



Tecan AC Extraction Plate™ for sample preparation

Efficiency of extraction: the influence of pH

Introduction

LC/MS quantification of small molecule analytes by LC/MS in biological samples, such as serum, plasma, urine or blood, is generally only performed after a sample clean-up step. The reason for this is that specific matrix constituents like proteins and phospholipids might negatively affect the analysis, and in the worst case, clog the column, leading to high LC back pressure. The innovative Absorption Chemistry (AC) Extraction Plate Plate with TICE™ technology, featuring wells with a proprietary TICE coating, simplifies the sample preparation step, and requires only minimal liquid manipulation and no precipitation, filtration, centrifugation or solvent evaporation steps.

For efficient extraction from a biological sample matrix into the TICE coating, it is important that the molecule is in a neutral state.

Many of the small analytes of interest have functional groups that will either be protonated or deprotonated, depending on the pH value of the solution. Not surprisingly, for these molecules, the extraction efficiency depends on the pH of the medium.

In this technical note, we provide background information on the pH-related factors that influence the distribution coefficient (or partition ratio) of a molecule between the TICE coating and the aqueous medium. Furthermore, we explain how the optimal extraction pH can be determined experimentally, or be approximated by calculation.

Partition ratio

When a sample is pipetted into a well of the AC Extraction Plate, the analyte begins to diffuse across the contact interface. The diffusion across the interface is reversible and continues until the equilibrium has been reached, at which point the rate of diffusion is equal in both directions. This specific diffusion process is called partitioning. At the process equilibrium, the analyte is distributed between the aqueous sample and the AC coating in a precise ratio of concentration (C) in each phase known as the partition ratio, or distribution constant, K. The partition ratio may also be referred to as the ratio of the solubility (S) of the analyte in each phase.

$$K_{AC} = \frac{C_{AC}}{C_{water}} = \frac{S_{AC}}{S_{water}}$$

As the partition ratio is a large number it is often given as the common logarithm of K, or $\log K_{AC}$. $\log K_{AC}$ defines the tendency of the analyte in its non-ionized form to migrate from water to the phase, just as $\log P$ defines the attraction of an analyte to octanol relative to water.

Apparent partition ratio

Most drugs used in medicine act as weak acids or bases in aqueous solution, sometimes as both. These drugs are ionized depending on their pKas and the pH of the solution. In their ionized state, drugs are usually poorly soluble in the AC phase and therefore may not partition into the coating. Because the partitioning of the ionizable drug depends on the solution pH, $\log K_{AC}$ may not accurately reflect the distribution between the two phases. $\log D$ is then used to define the partitioning process. At a pH where the drug is not ionized, $\log D_{AC} = \log K_{AC}$ holds. When the drug is ionized, or partially ionized, $\log D_{AC}$ is smaller than $\log K_{AC}$.

During an extraction of ionizable drugs the partitioning is governed by two coupled processes. The first process is the neutral drug species diffusing between the aqueous phase and the AC phase, and its (undisturbed) equilibrium state is defined by $\log K_{AC}$ (neutral species only). The second process, the protonation/deprotonation of the analyte, takes place solely within the aqueous phase. The degree of ionization depends upon the pKa of the drug and the solution pH.

Therefore, for this case, $\log K_{AC}$ alone is not sufficient for describing the partitioning process. To account for the mixture of coexisting species, $\log D_{AC}$ includes all species of the drug whether ionized or not. For a weak basic drug this can be displayed schematically as shown in Figure 1.

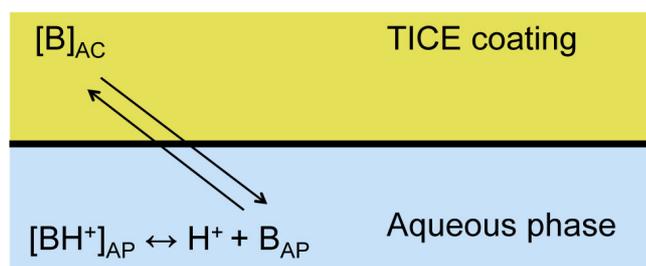


Figure 1 For a basic analyte, B, two simultaneous processes influence the partitioning ratio: protonation in the aqueous phase (horizontal) and diffusion of the neutral molecule into the TICE coating phase (vertical).

From Figure 1 it follows that $\log D_{AC}$ can be defined as:

$$\log D_{AC} = \log \left(\frac{[\text{Neutral solute}]_{AC}}{[\text{Neutral solute}]_{water} + [\text{Ionized solute}]_{water}} \right)$$

Optimization of the extraction pH

An extraction with the AC Extraction Plate can be optimized by finding a solution pH that minimizes or prevents ionization of the analyte. At this pH, $\log D_{AC} \approx \log K_{AC}$ and the extraction efficiency will be highest. The optimum extraction pH can be determined in two different ways, either experimentally or via calculation. Both methods are described below.

Determining $\log D_{AC}$ vs. pH value experimentally

The most accurate method for determining the optimal pH for an extraction is via an experimental procedure, which is more time consuming than the calculation method. $\log D_{AC}$ is determined using the Direct Extraction-Elution Method (DEEM) approach at various pH values. Plotting $\log D_{AC}$ vs. pH allows the identification of the pH value at which $\log D_{AC}$ is highest. This is the pH at which the extraction efficiency for that particular analyte will be highest. For more information about designing and performing a DEEM experiment, see reference [1]. Figure 2 shows a DEEM plot for phenylbutazone.

