

APPLICATION

A Simple Approach to Method Development using Analyte LogD

Dani Xing, Zeshan Aqeel, and Ryan Splitstone
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA



Dani Xing
Technical Specialist

Traveling is becoming one of my favorite things to do but I love finding a good place to eat or napping in the afternoon after reading a good book. I like too and Krav Maga (is that a verb?)! ...So I can keep eating :)

Introduction

Chemical compounds that tend towards London dispersion forces, in terms of dictating intermolecular interactions, are often characterized as lipophilic, non-polar, and hydrophobic. These terms are commonly related and often have a shared meaning, but not in all circumstances.

A compound's prevailing intermolecular force provides information in terms of the compound's absolute solubility. This solubility distribution indicates how easily a drug compound can be absorbed and delivered to a biological system. Therefore, a matrix was developed to better measure the relative distribution of a solute in a polar/non-polar solution.

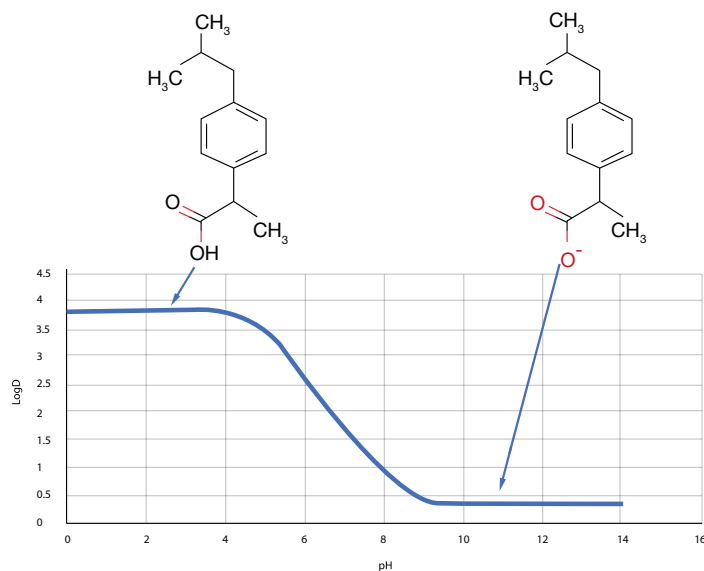
The distribution coefficient (D) is the ratio of the solute concentration of all species of a compound in a solution system of two immiscible phases, when at equilibrium. The ratio is taken with respect to the pH of the system and is logarithmically measured, typically in a biphasic system of n-octanol and water (**Figure 1**). LogD is taken with respect to pH because compounds with ionizable groups exist in solution as a mixture of different ionic forms that correspond to the specific compounds relative pK_a(s) of its ionizable groups, if applicable.

Figure 1.
The LogD expression

$$\text{LogD} = \text{Log} \left(\frac{[\text{Solute}]_{\text{Octanol}}^{\text{Ionized}} + [\text{Solute}]_{\text{Octanol}}^{\text{Un-ionized}}}{[\text{Solute}]_{\text{Water}}^{\text{Ionized}} + [\text{Solute}]_{\text{Water}}^{\text{Un-ionized}}} \right)$$

The overall concentration of the compound that is dissolved in the n-octanol is typically dominated by the un-ionized species of the compound. Conversely, the water phase typically favors the ionized species due to the increase in hydrophilic nature. Therefore, LogD can be graphically represented in respect to the pH of the system and depicts the changing solubility of a compound at different pH values (**Figure 2**).

Figure 2.
Ibuprofen's LogD in respect to pH



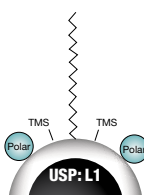
In terms of chromatography, LogD can provide useful information in terms of mobile phase pH selection and overall HPLC/UHPLC selectivity. Because the coefficients relate solubility and the interaction potential within an organic/aqueous solution, a correlation can be made to reversed phase HPLC/UHPLC.

