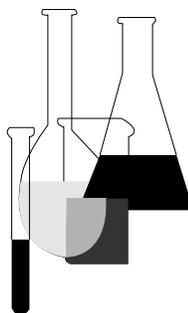




Product Properties Test Guidelines

OPPTS 830.7050

UV/Visible Absorption



INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

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OPPTS 830.7050 UV/Visible absorption.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source materials used in developing this harmonized OPPTS test guideline are the OPPT guideline under 40 CFR 796.1050 Absorption in aqueous solution: Ultraviolet/visible spectra and OECD guideline 101 UV-VIS Absorption Spectra.

(b) **Introductory information**—(1) **Guidance information.** (i) Molecular formula.

(ii) Structural formula.

(2) **Standard documents.** The spectrophotometric method is based on national standards and consensus methods which are applied to measure the absorption spectra.

(c) **Method**—(1)(i) **Introduction, purpose, scope, relevance, application and limits of test.** (A) The primary environmental purpose in determining the ultraviolet/visible (UV/VIS) absorption spectrum of a chemical compound is to have some indication of the wavelengths at which the compounds may be susceptible to photochemical degradation. Since photochemical degradation is likely to occur in both the atmosphere and the aquatic environment, spectra appropriate to these media will be informative concerning the need for further persistence testing.

(B) Degradation will depend upon the total energy absorbed in specific wavelength regions. Such energy absorption is characterized by both molar absorption coefficient (molar extinction coefficient) and band width. However, the absence of measurable absorption does not preclude the possibility of photodegradation.

(ii) **Definitions and units.** The *UV-VIS absorption spectrum* of a solution is a function of the concentration, c_i , expressed in moles per liter (mol/L), of all absorbing species present; the path length, d , of the spectrophotometer cell, expressed in centimeters; and the molar extinction coefficient, ϵ_i , of each species. The absorbance, A , of the solution is then given by:

$$A = d \sum_i \epsilon_i c_i$$

For a resolvable absorbance peak, the band width λ is the wavelength range, expressed in nanometers (nm = 10^{-9} m), of the peak at half the absorbance maximum.

(iii) **Reference substances.** (A) The reference substances need not be employed in all cases when investigating a new substance. They are provided primarily so that calibration of the method may be performed from time to time and to offer the chance to compare the results when another method is applied.

(B) Reference compounds appropriate for the calibration of the system are:

(1) Potassium dichromate (in 0.005 mol/L, H₂SO₄ solution) from J.A.A. Ketelaar, under paragraph (e)(2) of this guideline:

log ε	3.56	3.63	3.16	3.50
λ in nm	235	257	313	350

(2) Fluoranthene (in methanol) from *C.R.C. Atlas of Spectral Data*, under paragraph (e)(3) of this guideline:

log ε	4.75	4.18	4.73	3.91	3.92
λ in nm	237	236	288	339	357

(3) 4-nitrophenol (in methanol) from *C.R.C. Atlas of Spectral Data*, under paragraph (e)(3) of this guideline:

log ε	3.88	4.04
λ in nm	288	311

Refer to paragraph (e)(1) of this guideline for more information.

(iv) **Principle of the test method.** This method utilizes a double-beam spectrophotometer which records only the absorption differences between the blank and test solutions to give the spectrum of the chemical being tested.

(v) **Quality criteria—reproducibility and sensitivity.** (A) Reproducibility and sensitivity, need not be measured directly. Instead, the accuracy of the system in measuring the spectra of reference compounds will be defined so as to assure appropriate reproducibility and sensitivity. It is preferable to use a recording double-beam spectrophotometer to obtain the UV/vis spectrum of the test compound. Such an instrument should have a photometric accuracy of ±0.02 units over the absorbance range of 0 to 2 units. It should be capable of recording absorbances at wavelengths of 200 to 750 nm with a wavelength accuracy of ±0.5 nm. The cells em-

ployed with the instrument must necessarily be transparent over this wavelength range and must have a path length determined to within 1 percent. To ensure that the instrument is performing satisfactorily, spectra for test solutions of $K_2Cr_2O_7$ (for absorbance accuracy) and holmium glass (for wavelength accuracy) should be run periodically.

(B) In the event that a recording double-beam instrument is not available, it will be necessary to determine the absorbance of the test solution in a single-beam instrument at 5-nm intervals over the entire wavelength range and at 1-nm intervals where there are indicated absorbance maxima. Wavelength and absorbance tests should be done as with the double-beam instrument.

