

Sep 23, 2024

LogP / LogD shake-flask method

DOI

dx.doi.org/10.17504/protocols.io.e6nvw14kd1mk/v1

Yuriii Kheylik¹

¹Enamine Ltd

ASAP Discovery



Nick Lynch

Curlew Research, ASAP

OPEN  ACCESS



DOI: dx.doi.org/10.17504/protocols.io.e6nvw14kd1mk/v1

Protocol Citation: Yuriii Kheylik 2024. LogP / LogD shake-flask method. [protocols.io](#)

<https://dx.doi.org/10.17504/protocols.io.e6nvw14kd1mk/v1>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

Created: August 09, 2024

Last Modified: September 23, 2024

Protocol Integer ID: 106086

Keywords: 1-octanol, LogD, Lipophilicity, LogP , HPLC system, ADMET, Bioavailability

Disclaimer

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to **protocols.io** is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with **protocols.io**, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

Abstract

Lipophilicity is possibly the most important physicochemical parameter for any potential drug candidate. Lipophilicity measurements are valuable for understanding how drugs are dissolved in plasma and other aqueous biological fluids. Lipophilicity is typically accessed as the distribution of the tested compound between two solvents - typically non-aqueous organic (1-octanol) and aqueous (pH-buffered water), and then LogP is expressed as a Log of the concentration ratio between two phases. LogP is widely used in cheminformatics and is a component of Lipinski's "rule of five", which is a golden standard to evaluate a drug-likeness of a compound. According to this rule, the successful drug candidate should possess LogP value not greater than 5.

LogD is a distribution coefficient widely used to measure the lipophilicity of ionizable compounds, where the partition is a function of the pH.

For nonionizable compounds LogP = LogD throughout pH range, whereas for ionizable compounds LogD takes into account the partition of both ionized and non-ionized forms. LogD is more convenient for practical measurements, as it takes into account solution pH, which is important for the analysis of the drug candidate properties in various biological media with different pH values. The shake flask method is considered the gold standard technique for determining log D.

Guidelines

Calculations of the distribution coefficients (D) for determine LogD:

Calculations of the distribution ratios were carried out using the equation below.

$$D = \frac{d_o \cdot S_o}{d_p \cdot S_p}$$

where:

S_o - peak area of the analyte in octanol phase

S_p - peak area of the analyte in PBS buffer

d_o - dilution coefficient for octanol phase

d_p - dilution coefficient for aqueous phase

LogD - it represents the logarithm (log10) of the distribution coefficient (D) of a molecule.

Materials

Equipment:

1. Liquid handling robots Neon 100 by Xiril AG (Switzerland)
2. Shimadzu Prominence HPLC Systems (Japan)
3. Hybrid triple quadrupole/linear ion trap mass-detector 4000 QTRAP with Turbo V ion source (AB Sciex, Canada)
4. VWR Membrane Nitrogen Generators N2-04-L1466, nitrogen purity 99.9%+ (VWR, USA)
5. MTR22 Multi Mix Rotator (UNICO, USA)
6. Laboratory Centrifuge, Sigma 4-15C, Qiagen (SIGMA GmbH, Germany)
7. Water purification system Millipore Milli-Q Gradient A10 (Millipore, France)
8. Multichannel Electronic Pipettes 2-125 µL, 15-1250 µL, Matrix (Thermo Scientific, USA)

Material:

1.  Phosphate buffered saline powder, pH 7.4, for preparing 1 L solutions **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P3813**
2.  1-Octanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #472328**
3.  Acetonitrile **Merck MilliporeSigma (Sigma-Aldrich) Catalog #34851**
4.  Dimethyl sulfoxide (DMSO) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #34869**
5.  Blank and Alphanumeric Storage Tubes **Thermo Fisher Scientific Catalog #4170**
6. 1.4 ml microtubes in multiracks (ThermoScientific, USA; Cat # 4253)

Safety warnings

-  Always wear appropriate PPE for this protocol
Refer to Material Safety Data Sheets for additional safety and handling information.

