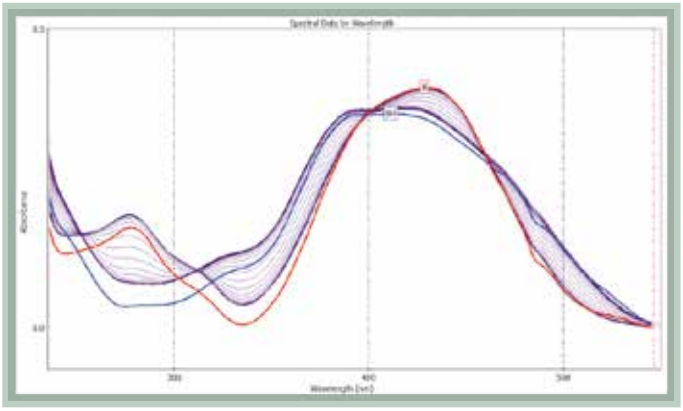
Application note 02/11

pK_a measurements in 15 minutes – the PionT3 Fast UV pK_a method

Understanding the ionisation properties of a molecule is an essential requirement for drug development. PionT3 technology provides the capability to perform pK_a measurements in 15 minutes using only 5 µL of a 10 mM stock solution. This method requires that the molecule exhibits discernable UV absorption changes on ionisation and possesses an intrinsic aqueous solubility greater than 30 µM. Compounds having a lower aqueous solubility can be accommodated using a cosolvent assay.



Phenazopyridine Spectra

The PionT3 Fast UV pK_a method has been validated against results reported in the literature and produces results that are of comparable quality to those obtained by traditional methods. Measurement repeatability is typically within 0.01 pK_a units. A complete summary of the data collected in this study is available on request.

Experimental

The PionT3 Fast UV pK_a method is based on the spectrophotometric (UV-metric) titration method reported elsewhere[1]. In essence, a dilute sample solution is titrated with acid or base and UV/vis spectra are collected. The spectra obtained for phenazopyridine are shown in Figure 1. Provided that the UV spectra of the neutral and ionized species are different, the pK_a of the sample can be calculated from the pH readings and UV spectra collected. This method is not suitable for compounds which show no UV change.

Titration speed is limited by the need to wait for the pH to stabilize after each titrant addition before a data point can be collected. In a traditional UV-metric titration, a simple buffer such as phosphate or TRIS may be used to reduce the stabilization time, but in these cases a typical titration will take up to 45 minutes to complete.

The PionT3 Fast UV pKa method uses a proprietary linear buffer system adapted from the literature[2] to achieve much more rapid equilibration. A titration covering the pH range 2 - 12 can be completed in 5 minutes. Fast UV pK_a assays consist of three titrations of the same sample aliquot, allowing confirmation of the pK_a result.

The method was validated using a test set of 20 compounds, incorporating 15 compounds which display strong spectroscopic changes (warfarin, hydrochlorothiazide, chlorzoxazone, amiloride, furosemide, diazepam, flumequin, benzocaine, quinine, papverine, labetalol, terbutaline, phenazopyridine, bendroflumethiazide and tolmetin) and 5 compounds which undergo weaker spectroscopic changes (naproxen, ketoprofen, nortryptiline, promethazine and fluoxetine). Samples were prepared as 10 mM stock solutions in DMSO and a fixed aliquot size of 5 µL was used. A standard assay template provided in the PionT3 software was used for all experiments.

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"PionT3 technology provides the capability to perform rapid pK_a measurements in 15 minutes using only 5 μL of a 10 mM stock solution."

Results

A comparison of the results obtained using the Fast UV pK_a method with the literature[1,3,4,5,6,7] is shown in Figure 2. The results showed excellent agreement with the literature, as demonstrated by the correlation coefficient (R^2) of >0.99 and the absence of any outliers. Reproducibility was very good; standard deviations over 10 assays of 0.01 or less were obtained for 14 of the compounds. In all cases, including the compounds which displayed only weak UV changes, the standard deviations were no higher than 0.04.

High resolution data of approximately 50 data points per titration were collected. For the strongly UV-absorbing compounds, the data quality was comparable to the results obtained using the traditional method. With PionT3 data processing, compounds which exhibit relatively minor spectral differences between the ionised and neutral species can also yield excellent data.

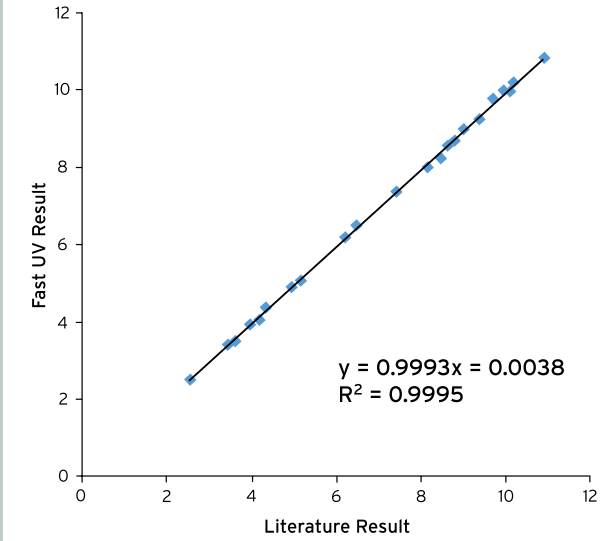

Figure 2. Literature vs Measured pK_a

Conclusion

The results obtained from the Fast UV pK_a method are of comparable quality to those of traditional titration techniques, showing excellent agreement with the literature (R^2 = >0.99) and a high level of repeatability. It can be successfully applied even to compounds which display weak spectroscopic changes on ionisation.

The Fast UV pK_a method can provide data where other methods may fail, for example for very insoluble compounds which can be maintained in a supersaturated state during the titration or for unstable compounds which can be analysed prior to decomposition.

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