# ●●● Evolution of Lipophilicity Assays Within the BRo5 Space ●●●

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## Abstract:

As the chemical space expands to more Beyond Rule of 5 compounds, the question arises of which assay is best to accommodate increasing lipophilicity values. The gold-standard assay of shake flask logD, the chromatographic ElogD, and novel AlphalogD all have their pros and cons when it comes to solubility issues, column technology, and lipophilicity range. The need for review of these current methods is a priority to have a reliable, high-throughput, and sustainable way to measure logD/P of large molecules.

## Introduction:

Lipophilicity is a key pillar in understanding a compound's physicochemical properties and furthering its development throughout drug discovery. Measuring logD and logP of basic, acidic, and neutral compounds requires accurate assays that produce reliable data. Shake flask logD is the gold-standard method when it comes to measuring lipophilicity values between 0.5 to 3.4, but it is limited by poor solubility of large molecules. The use of chromatographic lipophilicity assays, such as ElogD and AlphalogD, mimics octanol-water partitioning using an octanol-saturated organic mobile phase on a $C_{16}$-amide support. ElogD requires predicted lipophilicity values to determine the organic range. AlphalogD is an optimization based on experimental values only. The comparisons between ElogD and AlphalogD show a good correlation due to similar column chemistry, but ion-pairing, aggregation, and misprediction can explain outliers. AlphalogD solves the solubility issues seen in the other assays, but the demand for higher organic ranges is not always beneficial. Prioritizing further optimization has become more apparent to solve challenges attributed to complex interactions while improving data analysis and interpretation. The need for continuous improvement and evaluation of techniques to measure lipophilicity is necessary to study the chemical space expansion.

## Shake Flask

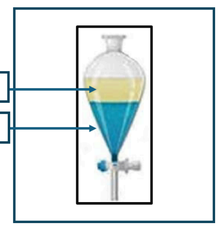
**Method**

logD = log{(solute)octanol / [(ionized solute)water + (neutral solute)water]}

- Non-miscible two-phase system of octanol and aqueous buffer at a specific pH.
- Quantification of solute amount in each phase.
- 95% confidence for lipophilicity between 0.5 and 3.4.

**Limitations**

- Compound solubility in aqueous or organic phase.
- Sensitivity of quantitative method – LC/MS/MS is the most sensitive.
- 95% of confidence in data reliability for:
  - -2.0 < LogD < 3.4, by CLND quantification.
  - -1.0 < LogD < 2.8, by UV quantification.
  - -1.0 < LogD < 3.1, by MS/MS quantification.




## HPLC ElogD

- **Mobile Phases:**
  - Aqueous: 20mM MOPS pH 7.4 in 0.05% octanol saturated water with 0.15% n-decylamine.
  - Organic: 0.25% octanol saturated methanol.
- Use of ion-pairing agent (MOPS) to enhance ionization.
- Dependent on lipophilicity prediction.

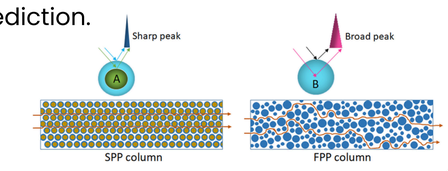
[1]

| Method range | $ELogD_{oct}$ range | Flow-rate (mL/min) | % MeOH |
|---|---|---|---|
| Low | <1 | 0.5 | 15,20,25 |
| Middle | 1–3 | 1 | 40,45,50 |
| High | >3 | 2 | 60,65,70 |

## HPLC AlphalogD

- **Mobile Phases:**
  - Aqueous: 50mM Ammonium acetate pH 7.4 in 0.05% octanol saturated water.
  - Organic: 0.25% octanol saturated methanol
- Support built on Semi-Porous Particles (SPP):
  Solid core covered with a thin layer of porous modified $C_{16}$-amide particles.
- No need for lipophilicity prediction.