Preparation of auxiliary solutions

1 1 litre PBS: 0.01 M Phosphate buffer

In a bottle for reagents with a cap of 1L capacity, place:

- contents of  1 L sachet with phosphate buffer,
-  1 L of water, mix thoroughly, and filter through a membrane filter with a pore diameter of 0.45 µm.

Note

The shelf life of the solution is 2 weeks when stored at  5 °C .

2 100 ml of A40 solution (extract thinner):

In a bottle for reagents with a lid with a capacity of 100 ml, place:

-  60 mL of H₂O,
-  40 mL of AcN and mix thoroughly.

Note

The shelf life of the solution is 3 months when stored at  Room temperature .

3 100 ml of solution A5 (extract thinner):

In a bottle for reagents with a cap of 100 ml, place:

-  95 mL H₂O,
-  5 mL of AcN and mix thoroughly.

Note

The shelf life of the solution is 1 month when stored at  Room temperature .

4 1-octanol & PBS mix:

1d

The 1-octanol and pH 7.4,  0.01 Molarity (M) , shake phosphate buffer together for  24:00:00 and allow the phases to separate before use.

5 Preparation of 10 mM stock compound:

Prepare  10 millimolar (mM) stock solutions of test substances in DMSO. As a rule,  100 µL of solution is enough.

Sample preparation

2h 2m

6

Note

Sample preparation is carried out using a Neo 100 robotic station from Xiril.

7 Preparation for mixing 1-octanol, PBS, and 10 millimolar (mM) stock solution.

- Below, fig. 1 shows the placement of microtubes on a plate to prepare 5 test and reference compounds.



Fig. 1 Location map of samples for 5 test and reference compounds

- 8 The plate is installed in position "G" of the robotic station. Test and reference stock compounds should be placed in special holders, as shown in Fig. 2.

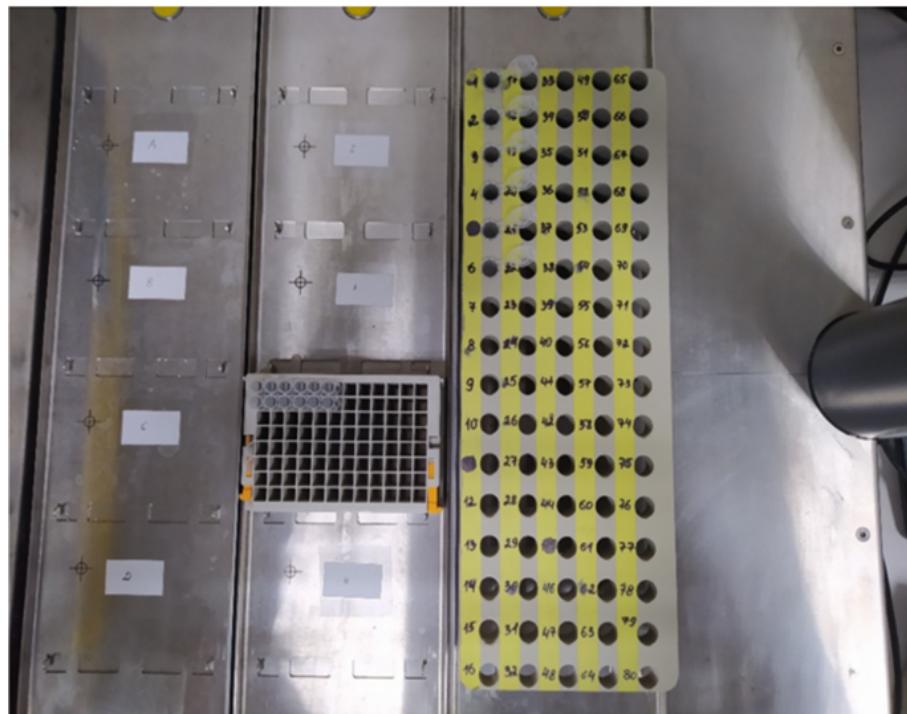


Fig. 2 Location map for test and reference stock compounds

- 9 Plastic vials type "Falcon 15 mL" with PBS, 1-Octanol, and A40, and vials for a drain on the automatic station should be placed as shown in Fig. 3.