(2) Description of the test procedure—(i) Preparation of test solutions. (A) Solutions should be prepared by accurately weighing an appropriate amount of the purest form of the test substance available. This should be made up in a concentration which will result in at least one absorbance maximum in the range 0.5 to 1.5 units.

(B) The absorption of a compound is due to its particular chemical form. It is often the case that different forms are present, depending on whether the medium is acidic, basic, or neutral. Consequently, spectra under all three conditions are required where solubility and concentration allow. Where it is not possible to obtain sufficient concentrations in any of the aqueous media, a suitable organic solvent should be used (methanol preferred).

(C) The acid medium should have a pH of less than 2, and the basic medium should be at least pH 10. The solvent for the neutral solution, and for preparing the acidic and basic ones, should be distilled water, transparent to UV radiation down to 200 nm. If methanol must be used, acidic and basic solutions can be prepared by adding 10 percent by volume of HCl or NaOH in aqueous solution ($[HCl], [NaOH] = 1 \text{ mol/L}$).

(D) In theory, all chemical species other than that being tested are present in both beams and would therefore not appear in the recorded spectrum of a double-beam instrument. In practice, because the solvent is usually present in great excess, there is a threshold value of wavelength below which it is not possible to record the spectrum of the test chemical. Such a wavelength will be a property of the solvent or of the test medium. In general, distilled water is useful from 200 nm (dissolved ions will often increase this), methanol from 210 nm, hexane from 210 nm, acetonitrile from 215 nm, and dichloromethane from 235 nm.

(ii) **Blank solutions.** A blank must be prepared containing the solvent and all chemical species other than the test chemical. The absorption spectrum of this solution should be recorded in a manner identical to that of the test solution and preferably on the same chart. This “baseline” spec-

trum should never record an absorbance reading varying more than ± 0.05 from the nominal zero value.

(iii) **Cells.** Cell pathlengths are usually between 0.1 cm and 10 cm. Cell lengths should be selected to permit recording of at least one maximum in the absorbance range of 0.5 to 1.5 units. Which set of cells should be used will be governed by the concentration and the absorbance of the test solution as indicated by the Beer-Lambert law. The cells should be transparent over the range of the spectrum being recorded, and the pathlengths should be known to an accuracy of at least 1 per cent. Cells should be thoroughly cleaned in an appropriate manner (chromic acid is useful for quartz cells) and rinsed several times with the test or blank solutions.

(iv) **Performance of the test.** Both cells to be employed should be rinsed with the blank solution before filling. The instrument should be set to scan at a rate appropriate for the required wavelength resolution and the spectrum of the blank recorded. The sample cell should then be rinsed and filled with the test solution and the scanning repeated, preferably on the same chart, to display the baseline. The test should be carried out at 25 °C.

(d) **Data and reporting—(1) Treatment of results.** (i) The molar absorption coefficient, ϵ , should be calculated for all absorbance maxima of the test substance. The formula for this calculation is:

$$\epsilon = A/c_i \times d$$

where the quantities are as defined at paragraph (d)(1)(ii) of this guideline.

(ii) For each peak which is capable of being resolved, either as recorded or by extrapolated symmetrical peaks, the bandwidth should be recorded.

(2) **Test report.** (i) The report should contain a copy of each of the three spectra (three pH conditions). If neither water nor methanol solutions are feasible, there will be only one spectrum. Spectra should include a readable wavelength scale. Each spectrum should be clearly marked with the test conditions.

(ii) For each maximum in each spectrum, the ϵ value and bandwidth (when applicable) should be calculated and reported, along with the wavelength of the maximum. This should be presented in tabular form.

(iii) The various test conditions should be included, such as scan speed, the name and model of the spectrophotometer, the slit width (where available), cell type and path length, the concentrations of the test substance, and the nature and acidity of the solvent medium. A recent test spectrum on appropriate reference materials for photometric and wavelength accuracy should also be submitted (see paragraph (d)(2)(i) of this guideline).

(e) **References.** The following references should be consulted for additional background material on this test guideline.

(1) Milazzo, G. et al., *Analytical Chemistry*, 149:711 (1977).

(2) Katelaar, J.A.A., *Photoelectric Spectrometry Group Bulletin 8* (Cambridge, 1955).

(3) Chemical Rubber Company, *Atlas of Spectral Data* (Cliffland, Ohio).

(4) *Organization for Economic Cooperation and Development*, Guidelines for The Testing of Chemicals, OECD 101, UV-VIS Absorption Spectra, OECD, Paris, France.