cyprotex AN EVOTEC COMPANY

*In vitro* **ADME & PK**

# $pK_a$ and log P

## Background Information

'$pK_a$ affects solubility, permeability, log D and oral absorption by modulating the distribution of neutral and charged species.'

[1]Di L and Kerns EH. (2003) *Current Opinion in Chemical Biology* **7**; 402-408.

- The $pK_a$ is the pH at which the molecule is 50% protonated.
- Log P (or partition co-efficient) is a measure of the lipophilicity of a compound.
- Cyprotex's $pK_a$ and log P determination uses UV-metric and pH-metric technology developed by Sirius, which is considered to be a 'gold standard' method for determining these properties.
- In UV-metric methods, the $pK_a$ is measured by analysing changes in multi-wavelength UV spectra during acid-based titration of the sample. UV-metric $pK_a$ methods work for compounds with pH-sensitive chromophores.
- In pH-metric methods, $pK_a$ is measured by titrating a solution of the sample in water or solvent with acid and base, and calculating the $pK_a$ from the shape of the titration. pH-metric methods work for any ionisable compound, but require more sample than UV-metric methods.
- The pH-metric method is also used to measure log P in a two-phase acid-base titration in the presence of octanol.

## Protocol

**Method**
Fast UV titration for $pK_a$
UV-metric titration for $pK_a$
Potentiometric (pH-metric) titration for $pK_a$ and log P

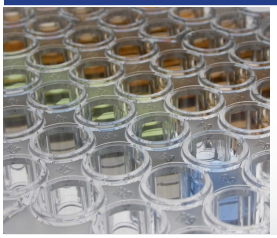
**Instrument**
SiriusT3

**Test Article Requirements**
3-5 µL of 10 mM stock solution (UV-metric)
1 mg solid compound (pH-metric)

**Partition Solvent used for Log P Determination**
n-Octanol (others available on request)

**Data Delivery**
$pK_a$
log P (optional)
Standard error
RMSD
Calculated log D at $pH_{7.4}$
(based on $pK_a$ and log P)

**log P is determined** from the shape of titration curves obtained in dual-phase titrations.

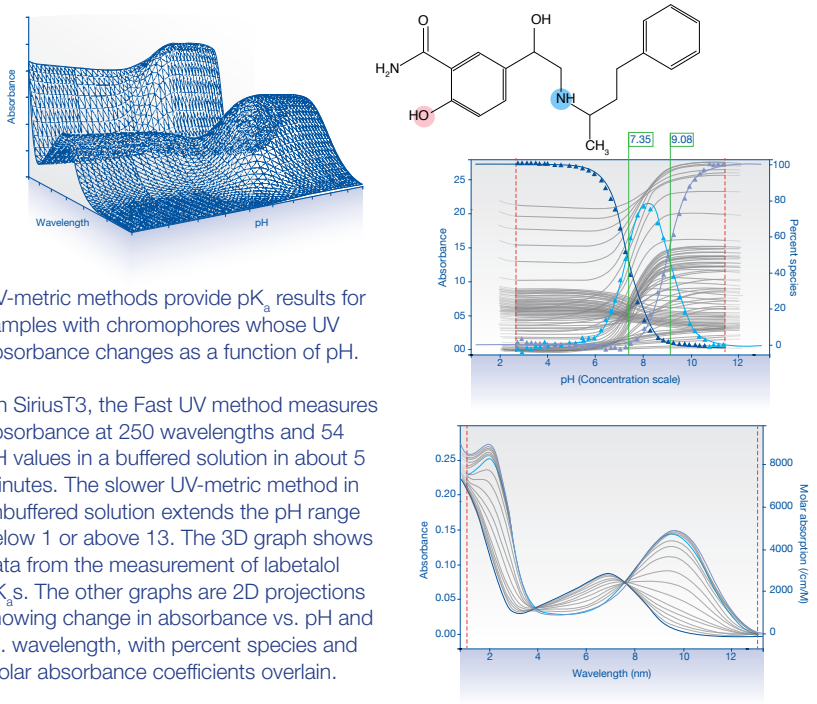


## $pK_a$ and log P

$pK_a$ measurements are determined using the SiriusT3 instrument from Sirius-Analytical using either a UV-metric or pH-metric approach.

