APPLICATION

A Simple Approach to Method Development using Analyte LogD

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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 hike 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 compounds absolute solubility. This solubility distribution provides an indication of 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.

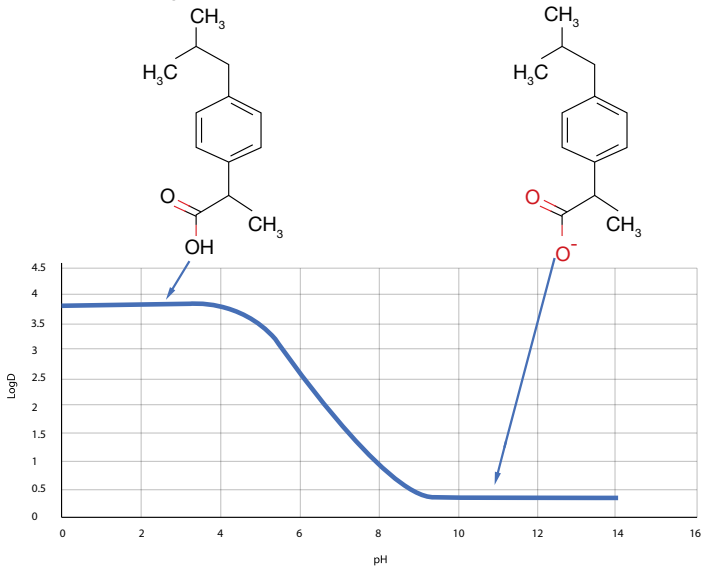
The distribution coefficient (D) is the ratio of the solute concentration of all species of a compound in a solution system of two immiscible phases, when at equilibrium. The ratio is taken in respect to the pH of the system and is logarithmically measured, typically in a biphase of n-octanol and water (**Figure 1**). LogD is taken in 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 p*K*a(s) of its ionizable groups, if applicable.

Figure 1.
The LogD expression

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

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



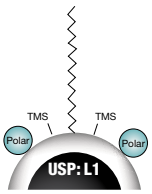
In terms of chromatography, LogD can provide useful information in terms of mobile phase pH selection and overall HPLC/UHPLC selectivity. Because the coefficients relate solubility and the interaction potential within an organic/aqueous solution, a correlation can be made to reversed phase HPLC/UHPLC.

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 p*K*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

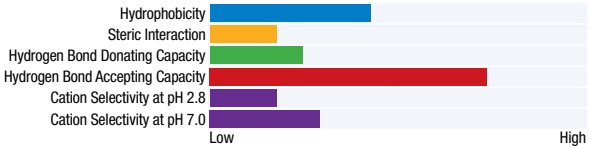
Ibuprofen standards were acquired from Sigma-Aldrich® (Saint Louis, MO). Buffers were prepared using potassium phosphate dibasic salt obtained from Fisher Scientific® (Hampton, NH) and adjusted to pH 2 and 8 with phosphoric acid and potassium hydroxide, respectively. The potassium buffer was prepared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

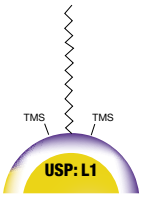
The column dimension selected was 50 x 2.1 mm with a particle size of 2.6 µm. The phases utilized were the Kinetex XB-C18, Kinetex EVO C18, and Kinetex Polar C18 and all tests were run on an Agilent® 1100 HPLC (Palo Alto, CA) with a flow rate of 2.0 mL/min. UV/Vis detection was used at 310 nm, no reference 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

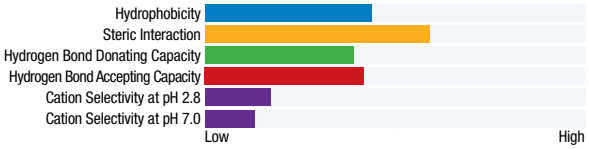
Combined C18 and polar modified surface that provide polar and non-polar retention alongside 100 % aqueous stability.

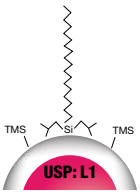




Kinetex EVO C18

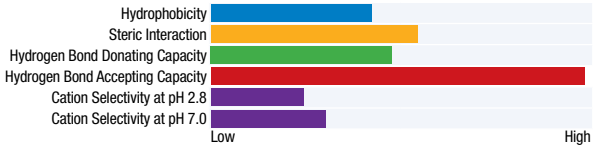
Novel pH 1-12 stable C18 that delivers robust methods and improved peak shape for bases.