In this example, an ibuprofen standard was obtained and screened on 4 different reversed phase Kinetex[®] core-shell columns at two separate pH's, 2.0 and 8.0. The values of mobile phase pH were selected to correspond to ibuprofen's pK_a of 4.9 and identified by referencing Figure 2. The purpose of the selection of two distinct pH values was to demonstrate the solubility implications of the fractional composition of the ionized vs. unionized species of the drug. Furthermore, we demonstrated how LogD is a useful and fast indicator in determining the most appropriate mobile phase pH for a given method.

Materials and Methods

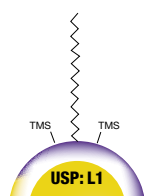
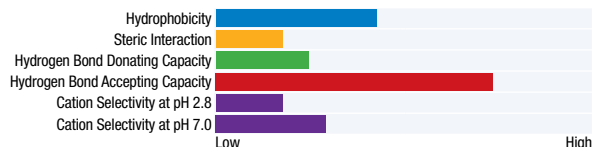
Ibuprofen standards were acquired from Sigma-Aldrich[®] (Saint Louis, MO). Buffers were prepared using potassium phosphate dibasic salt obtained from Fisher Scientific[®] (Hampton, NH) and adjusted to pH 2 and 8 with phosphoric acid and potassium hydroxide, respectively. The potassium buffer was prepared to a 20 mM concentration.

The column dimension selected was 50 x 2.1 mm with a particle size of 2.6 μm. The phases utilized were the Kinetex XB-C18, Kinetex EVO C18, and Kinetex Polar C18 and all tests were run on an Agilent[®] 1100 HPLC (Palo Alto, CA) with a flow rate of 2.0 mL/min. UV/Vis detection was used at 310 nm, no reference wavelength was used in this experiment. The column temperature was set at 30 °C with a 6-position column selector. Injection volume was set at 10 μL with an injection concentration of 0.1 mg/mL.



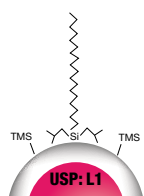
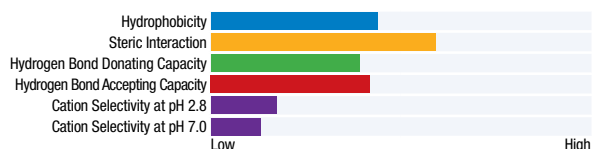
Kinetex[®] Polar C18

Combined C18 and polar modified surface that provide polar and non-polar retention alongside 100% aqueous stability.



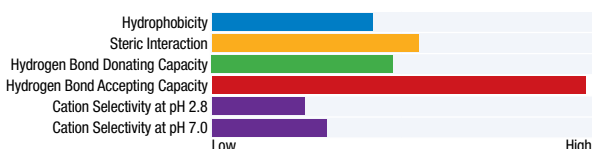
Kinetex EVO C18

Novel pH 1-12 stable C18 that delivers robust methods and improved peak shape for bases.



