# A Robust, Viable, and Resource Sparing HPLC-Based LogP Method Applied to Common Lipophilic Drugs to Help in Expanding *in Silico* Training Datasets

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## Abstract

Reliable, experimentally determined partition coefficient P (logP) for most drugs is often unavailable in the literature. Many values are from *in silico* predictions and may not accurately reflect drug lipophilicity. In this study, a robust, viable, and resource-sparing method to measure logP was developed using reverse-phase high-performance liquid chromatography (RP-HPLC). The logP of twelve common drugs was measured using calibration curves at pH 6 and 9 that were created using reference standards with well-established logP. The HPLC method reported here can be used for high throughput estimation of logP of commonly used drugs. LogP values here showed general agreement with the other few HPLC-based literature logP values available. Additionally, the HPLC-based logP values found here agreed partially with literature logP values found using other methodologies (± 10%). However, there was no strong agreement since there are few experimentally determined literature logP values. This paper shows a facile method to estimate logP without using octanol or computational approaches. This method has excellent promise to provide reliable logP values of commonly used drugs available in the literature. A larger pool of reliable logP values of commonly drugs has the promise to improve the quality of medicinal chemistry, pharmacodynamic, and pharmacokinetic training sets, and models.

## Introduction and Objectives

- LogP is a critical lipophilicity index for medicinal chemistry, dissolution, pharmacokinetics, and pharmacodynamics modeling (1)
- **Issues:**
  - Reliable, experimentally determined logP for most drugs are often unavailable in the literature but are essential for future logP computer model training sets
  - The classic octanol/water shake-flask experiment is labor intensive, requires lengthy experimental duration, and requires large amounts of compounds
- **Goal:** This study aimed to measure the logP of twelve drugs commonly used in non-clinical and clinical studies using a time and resource-sparing RP-HPLC method
- **Impact:** Use of HPLC-based logP methods can allow for more frequent measurement and reporting of logP values of drugs.

## Overall Approach and Materials

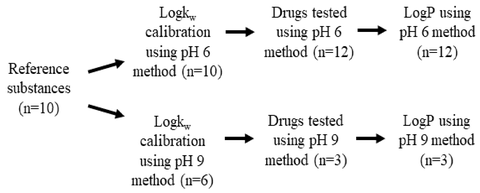


Figure 1 - Scope of studies to measure HPLC-based drug logP at pH 6 and 9.

**Approach**

- Measure logP through RP-UPLC + PDA detection by using a calibration curve with retention time of compounds with known experimental LogP (2-4)
- Lipophilicity can be determined by LC based on the partitioning of the compound between polar mobile phase and apolar column

| Tested drugs | BCS class | MW (g/mol) | pK$_a$ (base/acid)[a] | Literature LogP |
|---|---|---|---|---|
| **Rivaroxaban** | II | 435.88 | 10.87 (acid) | 2.08 |
| **Carbamazepine** | II | 236.27 | 10.86 (acid) | 2.63 |
| **Albendazole** | II | 265.33 | 3.83 (base), 9.94 (acid) | 3.07 |
| **Ibuprofen** | II | 206.29 | 4.54 (acid) | 3.51 |
| **Griseofulvin** | II | 352.76 | None (neutral) | 3.53 |
| **Isavuconazole** | I | 437.47 | 3.28 (base) | 3.56 |
| **Ketoconazole** | II | 531.43 | 4.22 (base), 6.15 (base) | 4.34 |
| **Ritonavir** | IV | 720.95 | 4.46 (base) | 4.94 |
| **Fenofibrate** | II | 360.83 | None (neutral) | 5.19 |
| **Posaconazole** | II | 700.8 | 4.67 (base) | 5.36 |
| **Itraconazole** | II | 705.6 | 4.57 (base) | 5.66 |
| **Lapatinib** | II | 581.06 | 6.53 (base) | 6.0 |

Table 1 - Physicochemical properties of ten OECD reference substances for logP calibration curves. All reference substances were in neutral form at pH 6 and 9.

| Reference Substances | MW (g/mol) | LogP[3] |
|---|---|---|
| **4-Methylbenzyl alcohol** | 122.16 | 1.6 |
| **Methyl benzoate** | 136.15 | 2.1 |
| **Atrazine** | 215.68 | 2.6 |
| **Benzophenone** | 182.22 | 3.2 |
| **Phenyl benzoate[a]** | 198.22 | 3.6 |
| **Benzyl benzoate[a]** | 212.25 | 4.0 |
| **Butylbenzene[a]** | 134.22 | 4.6 |
| **Bibenzyl[a]** | 182.27 | 4.8 |
| **Triphenylamine[a]** | 245.33 | 5.7 |
| **Dichlorodiphenyltrichloroethane (DDT)[a]** | 285.59 | 6.5 |

Table 1 - Physicochemical properties of ten reference substances for logP calibration curves. All reference substances were in neutral form at pH 6 and 9. All ref substances were analyzed at pH 6.
a) Reference substances analyzed at pH 9.