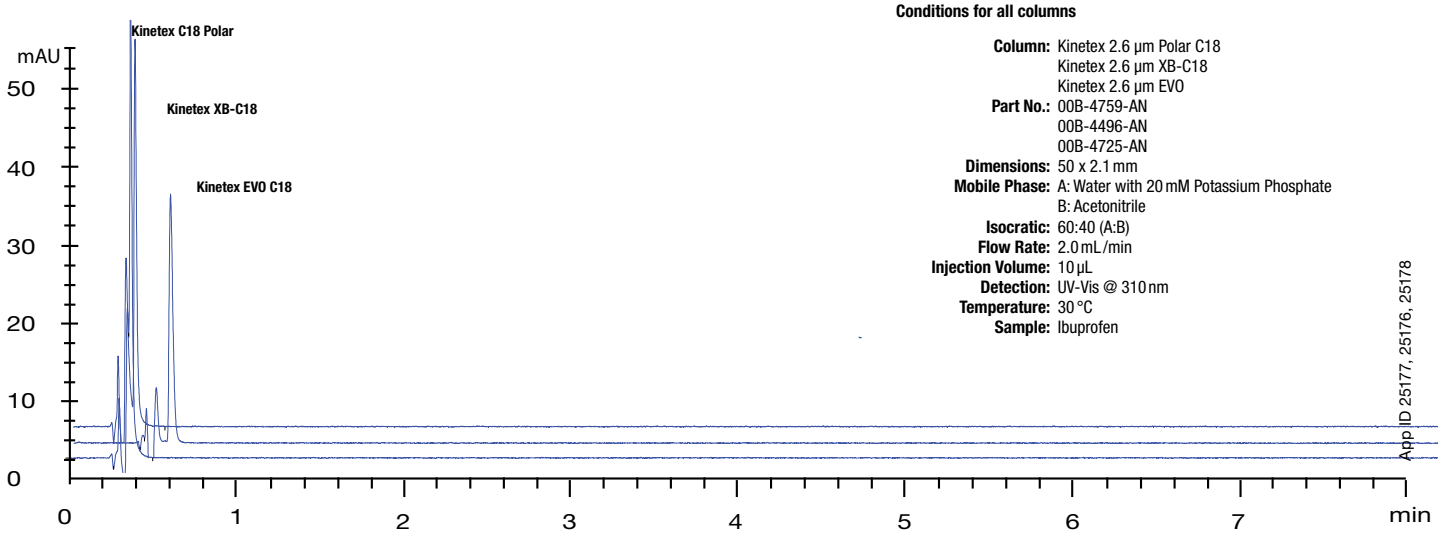
Kinetex XB-C18

Di-isobutyl side chains differentiate this C18 column. Low ligand density and an inactive surface make 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

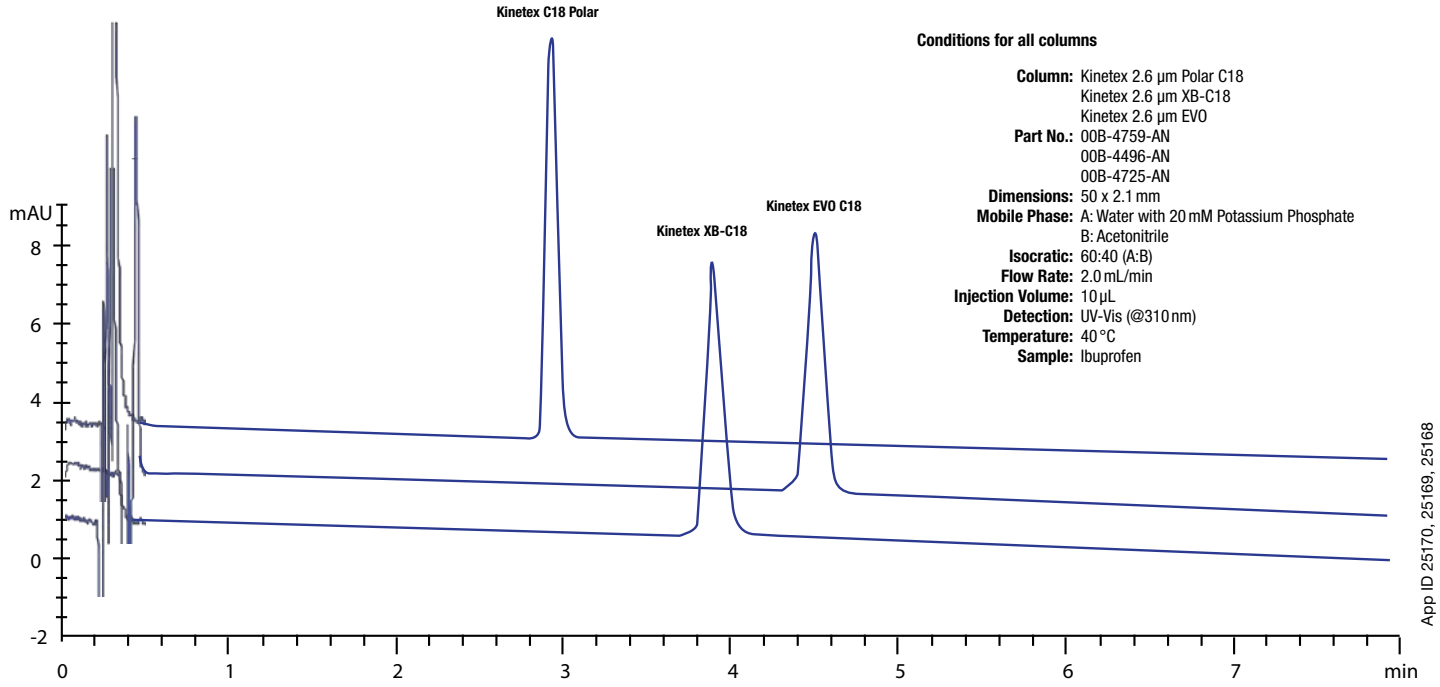


Conditions for all columns

Column: Kinetex 2.6 µm Polar C18
Kinetex 2.6 µm XB-C18
Kinetex 2.6 µm EVO
Part No.: 00B-4759-AN
00B-4496-AN
00B-4725-AN
Dimensions: 50 x 2.1 mm
Mobile Phase: A: Water with 20 mM Potassium Phosphate
B: Acetonitrile
Isocratic: 60:40 (A:B)
Flow Rate: 2.0 mL/min
Injection Volume: 10 µL
Detection: UV-Vis @ 310 nm
Temperature: 30 °C
Sample: Ibuprofen

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.



Kinetex Phase	Retention Time (min)	Efficiency	Asymmetry	Peak Height	Void Time (min)
XB-C18	3.88	8458	0.76	6.94	0.26
EVO C18	4.49	9814	0.91	6.62	0.37
Polar C18	2.93	9259	0.86	9.85	0.25

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 p*K*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 formed with a p*K*a value of 4.9. Under isocratic mobile phase conditions of 60:40 (Aqueous/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 µm 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

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