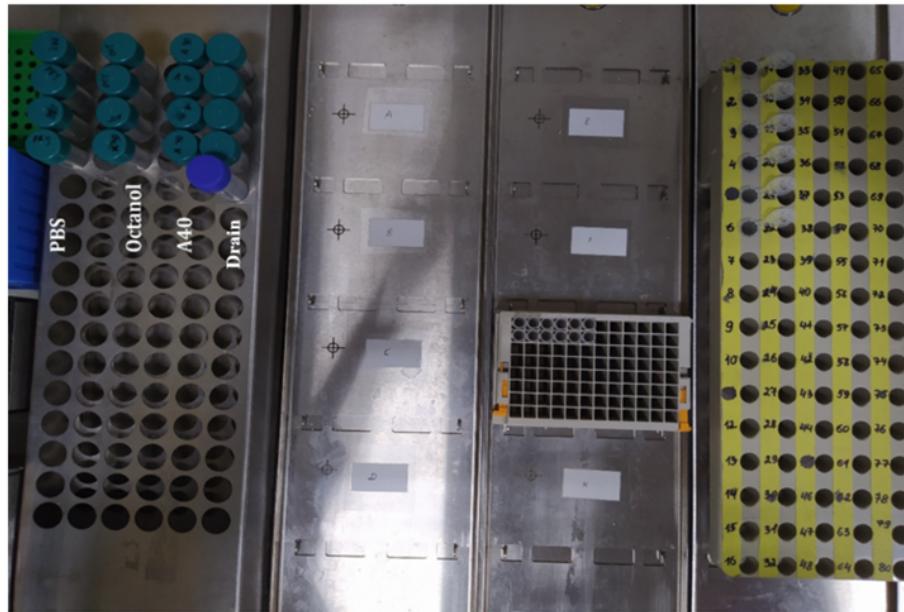


Fig. 3. Placement of vials with PBS, 1-octanol, A40, and drain on the automatic station Neo 100 of AG Xiril

- 10 Place the box with tips 1000 µl and 200 µl in the indicated positions as shown in Fig. 4.