## Methodology

$$k = (T_r - T_0)/T_0$$

- k = capacity factor
- Tr = retention time of reference standard or test drug
- T0 = retention time of unretained compound (uracil)

$$logk = logk_w - S * \varphi$$

- Logkw = log of the retention factor at 100% water → extrapolated value from regression
- S = slope of regression

$$logP = a * logk_w + b$$

- LogP is correlated with log of the retention factor at 100% water
- Calibration curve with reference substances relating known logP and experimental logkw

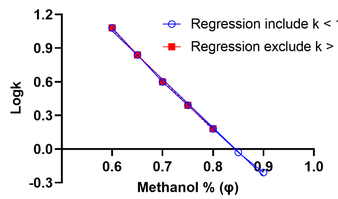


Figure 2 - Plot of logk vs methanol percentage (φ) for reference substance benzyl benzoate at pH 6. Blue line and symbol denotes regression which includes k < 1. Red line and symbol denotes regression which includes k ≥ 1.

- For each reference substance and test drug → least five proportions of methanol were tested
- The percentage of methanol in the mobile phase varied from 45 to 90% depending on the compound being analyzed
- For each reference substance and drug, five to seven retention factor values were collected

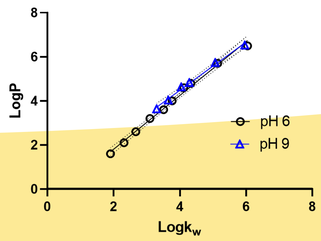


Figure 3- LogP calibration curves for pH 6 and pH 9. Black circles denote calibration curve of ten reference substances with logP ranging from 1.6 to 6.5 at pH 6. Blue triangles denote calibration curve of six reference substances with logP ranging from 3.6 to 6.5 at pH 9.

## Results

| Test drugs | Literature logP or logP$_{oct}$ | HPLC-based logP (pH 6) | HPLC-based logP (pH 9) |
|---|---|---|---|
| **Rivaroxaban** | 2.08[a] | 3.005 | ---- |
| **Carbamazepine** | 2.3[d], 2.63[b] | 2.699 | ---- |
| **Albendazole** | 3.07[a] | 3.359 | ---- |
| **Ibuprofen** | 3.3[d]; 3.47*[c], 3.51[b] | 3.566* | ---- |
| **Griseofulvin** | 3.53[a] | 3.566 | ---- |
| **Isavuconazole** | 3.56[d] | 4.934 | ---- |
| **Ketoconazole** | 4.34[b] | 5.068 | 5.179 |
| **Ritonavir** | 4.94[a] | 5.825 | ---- |
| **Fenofibrate** | 5.19[a] | 5.422 | ---- |
| **Posaconazole** | 5.36[a] | 6.716 | ---- |
| **Itraconazole** | 5.66[d,e] | 6.814 | 6.888 |
| **Lapatinib** | 6.0[d,f] | 5.971 | 5.880 |

Table 2 - LogP values of twelve test drugs. LogP values were from the literature, in silico prediction using ADMET Predictor (S+logP, MlogP), and HPLC-based logP generated here. Literature logP values involved computer calculation and/or experimental tests. HPLC-based logP here tended to be higher than literature logP and in silico predictions.
a) *in silico*; b HPLC; c) Thin-layer chromatography; d) Shake-flask or slow-stirring method; e) US FDA package insert; f) US FDA review documents. * ionization > 90%.

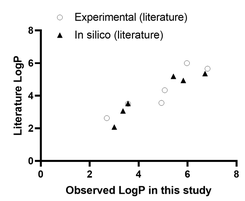


Figure 4 - Correlation of logP reported in the literature and observed logP values in this study (r2=0.931, p-values < 0.0001). Literature was either experimental or in silico.

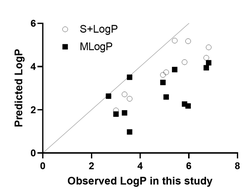


Figure 5 - Relationship between observed HPLC-based logP in this study and predicted logP from S+logP and MlogP in silico methods. S+logP were closer than MlogP predictions to our observed logP. MlogP values were smaller than S+logP predictions.

## Conclusions

- Results indicate that the HPLC-based method to measure drug logP was viable and robust.
- Drug logP values were obtained in a reasonably facile and resource sparing manner, with less than 5 mg of each drug needed.
- Logkw values here of the four reference substances with literature logkw values agreed, supporting robustness of the method
- The HPLC-based method exhibited robustness via leave-one-out analysis in estimating logP, no practical impact of including or excluding retention factors less than one on logkw, and no practical impact of pH on logP.
- There was partial agreement between logP values here and literature logP. Six of the twelve test drugs showed HPLC-based logP values here that were similar (within 10%) to literature logP. Use of HPLC-based logP methods perhaps can allow for more frequently measurement and reporting of logP values of drugs.

## Bibliography

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