can also be obtained when solubility is determined, versus pH. There are, however, some important limitations to this method. Compounds must remain soluble, especially standards, over the duration of the assay, in whatever solvent system is used. Certain compounds, if not soluble in 20% aqueous acetonitrile (ACN), may produce visible precipitate and cloudiness, which can interfere with the UV spectroscopic analysis. If the precipitate is found, other analytical methods, such as HPLC and LC/MS/MS, may be used. Some compounds, on visual inspection, may contain color-producing chromophores, so the spectral range should be increased beyond 500 nm. The fact that the sample is made up in a 5% (v/v) DMSO solution may result in an overestimation of the compound's solubility in a purely aqueous solution. Lowering the amount of DMSO (e.g., 0.5%) may improve the correlation between the aqueous solubility method and the shake-flask method. If the compound is less than 95% pure, the UV spectroscopy method may not be suitable. A complex mixture would require some sort of chromatographic separation prior to analysis. It is essential that the compounds being investigated have sufficient UV spectroscopic molar absorptivities (extinction coefficients) to provide the requisite analytical sensitivity.

With these limitations in mind, the assay is still well suited as a high-throughput tool for a number of compound-screening applications, including the determination of structure–solubility relationships and establishing appropriate dosing concentration ranges for subsequent in vitro testing programs.

This method also allows the use of multiple-wavelength measurement and estimation of solubility. By analyzing the compound using spectroscopy at six wavelengths, the relative solubility, in the form of a screening ratio, can be calculated using the ratio of the pre- and postfiltered test samples. The calculated screening ratio provides a fast method for identifying compounds that are highly, moderately, or marginally soluble in aqueous solutions. As the screening ratio approaches unity, the sample approaches the upper limit of solubility—500 μM—as measured by the assay. If the screening ratio has a value less than 1 but greater than 0.5, the solubility of the compound is known to be between 100 and 500 μM. A screening ratio of less than 0.5 indicates that the compound's solubility is likely to be less than 100 μM.

$$\text{If: } \frac{(\sum \text{AU at 280, 300, 320, 340, 360 nm}) - (\text{AU at 800 nm})\,\text{Filtrate}}{(\sum \text{AU at 280, 300, 320, 340, 360 nm}) - (\text{AU at 800 nm})\,\text{Standard}} \approx 1.00 \tag{4.66}$$

$$\text{If: } \frac{(\sum \text{AU at 280, 300, 320, 340, 360 nm}) - (\text{AU at 800 nm})\,\text{Filtrate}}{(\sum \text{AU at 280, 300, 320, 340, 360 nm}) - (\text{AU at 800 nm})\,\text{Standard}} \leq 0.5 \tag{4.67}$$

Then: Aqueous solubility ≤ 100 μM

$$\text{If: } \frac{(\sum \text{AU at 280, 300, 320, 340, 360 nm}) - (\text{AU at 800 nm})\,\text{Filtrate}}{(\sum \text{AU at 280, 300, 320, 340, 360 nm}) - (\text{AU at 800 nm})\,\text{Standard}} < 1.00 \text{ and } > 0.5$$

Then: 100 μM < Aqueous solubility ≤ 500 μM

(4.68)