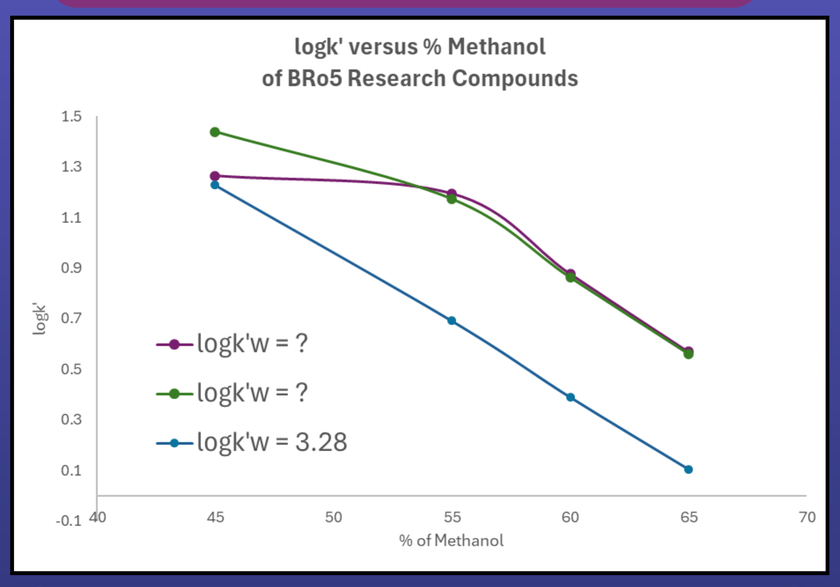
**Comparison of Semi-Porous Particles (SPP) with Fully Porous Particle [3]**




## Method Commonalities

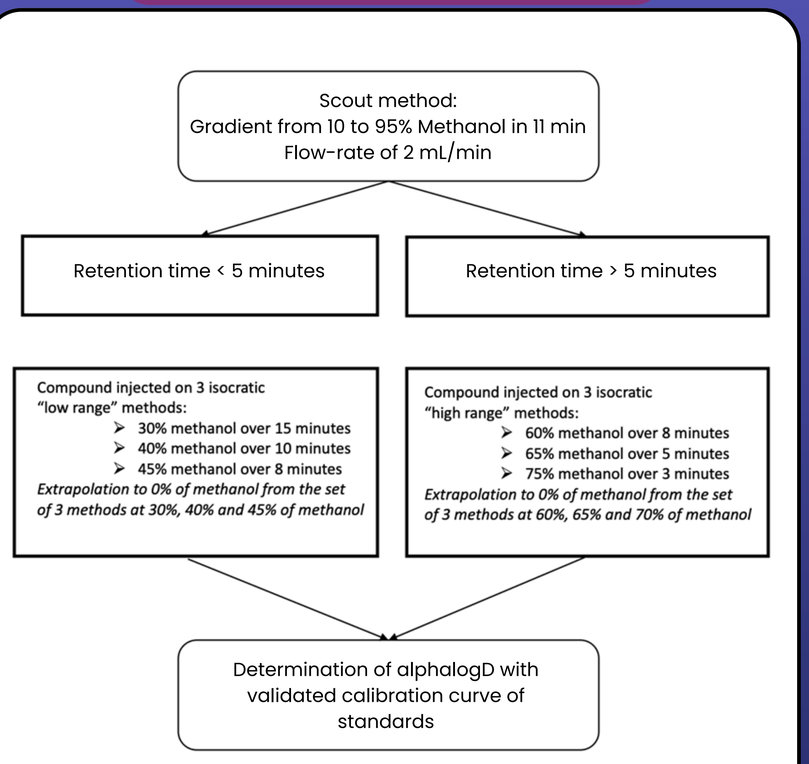
- Chromatographic reversed phase HPLC on $C_{16}$-amide support, for basic and neutral compounds.
- Dipole-dipole interactions between amide group and H-bond donor solutes, for low solubility range
- Capture retention time at different contents of organic solvents,
  - Calculate $logk' = log[(t_R - t_0)/t_0]$.
  - Extrapolation at 0% of organic solvent: logk'w.
  - Calculate logD from logk'w.

