Rapid Determination of Lipophilicity: Exploration and Establishment of Reversed-Phase Liquid Chromatography (RPLC) Methods

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Abstract

The lipophilicity (Log P or Log D) of a compound plays a crucial role in drug development. It serves as a valuable parameter for evaluating various properties of compounds, including absorption, distribution, metabolism, and excretion-toxicity (ADMET). Understanding the lipophilic nature of a compound provides valuable insights into its pharmacokinetic behavior and aids in optimizing drug formulations for improved efficacy and safety.

There are several commonly used methods for detecting Log P values of compounds. The choice of method depends on factors such as the required accuracy of Log P values and the stage of the project (discovery, pre-clinical development, or clinical development). Among these methods, the shake-flask method, although low throughput, is widely considered the gold standard due to its precision. However, accurately determining the lipophilicity values of highly lipophilic compounds (Log P > 5) ~~using it is~~ remains challenging using this method due to the extremely low solubility of compounds in the aqueous phase. To address this issue, the RPLC (reversed-phase liquid chromatography) method offers a wider measuring range. Moreover, this method is more efficient and user-friendly compared to the shake-flask method. With the increasing proportion of highly lipophilic compounds in drug discovery and development, the development of RPLC methods has gained significant interest among researchers.

In this study, we explored and established two RPLC methods that can efficiently and accurately detect the Log P values of compounds. The predictive ability of method was investigated with linear correlation coefficients. Based on considerations of cost, time efficiency, and predictive ability, we believe that RPLC methods are promising choices for predicting the lipophilicity of compounds.

Objective

Log P or Log D is a pivotal parameter in lipophilicity measurement. Log P more directly reflects the general trend of lipophilicity. Current methods for Log P measurement include computer simulation, the shake-flask method and RPLC method (Table 1). Among those methods, the shake-flask method is widely considered the gold standard. Compared with the shake-flask method, the RPLC method offers several advantages particularly the wide prediction range.

This study aimed to establish RPLC methods to detect the Log P of compounds efficiently and accurately. The RPLC methods are good candidates for predicting the lipophilicity of compounds based on multiple considerations including cost, speed and predictive ability.

Table 1. Comparison of different Log P measurement methods

Method	Prediction range (log value)	Interferences	(Measure) Speed	Reproducibility	Predictive ability
Computer simulation	Broad	–	Rapid	–	★
Shake-flask	−2–4	• Not applicable to degradable compounds • Demand for the high sample purity	Slow	★★	★★★
RPLC	0–6	• Mild • Insensitive to impurities	Rapid	★★★	★★

Method

The basic steps for measuring the Log P value of a compound using RPLC are as follows:

1. Calculate the capacity factors (k) of selected reference compounds in advance. The logarithms of the capacity factors are then plotted against the respective Log P values of the selected reference compounds. This equation is referred to as the standard equation.
2. Substitute the capacity factors of the test compounds into the standard equation to determine their Log P values.

We chose the following compounds as reference compounds (**Table 2**).

Table 2. List of Reference Compounds

Compounds	Log P
4-Acetylpyridine	0.5
Acetophenone	1.7
Chlorobenzene	2.8
Ethylbenzene	3.2
Phenanthrene	4.5
Triphenylamine	5.7

We used a HPLC (Shimadzu, LC-6AD) and a column (waters, 5 μm, 250 mm × 4.6 mm) were used for this study. Methanol and 10 mM Phosphate-buffered saline (PBS) with a pH of 7.4 were used as organic and aqueous phases, respectively. Based on these chromatographic parameters, we established the RPLC Lipophilicity Determination **Method 1** (hereinafter referred to as Method 1).