Kinetex XB-C18

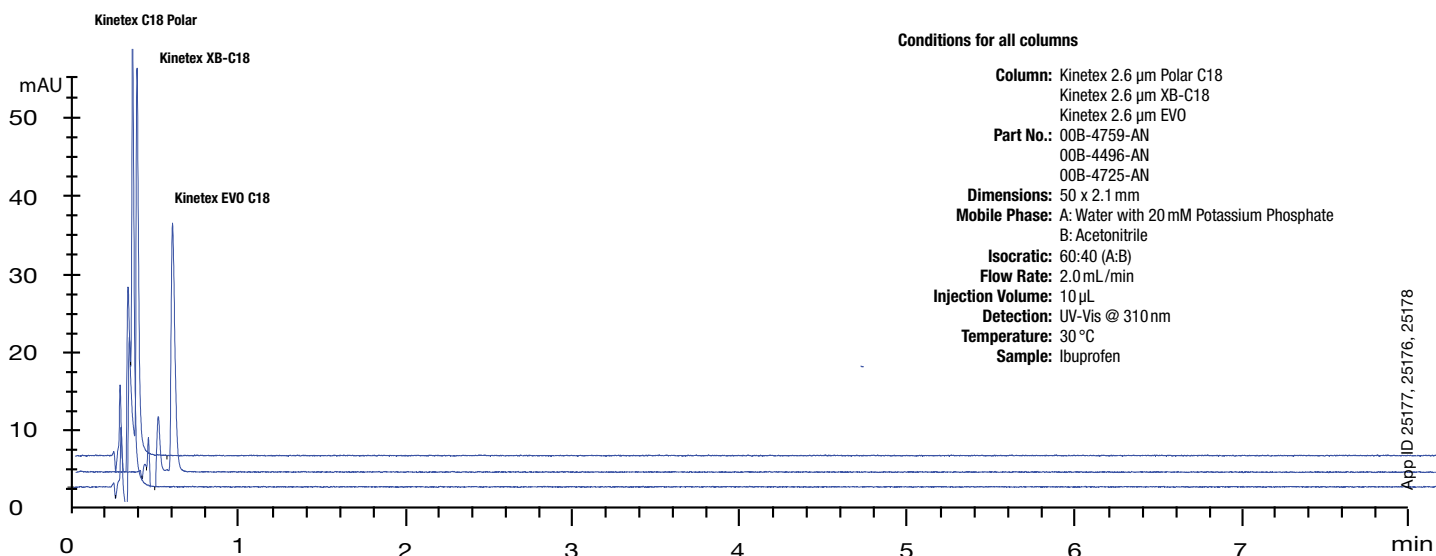
Di-isobutyl side chains differentiate this C18 column. Low ligand density and an inactive surface makes this column a great hydrogen acceptor. This phase will demonstrate improved peak shape for basic compounds and increased retention of acids.



Results

Figure 3.

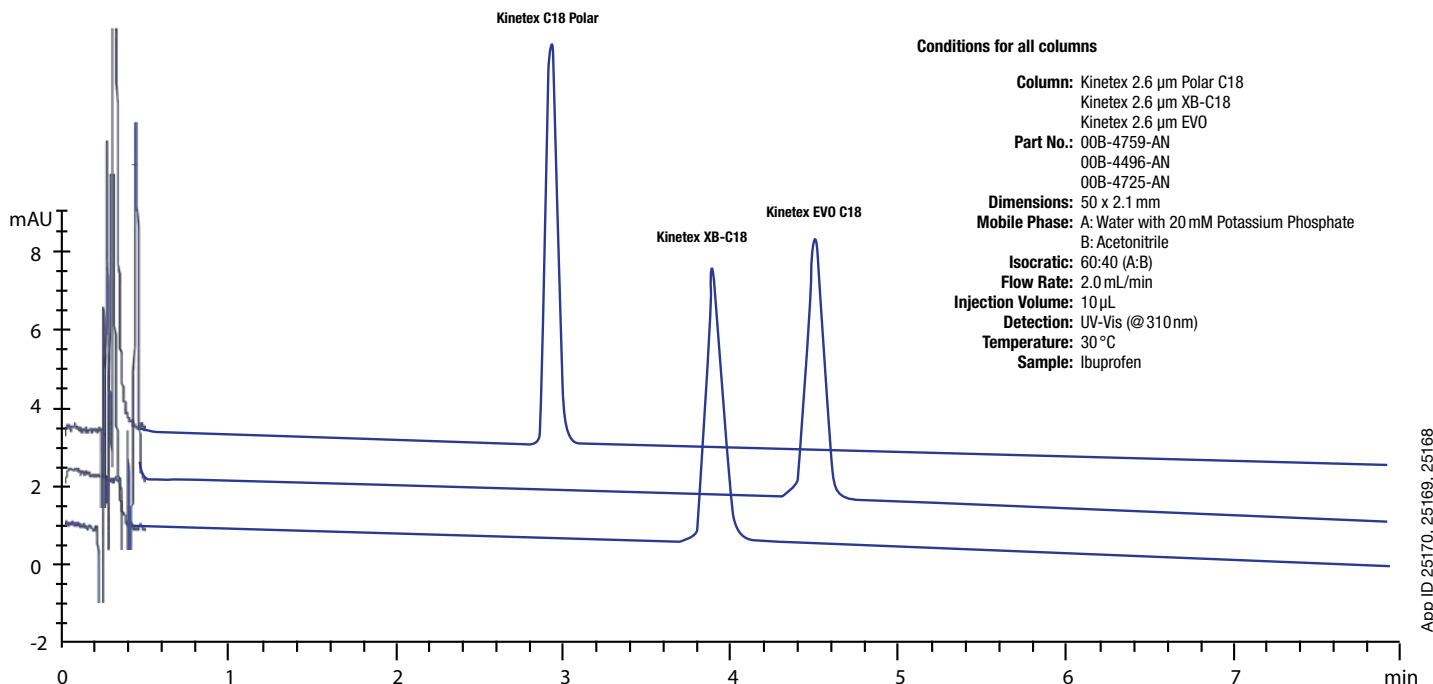
Overlay of ibuprofen standard at 0.1 mg/mL over three different Kinetex phase selectivities at pH 8.0