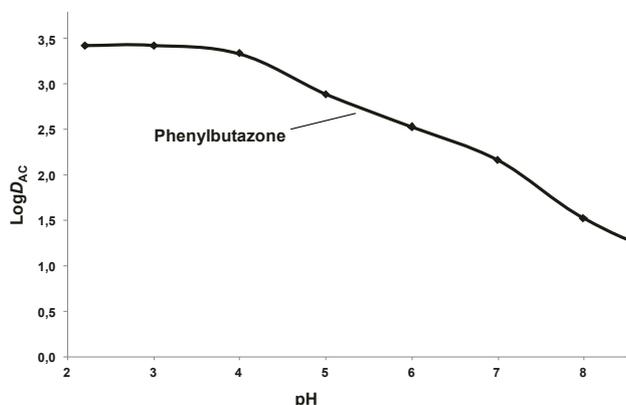


Figure 2 Phenylbutazone was extracted with Tecan's AC Extraction Plate at different pH values, and $\text{Log}D_{AC}$ was plotted against pH.

From Figure 2 it can be derived that a pH value ≤ 4 should give an excellent extraction.

Determining $\text{Log}D_{AC}$ vs. pH value by calculation

For a given compound, $\text{Log}D_{AC}$ is related to $\text{Log}K_{AC}$ and pKa by the following equations:

$$\text{Log}D_{AC} = \text{Log}K_{AC} - \text{Log} [1 + 10^{(\text{pH} - \text{p}K_a)}] \text{ for an acid}$$

$$\text{Log}D_{AC} = \text{Log}K_{AC} - \text{Log} [1 + 10^{(\text{p}K_a - \text{pH})}] \text{ for a base}$$

pKa is determined experimentally, and may be different depending on the type of organic solvent present in the aqueous solution and the exact ratio of water vs. organic solvent. Plotting $\text{Log}D_{AC}$ vs. pH allows for the determination of a pH range where $\text{Log}D_{AC}$ is equal to $\text{Log}K_{AC}$. An example for phenylbutazone at three literature values of pKa is shown in Figure 3.

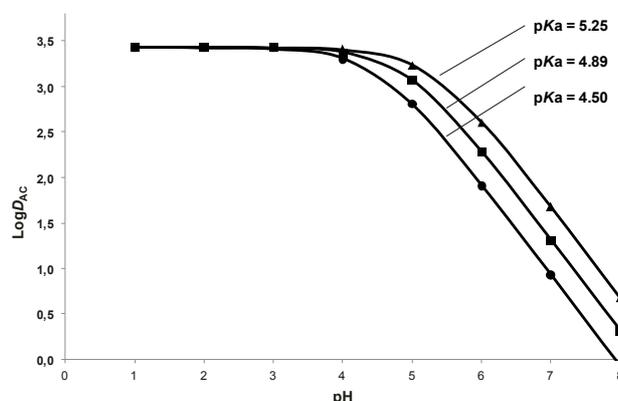


Figure 3 Calculated $\text{Log}D_{AC}$ values for phenylbutazone plotted against three different pH values.

From Figure 3 it can be derived that maintaining a pH value ≤ 4 should give the best extraction, as $\text{Log}D_{AC} \approx \text{Log}K_{AC}$.

Estimating $\text{Log}D_{AC}$ by using $\text{Log}D_P$ calculators

Tools are available to assist in estimating $\text{Log}P$ and $\text{Log}D$, including online calculators. An example of an online calculator can be found on the website mentioned in reference [2].

The calculators are 'estimators' and may produce results slightly different from experimental values. Regardless, it has been found that the $\text{Log}D_P$ plots obtained can be useful in estimating the best pH conditions for an extraction. Figure 4 is a plot obtained using the online calculator by ChemAxon [2].

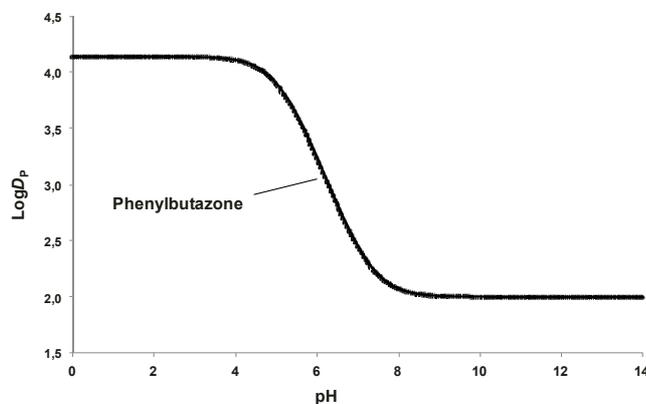


Figure 4 Calculated $\text{Log}D_P$ values for phenylbutazone plotted against pH values. $\text{Log}D_P$ values were calculated by using an online tool [2].

Conclusion

As shown in this technical note, the efficiency of the TICE coating for ionizable compounds varies with the pH value of the solution. There are two different approaches for finding the optimum pH for sample clean-up with the AC Extraction Plate, the analyst can use two different approaches. The first option is an experimental procedure based on the DEEM approach, which delivers the most accurate values. Alternatively, the optimum pH value can also be estimated by calculation, employing an online calculator.

References

1. White paper: Technical background to the Tecan AC Extraction Plate™.
<http://www.tecan.com/consumables>
2. ChemAxon online tool for estimating LogD_P values.
<http://www.chemicalize.org/>

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