Background Information

- 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.

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.

### pKₐ and log P

pKₐ 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 pKₐ values.

UV-metric methods provide pKₐ 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 pKₐs. 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 pKₐ 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 pKₐ, and the calculated points are fitted to the measured curve by manipulating the pKₐ value. The pKₐ that provides the best fit is taken to be the measured pKₐ. pH-metric methods will measure all pKₐs 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 pKₐ 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