The ultraviolet-visible (UV-Vis) spectral analysis is one of the first steps in phototoxicity assessments to determine the absorption of test compounds. Absorption within the range of UV and visible light (200-700 nm) is considered significant, as defined by a molecular extinction coefficient (MEC) >1000 M cm⁻¹, triggering potential phototoxicity considerations. Determination of the absorption spectra and MEC, when possible, is described in OECD Test Guideline (TG) 101: UV-Vis Absorption Spectroscopy. Adopted in 1983, TG 101 provides guidance on evaluation, with defined molecular weight that is soluble in water or methanol, and analysis of absorbance with a spectrophotometer.

The growing use of botanicals, extracts, and complex mixtures without defined molecular weights in various industries calls for testing, depending upon the methodologies described in OECD TG 101. With the limited solvents described in TG 101 (water and methanol), novel chemistries need evaluation, and solubility as a critical component of the assay, additional solvents were investigated for use. Failure to achieve full solubility can produce interference with the absorbance readings (i.e., fiber effects or diminished absorbance values). Further, challenges may arise when selecting appropriate concentrations for complex mixtures to produce a reliable spectra. Guidance on selecting significant absorption using an absorbance threshold was presented in Nahidb, et al. (2015). Here we present approaches taken to adopt the OECD TG 101 UV-Vis assay.

Experimental Design

Test compounds were prepared at multiple concentrations in three different solvent buffer systems (acidic, basic and neutral) (Figure 1), and then absorbance (Optical Density (OD)) determined at wavelengths of 230 to 800 nm in 2 cm increments using a Tecan® Infinite M Nano (Figure 2). Spectral scans and OD values of selected peaks were analyzed using Magellan® Reader Software, and Molecular Extinction Coefficient (MEC) values were calculated using peak absorbance and molality. When MEC could not be determined, peak absorbance values and associated wavelength were presented.

Evaluation of Results

Compounds with MEC values >1000 M cm⁻¹ are considered to have significant light absorption. For compounds without defined molecular weights, an alternative evaluation using an absorption threshold of OD 1.0, as described in Nahidb, et al. (2015), was incorporated.

Evaluation of Solvents Using Chlorpromazine

Chlorpromazine was prepared at concentrations of 0.001 and 0.005 M in methanol, water, hexane, acetone, acetone, DMSO, HEOS, ethanol, 30% methanol in water, and 30% acetone in water (all buffers pH 7.0 ± 1.0). Spectral analysis of selected absorbances are presented in Figure 4, with MEC values for all solvents in Table 1. Determination of Weight/Volume Concentrations using p-Methoxycinnamaldehyde

Two fragrance compounds, p-methoxycinnamaldehyde and Acetovanillone, were prepared at several concentrations to estimate an appropriate mg/mL concentration for evaluation using the alternate evaluation threshold. The compounds were dissolved at a noted solvent (DMSO), 0.001, 0.005, and 0.010 mg/mL in water. Absorbance within the limit of the plate reader (e.g. OD 400) was analyzed (Figure 6) to determine concentrations where a “fiber” effect (e.g., producing flat lined responses with higher OD values with expected drift in absorbance concentrations as concentrations occurred) occurred. Absorbance at 290 nm and 306 nm was determined for each concentration (Table 3).

Conclusion & Future Directions

The UV-Vis assay is a crucial first step for screening of test compounds prior to evaluation in more complex and costly test systems. As the industry formulations change over time, and utilizing more complex and novel test compounds, an evolving approach using the UV-Vis assay is needed. We have further adapted the initial guidance to address through materials, of limited solubility, and evaluation of complex compounds.

Solubility is a critical component of this assay, as well as the solvent. The solubility of the test compound may impact the absorbance, as well as MEC, and ultimately the determination if additional phototoxicity testing is needed.

The work by Nahidb, et al. (2015) provide an alternate approach using an absorption threshold to investigate complex mixtures and substances without defined molecular weights. A larger subset of materials covering a wider variety of the industry sector may further elucidate the target concentrations that may be needed.

REFERENCES


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Table 3. Molar extinction coefficients for the compounds listed above; the compounds were dissolved at 0.001 M in water for all solvents except for Acetovanillone, which was dissolved at 0.005 M in water.