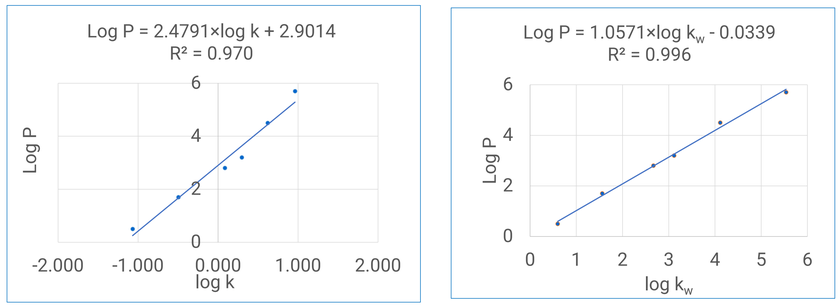
To eliminate this interference from the organic modifiers, we established the RPLC Lipophilicity Determination Method 2 (from now on referred to as **Method 2**).

Results and Discussion

The "Partition Coefficient (n-octanol/water), High-Performance Liquid Chromatography (HPLC) Method" from the Organization for Economic Co-operation and Development (OECD) Guidelines for the Testing of Chemicals (2004 edition) outlines the scope of application and requirements for determining Log P using HPLC [1]. It states that RPLC has a distinct advantage in determining the Log P of highly lipophilic compounds. Relevant studies [2] have also reported that this method is suitable for new modalities with high lipophilicity, such as proteolysis targeting chimeras (PROTACs).

In **Method 1**, Standard **Equation (1)** resulted in the linear correlation coefficient (R^2) of 0.97 (**Figure 1a**), which meets the regulatory requirements. In Method 2, log k in **Equation (1)** was replaced with log k_w (capacity factor of the compound in the absence of organic modifiers) to derive the standard equation (**Equation (2)**). For each reference compound, an equation relating log k and methanol content (φ) was established under three mobile phase gradient conditions (**Equation (3)**), with the intercept representing the log k_w value. As shown in **Figure 2b**, the R^2 value for **Equation (2)** is 0.996, indicating that **Method 2** has better predictive ability than **Method 1**. Therefore, more accurate lipophilicity values could be obtained using **Method 2** during the late stages of drug development.

$\text{Log P} = a \times \log k + b$ (1) $\quad \text{Log P} = a \times \log k_w + b$ (2) $\quad \log k = S\varphi + \log k_w$ (3)



(a) (b)

Figure 1. Linear fitting of the standard equation using a). Method 1 and b). Method 2

Twenty-one compounds with various lipophilicity were selected to measure log P values. For Method 1, the log P values of eighteen compounds are consistent with those reported in the literature [1,3], with differences within 0.5 log units. The remaining three compounds were retested with Method 2, producing data consistent with the literature values.

Table 3. Comparison of Methods 1 and 2 and the shake-flask method

Method	Standard equation	Correlation coefficient	Run time per compound	Cost/speed	Application scenario
1	Log P = a × log k + b	0.970	Within 0.5 h	Low/fast	Early drug screening of more than 30 compounds Presence of time constraints
2	Log P = a × log k_w + b	0.996	2–2.5 h	Moderate /slow	Drug screening in late stages of drug development Absence of time constraints
Shake-flask	NA	NA	4 h	High/slow	Absence of time constraints

Conclusion

Lipophilicity can be measured using different methods, such as shake-flask or RPLC methods. The shake-flask method is a direct experimental method and is considered the gold standard. The RPLC method is an indirect method that provides a wide prediction range. We developed two RPLC methods for lipophilicity measurement. Methods 1 and 2, along with the shake-flask method are suitable for determining compounds' lipophilicity in different stages of the projects (**Table 3**). We trust that the two RPLC methods will provide significant benefits to medicinal chemists when assessing medium or large-size compound libraries to study their structure-activity relationships.

References

[1] OECD. Test No. 117: Partition Coefficient (n-octanol/water), HPLC Method.
[2] Pike A, Williamson B, Harlfinger S, Martin S, McGinnity DF. Optimising proteolysis-targeting chimeras (PROTACs) for oral drug delivery: a drug metabolism and pharmacokinetics perspective. *Drug Discov Today*. 2020; 25(10):1793-1800.
[3] Bharate SS, Kumar V, Vishwakarma RA. Determining Partition Coefficient (Log P), Distribution Coefficient (Log D) and Ionization Constant (pKa) in Early Drug Discovery. Comb Chem High Throughput Screen. 2016; 19(6):461-469.

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