## Highlight of Hydrophobic Interactions

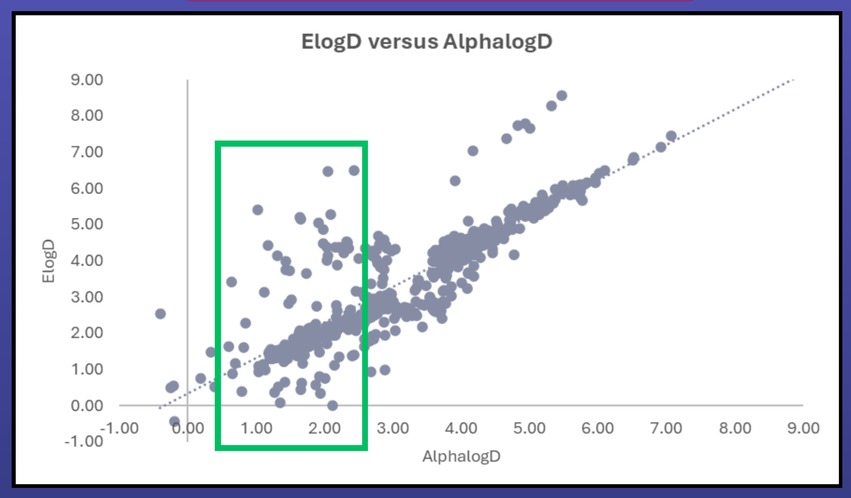



- Non-linearity: which part of the curve is correct?
- Which logk'w value to apply?
- Does lack of linearity indicate a potential change of conformation that highlights different interactions with the support and mobile phase?
- Does solubility impact hydrophobic interactions?

## AlphalogD Methodology




## Comparison of Assays




- 514 research compounds with varying molecular weights, unknown structures, and diverse origins
- Good correlation to each other, due to similar stationary phase chemistry
  - around 64% of the lipophilicity values are within the range of -0.5 to +0.5
- Correlation improves for compounds with higher logD values
- Higher variability in the low lipophilicity range, with around 24% of AlphalogD values that are lower than ElogD values

## AlphalogD Measurements for Large Molecules

| Name | Category | MW | Average AlphalogD (n=3) pH 7.4 | ACD predicted (logD pH 7.4) |
|---|---|---|---|---|
| Telaprevir | Linear peptide | 679.85 | 4.48 | 3.20 |
| Atazanavir | Linear peptide | 704.86 | 4.81 | 4.61 |
| Ritonavir | Linear peptide | 720.94 | 5.09 | 5.09 |
| Ledipasvir | Linear peptide | 889.00 | 6.99 | 6.38 |
| Everolimus | Macrocyclic peptide | 958.22 | 6.80 | 4.24 |
| Zotarolimus | Macrocyclic peptide | 966.21 | 6.45 | 3.55 |
| Temsirolimus | Macrocyclic peptide | 1030.29 | 7.00 | 4.12 |
| ARV-771 | PROTAC | 986.60 | 5.79 | N/A |
| dBET6 | PROTAC | 841.37 | 4.72 | N/A |
| Gefitinib-based PROTAC 3 | PROTAC | 934.51 | 6.40 | N/A |
| MZ1 | PROTAC | 1002.64 | 5.12 | N/A |

*Absence of literature values for high lipophilic molecules

## Discussion

**AlphalogD Strengths**

- Does not require predicted lipophilicity values, a "scout" run is utilized to determine organic range.
- Using AlphalogD as one of the descriptors to highlight chameleonicity; change of conformation in a specific environment.
- Higher throughput
- High lipophilicity = hydrophobic interactions.
- Low lipophilicity = hydrogen-bond interactions.

**Questions**

- When there are no literature values to compare to, which assay do we know is the best option?
- Above 60% of organic solvent, does solubility become the major phenomenon?
- Can AlphalogD be used at a pH lower than 7.4? Is this logP or logD?

## Future Steps

- Optimize organic ranges by combining ElogD and AlphalogD methods.
- Correlation to other physicochemical properties, such as intramoleuclar Hydrogen bonding (IMHB).
- AlphalogD method to be developed at pH 2.5 for acidic compounds.
- AlphalogD in an aprotic solvent; acetonitrile.

## References

[1] Katz, Daniel, et al. "AlphalogD Determination: An Optimized Reversed-Phase Liquid Chromatography Method to Measure Lipophilicity on Neutral and Basic Small and Beyond-Rule-of-Five Compounds." Journal of Chromatography A, vol. 1674, July 2022, https://doi.org/10.1016/j.chroma.2022.463146

[2] Lombardo, Franco, et al. "ElogDoct: A Tool for Lipophilicity Determination in Drug Discovery. 2. Basic and Neutral Compounds." Journal of Medicinal Chemistry, vol. 44, June 2001, https://pubs.acs.org/doi/10.1021/jm0100990

[3] Tellakula, Nancharaiah, et al. "Leveraging Superficially Porous Particle Technology to Develop High-Throughput HPLC Methods for Small-Molecule Drug Discovery". Chromatographia 86(6): 1-10, May 2023. 10.1007/s10337-023-04259-y.