

## Application Note:

# Monitoring Reverse Phase LC of Acetonitrile/Water in Pharma Applications

**Purpose:** To provide continuous monitoring of acetonitrile (ACN):water mobile phase gradients utilized in prep scale reverse phase chromatographic separation of active pharmaceutical ingredients with a ClearView db fiber optic filter photometer.

**Experimental:** A spectrophotometer was used to measure the NIR spectra of ACN:water mixtures to select wavelengths for our process photometer. Two meters of low-OH fiber optic cables were used and the samples were measured in a 10 mm quartz cuvette at 40 and 50°C.

**Results:** The resulting spectra are shown in Figure 1 for 10, 20, 30, 40 and 50 wt. % water in ACN. The main water peak near 1420 nm is off-scale for the 10 mm optical path selected. The alternative would be to use a 1 or 2 mm optical path to keep this peak on scale. The slightly different curves at each concentration reflect the effect of sample temperature on the water spectrum.

Two wavelengths were selected for calibration. The calibration model also includes a coefficient for sample temperature. The predicted vs. actual plot (Figure 2) shows an excellent calibration with an  $R^2$  of 0.9997 and a standard error of 0.3% water. The temperature coefficient indicates a correction of 0.15% water/°C. The ClearView db's long-term drift of <500  $\mu$ AU thus gives excellent precision.

**Discussion:** Our low cost ClearView db photometer can readily make this measurement using direct insertion fiber optic probes or flow cells for bypass streams. Our ClearView db photometer can measure two independent fiber optic probes at the same time decreasing the overall cost per sample point.

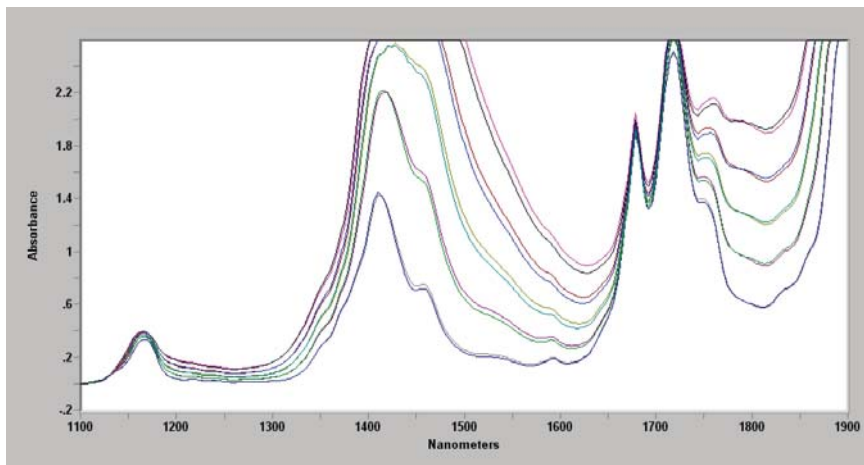


Figure 1

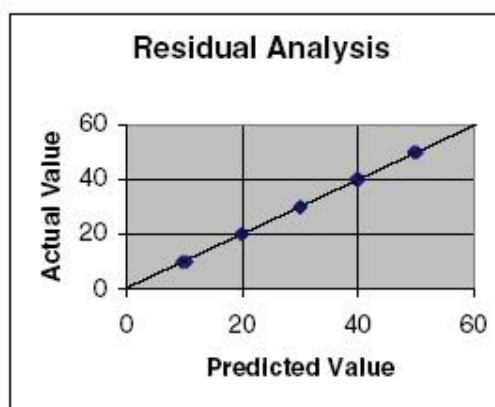


Figure 2

### ClearView® db Enclosure Options



Zpurge Unit  
Class I, Division 2

ExProof Unit  
Class I, Division 1

General  
Purpose Unit