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Determination of reversed-phase high performance liquid chromatography based octanol-water partition coefficients for neutral and ionizable compounds: Methodology evaluation

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ABSTRACT

Reversed-phase liquid chromatography (RPLC) based octanol-water partition coefficient (logP) or distribution coefficient (logD) determination methods were revisited and assessed comprehensively. Classic isocratic and some gradient RPLC methods were conducted and evaluated for neutral, weak acid and basic compounds. Different lipophilicity indexes in logP or logD determination were discussed in detail, including the retention factor $\log k_w$ corresponding to neat water as mobile phase extrapolated via linear solvent strength (LSS) model from isocratic runs and calculated with software from gradient runs, the chromatographic hydrophobicity index (CHI), apparent gradient capacity factor (k_g') and gradient retention time (t_g). Among the lipophilicity indexes discussed, $\log k_w$ from whether isocratic or gradient elution methods best correlated with logP or logD. Therefore $\log k_w$ is recommended as the preferred lipophilicity index for logP or logD determination. $\log k_w$ easily calculated from methanol gradient runs might be the main candidate to replace $\log k_w$ calculated from classic isocratic run as the ideal lipophilicity index. These revisited RPLC methods were not applicable for strongly ionized compounds that are hardly ion-suppressed. A previously reported imperfect ion-pair RPLC method was attempted and further explored for studying distribution coefficients (logD) of sulfonic acids that totally ionized in the mobile phase. Notably, experimental logD values of sulfonic acids were given for the first time. The IP-RPLC method provided a distinct way to explore logD values of ionized compounds.

1. Introduction

The logarithm of the octanol-water partition coefficient (logP) is a parameter of great importance in pharmaceutical industry and is accepted as one of the most relevant lipophilicity descriptors to be applied in absorption, distribution, metabolism, excretion and toxicity (ADMET) studies [1–3]. Although logP can be calculated via computer calculation methods, these calculation methods commonly exhibit rather large calculation errors and the accuracy is influenced by the models used [4–6]. As a classic logP prediction method recommended by the Organization for Economic Co-operation and Development (OECD), the reversed-phase liquid chromatography (RPLC) method [7] offers several practical advantages over the traditional shake-flask method (SFM) [8,9] and slow-stir method (SSM) [10], including speed, reproducibility, insensitivity to impurities and degradation products, broader dynamic range, on-line detection, and reduced sample handling and sample sizes.

Researchers have done a large number of theoretical and practical explorations aiming at the RPLC-based logP determination method [11–15]. Organic modifiers (methanol and acetonitrile), mobile phase additives (ion-suppressors to keep ionizable solutes in their neutral form and masking agents to eliminate silanophilic interactions) and stationary phases (silica- or polymer-based with different bonded phases) have been studied in detail by different research groups focusing on the classic isocratic RPLC method. For less time-consuming and higher throughput, some gradient RPLC methods were proposed showing satisfactory performance in determination of logP [16–22], which has become a very important research direction for logP measurement. Varied lipophilicity indexes were used to generate logP or distribution coefficient (logD) in isocratic and gradient methods. The retention factor $\log k_w$ parameter corresponding to neat water as mobile phase is considered a more reliable lipophilicity index than any arbitrarily selected

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isocratic logk in the classic isocratic method for logP measurement [11]. $logk_w$ is often extrapolated via linear solvent strength (LSS) model with at least four isocratic logk values at different concentration of organic modifier (φ) [23]. In some gradient methods, $logk_w$ values were calculated from gradient retention data via gradient retention equations [16–19,24]. In other gradient methods, the chromatographic hydrophobicity index (CHI) [20], apparent capacity factor (k_g') [21] and gradient retention time (t_g) [22] were employed as lipophilicity indexes. Details about the isocratic and gradient methods [11] are summarized in Supplementary material S1.

However, there might exist two problems which are worth discussing along with the developing of new RPLC methods. Firstly, although reviewed by many researchers including our group, these previously developed isocratic or gradient methods by different researchers were almost independent of each other. The experiments were conducted under different chromatographic conditions (chromatographic columns, mobile phases) with different model compounds. There were no cross comparisons of these isocratic and gradient methods under same chromatographic conditions with same model compounds. Users could not evaluate these newly reported methods objectively, which limits the application of these methods in real experiments for logP determination. Secondly, the applicable logP range was barely expanded with these emerging high-throughput RPLC methods. Although RPLC methods are able to predict logP values of highly hydrophobic compounds (logP > 4) which are difficult to be measured by SFM (logP from −2 to 4) [8], few existing experimental methods could be utilized to measure logP values of highly hydrophilic solutes (such as strongly ionizable ones that could not be suppressed by ion-suppressors). When a solute is dissociated in mobile phase, logD involving the contribution of all ionic species to the total hydrophobicity of the analyte is often used instead of logP. Early, Zou et al. [25] proposed that the ion-pair reversed-phase liquid chromatography (IP-RPLC) method could be used for measuring logD values of ionic compounds. They established a relationship between calculated logD value, retention factor (k) and solute charge for model sulphonic acids. However, in their work, experimental logD values were not used and no actual logD values of sulphonic acids were determined. Besides, those compounds with same net charge (n_e) but different ionizable groups (such as carboxyl and sulfonic groups) have not been investigated together. It is a pity that there were no follow-up studies and this method was paid little attention to by other researchers later on. As a possible method for expanding logD determination range, does the RP-IPLC method really deserve further studying?