App ID 25177, 25176, 25178

Kinetex Phase	Retention Time (min)	Efficiency	Asymmetry	Peak Height	Void Time (min)
XB-C18	0.35	2913	0.40	50.93	0.26
EVO C18	0.55	3194	0.58	33.39	0.39
Polar C18	0.33	2736	0.48	63.51	0.26

Figure 4. Overlay of ibuprofen standard at 0.1 mg/mL over three different Kinetex[®] phase selectivities at pH 2.0



App ID 25170, 25169, 25168

Discussion

A common drug product, ibuprofen, was analyzed under reversed phase conditions at pH 2.0 and 8.0 using a potassium phosphate buffer convention and acetonitrile as the organic solvent. The purpose of the example was to demonstrate the chromatographic implications of varied mobile phase pH conditions in respect to the analyte of interest's referenced pK_a value. In addition, we learned how LogD information can be used to identify the most appropriate mobile phase pH conditions for most general reversed phase separations.

In **Figures 3** and **4**, it was observed that retention and selectivity varied for all four phases. Overall, the retention times were found to be less than 5 minutes with the peak of interest eluting far from the void. This is acceptable for a 50 mm length column, where the goal of the experiment is to not only obtain good separation but also to optimize the runtime.

At pH 8.0, the analyte was primarily in its ionized form with a pK_a value of 4.9. Under isocratic mobile phase conditions of 60:40 (Water/Acetonitrile) retention of ibuprofen was just past the void volume of the system and column. Therefore, it is assumed that limited interaction transpired between the stationary phase, analyte of interest, and the mobile phase. With the analyte

of interest so close to the void, building a robust and reproducible method would be difficult without further optimization.

At pH 2.0, the analyte was in its neutral state and therefore is more hydrophobic, interacting more favorably with the hydrophobic ligand and reversed phase mobile phase. Consequently, the analyte most likely partitions more favorably into the organic layer, in this case, acetonitrile, giving way to the competition of the strong solvent for the analyte and the alkyl stationary phase. Increased selectivity for the analyte of interest was observed (**Figure 4**) and can be seen by the drastic increase in ibuprofen's retention time. At pH 2.0 and 8.0 for the same system, mobile phase ratios, flow rates, and additional running conditions were utilized. Therefore, the only variable adjusted was the mobile phase pH.

Conclusion

In conclusion, a mobile phase convention consisting of a 60% mixture of water with 20 mM potassium phosphate, pH adjusted to 2.0 with phosphoric acid and 40% acetonitrile provided the most suitable running conditions for this example. In comparison, a mobile phase mixture of 60:40 that was pH adjusted to 8.0 provided an analyte elution time too close to the relative void volume reported in **Figure 3**. In regard to the stationary phase performance, the Kinetex Polar C18 provided an improved peak shape and an acceptable retention time for a fast and efficient method.