### Figure 1

UV-metric method for measuring $pK_a$ values.

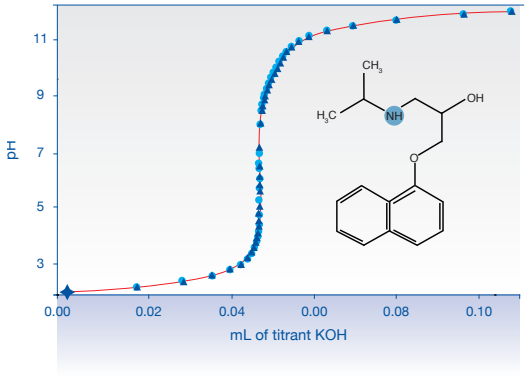



UV-metric methods provide $pK_a$ results for samples with chromophores whose UV absorbance changes as a function of pH.

On SiriusT3, the Fast UV method measures absorbance at 250 wavelengths and 54 pH values in a buffered solution in about 5 minutes. The slower UV-metric method in unbuffered solution extends the pH range below 1 or above 13. The 3D graph shows data from the measurement of labetalol $pK_a$s. The other graphs are 2D projections showing change in absorbance vs. pH and vs. wavelength, with percent species and molar absorbance coefficients overlain.

### Figure 2

pH-metric method for measuring $pK_a$ values.

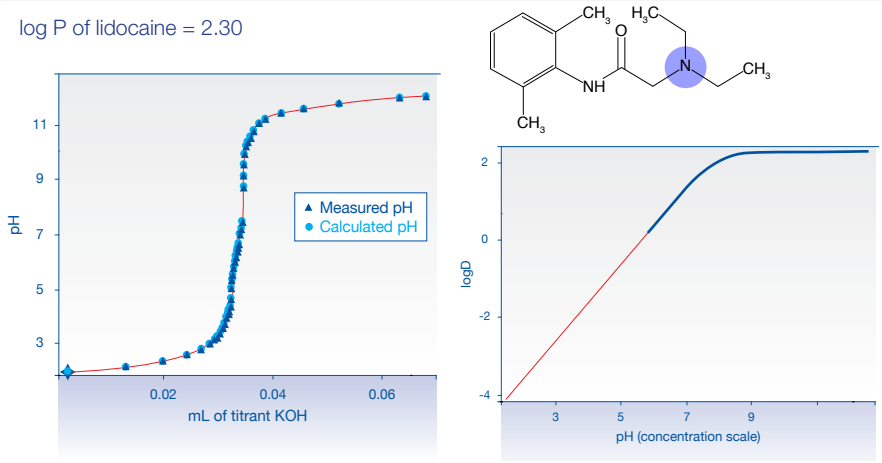



pH-metric methods are based on potentiometric acid-base titration. Results are obtained by a complex computational process. The pH of each point in the titration curve is calculated using equations that contain $pK_a$, and the calculated points are fitted to the measured curve by manipulating the $pK_a$ value. The $pK_a$ that provides the best fit is taken to be the measured $pK_a$. pH-metric methods will measure all $pK_a$s between 2 and 12, provided the sample is in solution throughout the experiment.

### Figure 3

pH-metric method for measuring log P values

log P of lidocaine = 2.30




In the pH-metric method for log P, a weighed sample is dissolved in a two-phase water-octanol system, and titrated over a pH range (typically 2 to 12 for bases and ampholytes, 12 to 2 for acids). Although the solution becomes opaque during stirring, the pH electrode continues to measure pH of the aqueous component of the solution. Results are obtained by a complex computational process. The pH of each point in the titration curve is calculated using equations that contain $pK_a$ and P, and the calculated points are fitted to the measured curve by manipulating the P value. The P that provides the best fit is taken to be the measured P value, which is reported as the logarithm, i.e. log P. As well as log P, the log D value as a function of pH is determined from the data.


**References**

[1] Di L and Kerns EH. (2003) *Current Opinion in Chemical Biology* **7**; 402-408.