ranging between 40 to 75%, in accordance with the OECD Guidelines. For each compound, the log k was determined (Equation (5)) at different proportions of methanol. At least five different percentages were used to obtain a linear relationship, and log k_w was obtained by extrapolation to 100% of pure water. The different percentages of methanol in the mobile phase were chosen based on the theoretical log P values for each xanthone derivative [225].

As an example of the xanthone derivative XD-10 (Figure 5), the log k_w obtained in both columns (C8 and C18) was 3.71.

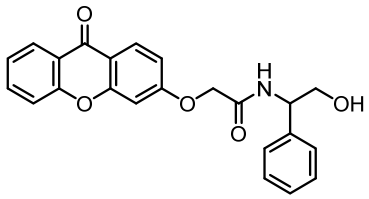

Figure 5. Chemical structure of xanthone derivative XD-10.

Figure 6a shows the chromatograms of XD-10 on the C18 column and with different proportions of methanol in the mobile phase. As expected, the retention time increased with the decrease of the percentage of methanol in the mobile phase.

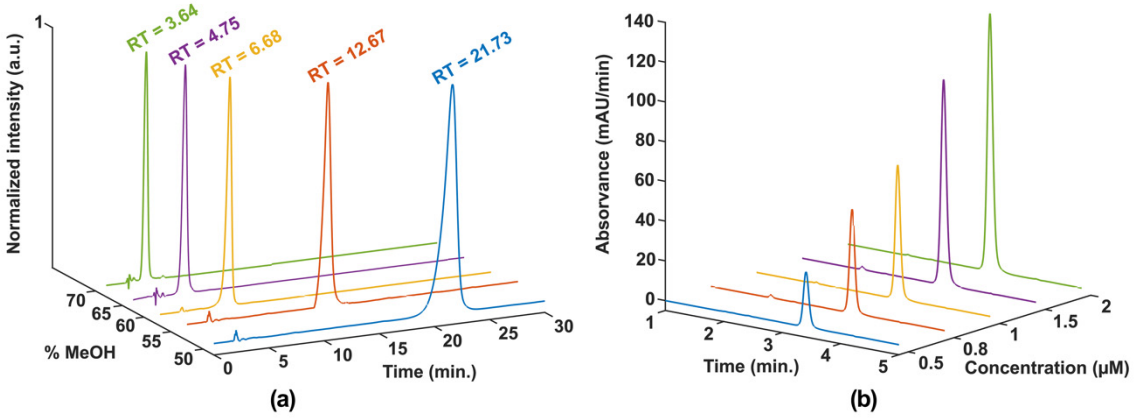

Figure 6. Chromatograms of XD-10. (**a**): RP-HPLC method on the C18 column with different proportions of MeOH in the mobile phase, a flow rate of 1.0 mL/min, and detection at 254 nm; (**b**): VALLME method coupled to HPLC, with different XD-10 concentrations present in the *n*-octanol phase (chromatographic conditions: water/MeOH (70:30 *v*/*v*) as mobile phase, the flow rate of 1.0 mL/min, and detection at 254 nm). (Reprint with permission from [225], Copyright (2018) ELSEVIER).

Similar to other xanthone derivatives, for XD-10, a linear correlation between the chromatographic parameter log k and the proportion of methanol in the mobile phase was obtained, with R^2 of 0.9967 and 0.9982 for C18 and C8 columns, respectively.

LC was also applied as an analytical method for direct quantification of the xanthone derivatives in the *n*-octanol phase after VALLME procedure [225]. For the analysis, a C18 hydrophobic silica-based column and a mixture of water/methanol (70:30 *v*/*v*) as a mobile phase were used. Once again, the theoretical log P values were the basis for the selection of the appropriate concentrations of the octanolic solutions for each xanthone derivative to fit the predictive concentrations in the *n*-octanol droplet into the linear zone of the calibration curve.

The external calibration curves for xanthone derivatives demonstrated good linear correlations. As an example, for XD-10 (Figure 5), a R^2 of 0.9933 was achieved. Figure 6b shows the chromatograms for XD-10 present in *n*-octanol phase after VALLME procedure with different concentrations. As expected, the concentration of XD-10 found in the *n*-octanol phase increases with the augment of the concentration. The log P values for all the compounds were calculated for each concentration using Equation (3) and fell within a maximum range of ±0.24 units, which is in accordance with the OECD acceptance criterion [32]. Moreover, the methodologies and data assembled in this study contributed to enlarging the knowledge of the lipophilicity of xanthone derivatives.

4. Conclusions

Lipophilicity exerts a huge influence in pharmacokinetic and pharmacodynamic behavior of drugs, thus motivating the intensive search for methods to properly assess this property. As discussed in this work, several methods have been developed to predict, measure, or estimate the partition coefficient between a nonaqueous and an aqueous phase. In silico approaches are quite useful to rapidly predict log P values, but for decision-making purposes, the predicted values should always be complemented with experimentally obtained values. Direct methods allow for directly measuring the log P values. However, the *n*-octanol/water partition determined by the traditional shake-flask method is labor-consuming, and it is not always the most appropriate method. A modification of the shake-flask method has been explored, but the absence of comparative studies concerning them makes it very difficult to identify the best approach. Indirect methods, namely RP-HPLC, have been proposed to circumvent the drawbacks of direct methods. The advent of UPLC enables an even faster determination of lipophilicity due to the significant improvement of chromatographic performance and efficiency. In addition, indirect measurements of lipophilicity provide a partial but incomplete model of the partition in the *n*-octanol/water system because the intermolecular interactions occurring in the retention in the reversed-phase column are similar but not identical. The case study described in this work neatly emphasizes the dual role of LC in the determination of Log P.

Besides the variability associated with the method itself, the variability of model used as a nonaqueous phase also hampers the comparation between partition coefficients values. In fact, isotropic and anisotropic lipophilicity are expressed in different and hard to compare scales. This problem could be solved soon by high-throughput measurements of anisotropic lipophilicity, such as IAM and MLC, which could provide the large datasets required to settle the proper correlations between the isotropic and anisotropic partition coefficient values.

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