APPLICATION

Ordering Information
Kinetex[®] Core-shell Columns

2.6 μ m Minibore Columns (mm)						SecurityGuard [™] ULTRA Cartridges [†]
Phases	30 x 2.1	50 x 2.1	75 x 2.1	100 x 2.1	150 x 2.1	3/pk
EVO C18	00A-4725-AN	00B-4725-AN	—	00D-4725-AN	00F-4725-AN	AJ0-9298
Polar C18	00A-4759-AN	00B-4759-AN	—	00D-4759-AN	00F-4759-AN	AJ0-9530
XB-C18	00A-4496-AN	00B-4496-AN	00C-4496-AN	00D-4496-AN	00F-4496-AN	AJ0-8782

for 2.1 mm ID

† SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000

Kinetex is available in additional dimensions and particle sizes.
www.phenomenex.com/Kinetex

Australia
 t: +61 (0)2-9428-6444
 auinfo@phenomenex.com

Austria
 t: +43 (0)1-319-1301
 anfrage@phenomenex.com

Belgium
 t: +32 (0)2 503 4015 (French)
 t: +32 (0)2 511 8666 (Dutch)
 beinfo@phenomenex.com

Canada
 t: +1 (800) 543-3681
 info@phenomenex.com

China
 t: +86 400-606-8099
 cninfo@phenomenex.com

Denmark
 t: +45 4824 8048
 nordicinfo@phenomenex.com

Finland
 t: +358 (0)9 4789 0063
 nordicinfo@phenomenex.com

France
 t: +33 (0)1 30 09 21 10
 franceinfo@phenomenex.com

Germany
 t: +49 (0)6021-58830-0
 anfrage@phenomenex.com

India
 t: +91 (0)40-3012 2400
 indiainfo@phenomenex.com

Ireland
 t: +353 (0)1 247 5405
 eireinfo@phenomenex.com

Italy
 t: +39 051 6327511
 italiainfo@phenomenex.com

Luxembourg
 t: +31 (0)30-2418700
 nlinfo@phenomenex.com

Mexico
 t: 01-800-844-5226
 tecnicomx@phenomenex.com

The Netherlands
 t: +31 (0)30-2418700
 nlinfo@phenomenex.com

New Zealand
 t: +64 (0)9-4780951
 nzinfo@phenomenex.com

Norway
 t: +47 810 02 005
 nordicinfo@phenomenex.com

Portugal
 t: +351 221 450 488
 ptinfo@phenomenex.com

Singapore
 t: +65 800-852-3944
 sginfo@phenomenex.com

Spain
 t: +34 91-413-8613
 espinfo@phenomenex.com

Sweden
 t: +46 (0)8 611 6950
 nordicinfo@phenomenex.com

Switzerland
 t: +41 61 692 20 20
 swissinfo@phenomenex.com

United Kingdom
 t: +44 (0)1625-501367
 ukinfo@phenomenex.com

USA
 t: +1 (310) 212-0555
 info@phenomenex.com

All other countries
Corporate Office USA 
 t: +1 (310) 212-0555
 info@phenomenex.com

www.phenomenex.com

Phenomenex products are available worldwide. For the distributor in your country, contact Phenomenex USA, International Department at international@phenomenex.com



Terms and Conditions
 Subject to Phenomenex Standard Terms and Conditions, which may be viewed at <http://www.phenomenex.com/TermsAndConditions>.

Trademarks
 Kinetex is a registered trademark and SecurityGuard, Axia, and MidBore are trademarks of Phenomenex.
 Agilent is a trademark of Agilent Technologies, Inc. Sigma-Aldrich is a registered trademark of Sigma-Aldrich Co., LLC. Fisher Scientific is a trademark of Fisher Scientific International, Inc.

Disclaimer
 Comparative separations may not be representative of all applications. Phenomenex is not affiliated with Agilent, Sigma Aldrich, or Fisher Scientific.
 Kinetex EVO is patented by Phenomenex. U.S. Patent Nos. 7,563,367 and 8,658,038 and foreign counterparts.
 FOR RESEARCH USE ONLY. Not for use in clinical diagnostic procedures.
 © 2018 Phenomenex, Inc. All rights reserved.