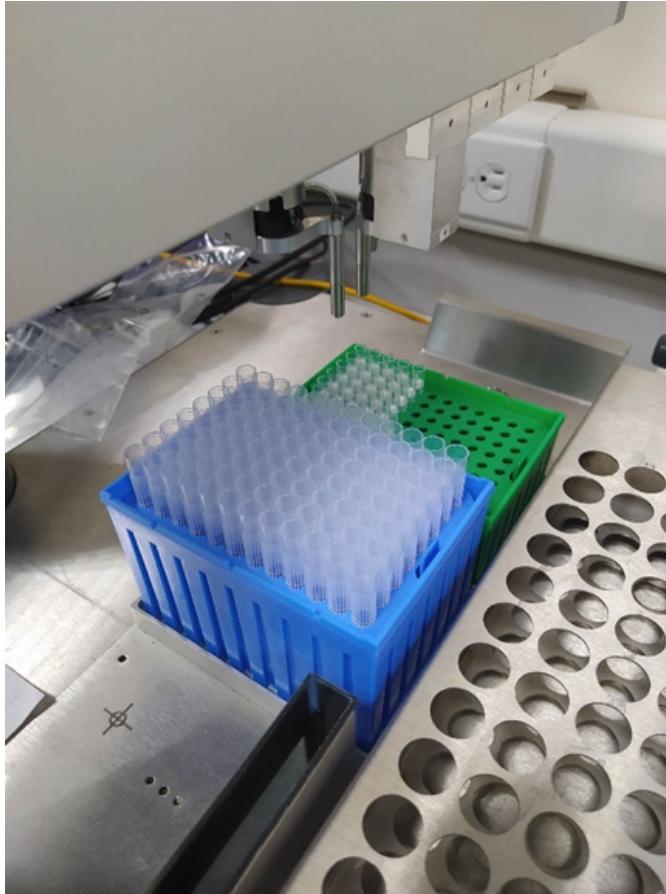


Fig. 4. Box with tips at the robotic station Neo 100

11 In the Lirix3 program Fig. 5, select the pre-created script for sample preparation.

- The first stage includes preparing  1 mL mix solution in a duplicate of 495:5:500 (v/v/v) PBS pH7.4/10mM stock/1-octanol.

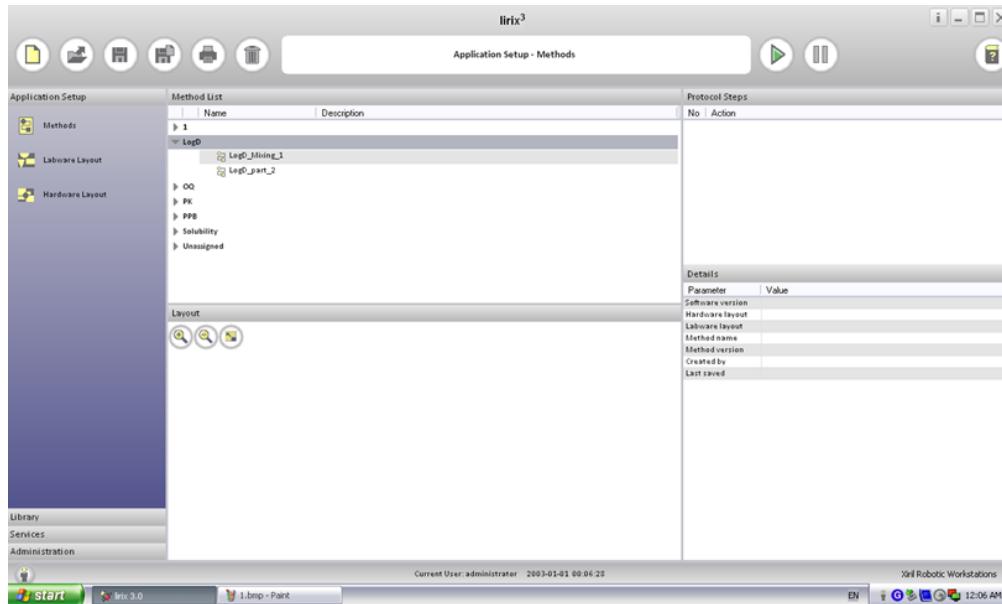


Fig. 5. Lirix3 software interface.

12 Close the microtubes with lids using a press, after mixing in the rotator for  02:00:00 at 30 revolutions per minute.

2h 2m



- Centrifuge plate for  3000 rpm, 00:02:00, and put it in the robotics station in place "G".

13 In position "D" of the robotic station, place a rack with microtubes of 0.7 µl at the rate of 6 microtubes per substance in duplicate. Place the microtubes on the plate as shown in Fig. 6.