Curiosity for answers to these two problems inspired us to revisit these logP determination methods. In this present work, an isocratic and some gradient RPLC methods with methanol or acetonitrile as organic modifier were firstly compared and discussed for determining logP or logD values of neutral, weak acid and basic compounds. Moreover, an IP-RPLC method was tested and further explored for studying logD values of strong acids which totally ionized in the mobile phase.

The contribution from silanol groups would influence logP determination of ionized compounds especially basic compounds. In most references, when determining logP or logD of basic compounds, mobile phase pH was often over 7.0, so polymer-based columns or end-capped silica-based columns were often used [11]. Besides, masking agents were often added in the mobile phase even end-capped silica-based columns were used because many of their solvent accessible silanol groups were still unprotected [26]. In various gradient methods for logP determination, silica-based columns [16,20,21] and polymer-based columns [22] were used. To avoid complicated retention behavior caused by mobile phase additives especially some silanol masking agents, we used

Table 1
Literature experimental log*P* and pK_a values of all investigated compounds.

No.	Compound	log*P*	No.	Compound	log*P*	pK_a	No.	Compound	log*P*	pK_a
1	Benzyl alcohol	1.10	25	Benzeneacetic acid	1.41	4.31	49	3,5-Dicarbomethoxybenzenesulfonic acid	NA	NA
2	Benzaldehyde	1.47	26	Benzoic acid	1.87	4.20	50	1-Naphthalenesulfonic acid	NA	NA
3	Anisole	2.11	27	2-Chlorobenzoic acid	2.05	2.94	51	2-Naphthalenesulfonic acid	NA	NA
4	Diphenylmethanol	2.67	28	2-Chlorophenol	2.15	8.35	52	3,5-Dichloro-2-hydroxybenzenesulfonic acid	NA	NA
5	Toluene	2.73	29	2-Bromobenzoic acid	2.20	2.85	53	4-Hydroxybenzenesulfonic acid	NA	NA
6	Chlorobenzene	2.89	30	4-Bromobenzeneacetic acid	2.31	4.19	54	2-Amino-1,4-benzenedisulfonic acid	NA	NA
7	Bromobenzene	2.99	31	3-Methylbenzoic acid	2.37	4.27	55	1,5-Naphthalenedisulfonic acid	NA	NA
8	1,2-Dimethylbenzene	3.12	32	4-Bromophenol	2.59	9.31	56	Benzenesulfonic acid	NA	NA
9	1,4-Dimethylbenzene	3.15	33	4-Chlorobenzoic acid	2.65	3.98	57	3-Sulfobenzoic acid	NA	NA
10	Ethylbenzene	3.15	34	3-Bromobenzoic acid	2.87	3.81	58	4-Sulfobenzoic acid	NA	NA
11	1,3-Dimethylbenzene	3.20	35	2,4-Dibromophenol	3.06	7.85	59	2-Amino-4-methylpyridine	0.56	7.38
12	Naphthalene	3.30	36	2-Naphthalenecarboxylic acid	3.28	4.16	60	4-Methoxyaniline	0.95	5.34
13	2-Chlorotoluene	3.42	37	2,6-Dibromophenol	3.36	6.80	61	Benzylamine	1.09	9.47
14	1,3-Dichlorobenzene	3.53	38	2,4,6-Trichlorophenol	3.69	6.21	62	2-Methoxyaniline	1.18	4.62
15	2-Methylnaphtalene	3.86	39	Pentachlorophenol	4.69	5.12	63	4-Ethoxyaniline	1.24	5.20
16	Biphenyl	4.01	40	2-Methylbenzeneacetic acid	NA	4.35	64	2,4-Dimethylaniline	1.68	4.70
17	1,3,5-Trichlorobenzene	4.19	41	2-Chlorobenzeneacetic acid	NA	4.07	65	N,N'-Dimethylbenzylamine	1.98	8.80
18	Diphenyl ether	4.21	42	1-Naphthaleneacetic acid	NA	4.24	66	4-Isopropylaniline	2.23	4.85
19	1,3,5-Tribromobenzene	4.51	43	3,5-Dimethylbenzoic acid	NA	4.30	67	N,N'-Dimethylaniline	2.31	5.07
20	Hexamethylbenzene	4.61	44	2,4,6-Tribromophenol	NA	5.95	68	Dibenzylamine	2.61	6.10
21	Pentachlorobenzene	5.18	45	5-Amino-2-nanphthalenesulfonic acid	NA	NA	69	β-Carboline	2.65	8.32
22	Hexabromobenzene	6.80	46	4-Methylbenzenesulfonic acid	NA	NA	70	N,N'-Diethylaniline	3.31	6.61
23	Benzyl chloride	NA	47	4-Chlorobenzenesulfonic acid	NA	NA	71	2-Amino-6-methylpyridine	NA	7.41
24	Pentabromotoluene	NA	48	2,4-Dimethylbenzenesulfonic acid	NA	NA	72	2,4-Dimethylpyridine	NA	6.85

All log*P* and pK_a values are literature experimental values obtained from database module of ACD/Labs software. NA: no literature experimental log*P* or pK_a values available.

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