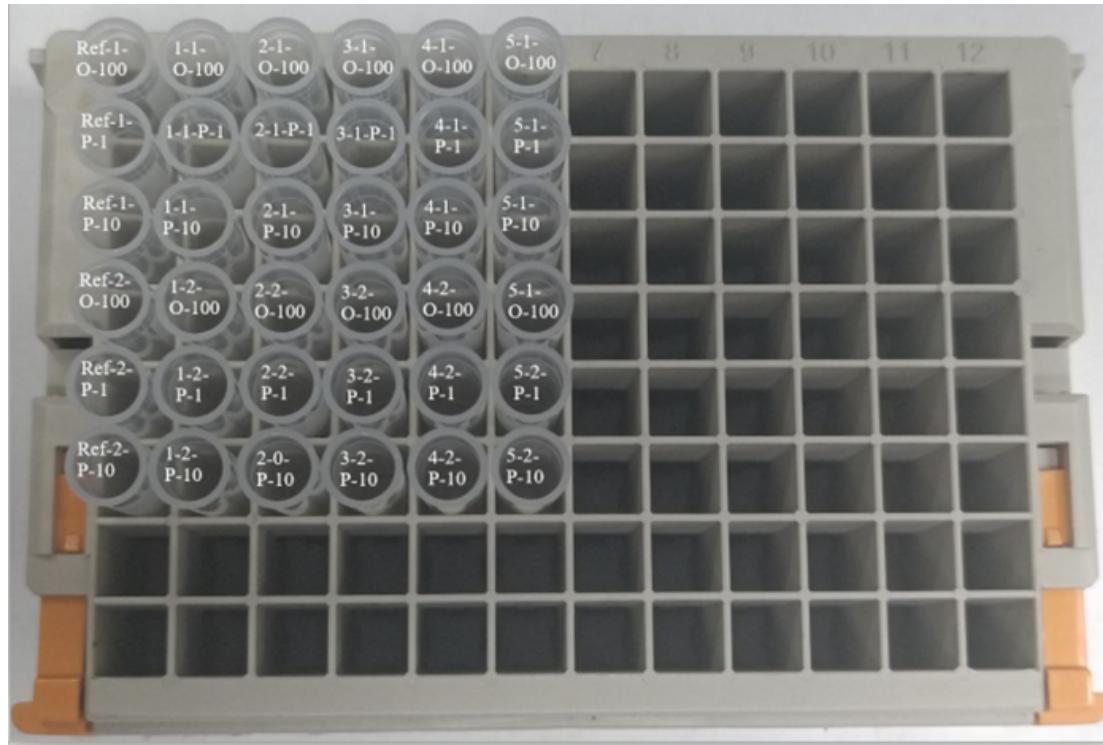


Fig. 6 Placement of test tubes on a rack for diluted phases.

- 14 Install a special guide bar on the rack for holding test tubes and positioning tips. The general layout of the boards at the robotic station is shown in Fig. 7.



Fig. 7. Placement of the tripods for separation 1-octanol from PBS and final sample dilution.

- 15 Open bottle drain and A40 (see Fig. 3).
- 16 In the Liric 3 starts script LogD part 2 (see Fig. 5). After that liquid handler provides the next operations:
 - 16.1 The first step is the dilution of 1-octanol in 100 times. In the microtubes "...-o-100" (see Fig. 5) plate position «D» add  990 µL A40 and  10 mL 1-octanol phase in the plate position «G», pipetting 5 times.
 - 16.2 The second step is to remove the excess 1-octanol phase to expose the aqueous phase.
 - 16.3 The next step is to transfer  300 µL PBS phase plate position «G» into microtubes "...-p-1" plate position «D»
 - 16.4 The final, dilution PBS phase is 10 times. Into microtubes "...-p-10" plate position «D» add  225 µL A40 and  25 µL PBS phase in the microtubes "...-p-1" plate position «G»,

pipetting 5 times.

- 17 Analyse both the samples (both phases) using an HPLC system coupled with a tandem mass spectrometer.



Protocol references

1. Analysis and Purification Methods in Combinatorial Chemistry, Chapter 17. Edited by Bing Yan. ISBN 0-471-210929-8 Copyright© 2004 by John Wiley & Sons, Inc .
2. OECD Guidelines for the Testing of Chemicals / Section 1: Physical-Chemical properties Test No. 117: Partition Coefficient (n-octanol/water), HPLC Method .
3. Combinatorial Chemistry & High Throughput Screening, 2001, 4, 511-519 High Throughput Log D Determination Using Liquid Chromatography-Mass Spectrometry. Dean M. Wilson, Xiaoli Wang, Erin Walsh, and Robyn A. Rourick .