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## Appendix II B. Ultraviolet and Visible Absorption Spectrophotometry

(Ph. Eur. method 2.2.25)

### Determination of absorbance

The absorbance ( $A$ ) of a solution is defined as the logarithm to base 10 of the reciprocal of the transmittance ( $T$ ) for monochromatic radiation:

$$A = \log_{10} \left( \frac{1}{T} \right) = \log_{10} \left( \frac{I_0}{I} \right)$$

$$T = I/I_0;$$

$$I_0 = \text{intensity of incident monochromatic radiation;}$$

$$I = \text{intensity of transmitted monochromatic radiation.}$$

In the absence of other physico-chemical factors, the absorbance ( $A$ ) is proportional to the path length ( $b$ ) through which the radiation passes and to the concentration ( $c$ ) of the substance in solution in accordance with the equation:

$$A = \epsilon cb$$

$$\epsilon = \text{molar absorptivity, if } b \text{ is expressed in centimetres and } c \text{ in moles per litre.}$$

The expression  $A_{1 \text{ cm}}^{1 \text{ per cent}}$  representing the specific absorbance of a dissolved substance refers to the absorbance of a 10 g/L solution in a 1 cm cell and measured at a defined wavelength so that:

$$A_{1 \text{ cm}}^{1 \text{ per cent}} = \frac{10\epsilon}{M_r}$$

Unless otherwise prescribed, measure the absorbance at the prescribed wavelength using a path length of 1 cm. Unless otherwise prescribed, the measurements are carried out with reference to the same solvent or the same mixture of solvents. The absorbance of the solvent measured against air and at the prescribed wavelength shall not exceed 0.4 and is preferably less than 0.2.

Plot the absorption spectrum with absorbance or function of absorbance as ordinate against wavelength or function of wavelength as abscissa.

Where a monograph gives a single value for the position of an absorption maximum, it is understood that the value obtained may differ by not more than  $\pm 2$  nm.

**Apparatus** Spectrophotometers suitable for measuring in the ultraviolet and visible range of the spectrum consist of an optical system capable of producing monochromatic radiation in the range of 200-800 nm and a device suitable for measuring the absorbance.

**Control of wavelengths** Verify the wavelength scale using the absorption maxima of *holmium perchlorate solution R*, the line of a hydrogen or deuterium discharge lamp or the lines of a mercury vapour arc shown in Table 2.2.25.-1. The permitted tolerance is  $\pm 1$  nm for the ultraviolet range and  $\pm 3$  nm for the visible range. Suitable certified reference materials may also be used.

Table 2.2.25.-1. – *Absorption maxima for control of wavelength scale*

241.15 nm (Ho)	404.66 nm (Hg)
253.7 nm (Hg)	435.83 nm (Hg)
287.15 nm (Ho)	486.0 nm (D $\beta$ )
302.25 nm (Hg)	486.1 nm (H $\beta$ )
313.16 nm (Hg)	536.3 nm (Ho)
334.15 nm (Hg)	546.07 nm (Hg)
361.5 nm (Ho)	576.96 nm (Hg)
365.48 nm (Hg)	579.07 nm (Hg)

**Control of absorbance** Check the absorbance using suitable filters or a solution of *potassium dichromate R* at the wavelengths indicated in Table 2.2.25.-2, which gives for each wavelength the exact value and the permitted limits of the specific absorbance. The table is based on a tolerance for the absorbance of  $\pm 0.01$ .

For the control of absorbance, use solutions of *potassium dichromate R* that has been previously dried to constant mass at 130 °C. For the control of absorbance at 235 nm, 257 nm, 313 nm and 350 nm, dissolve 57.0-63.0 mg of *potassium dichromate R* in 0.005 M *sulfuric acid* and dilute to 1000.0 mL with the same acid. For the control of absorbance at 430 nm, dissolve 57.0-63.0 mg of *potassium dichromate R* in 0.005 M *sulfuric acid* and dilute to 100.0 mL with the same acid. Suitable certified reference materials may also be used.

Table 2.2.25.-2

Wavelength (nm)	Specific absorbance $A_{1\text{ cm}}^{1\text{ per cent}}$	Maximum tolerance
235	124.5	122.9 to 126.2
257	144.5	142.8 to 146.2
313	48.6	47.0 to 50.3
350	107.3	105.6 to 109.0
430	15.9	15.7 to 16.1

**Limit of stray light** Stray light may be detected at a given wavelength with suitable filters or solutions: for example, the absorbance of a 12 g/L solution of *potassium chloride R* in a 1 cm cell increases steeply between 220 nm and 200 nm and is greater than 2.0 at 198 nm when compared with water as compensation liquid. Suitable certified reference materials may also be used.

**Resolution (for qualitative analysis)** When prescribed in a monograph, measure the resolution of the apparatus as follows: record the spectrum of a 0.02 per cent V/V solution of *toluene R* in *hexane R*. The minimum ratio of the absorbance at the maximum at 269 nm to that at the minimum at 266 nm is stated in the monograph. Suitable certified reference materials may also be used.

**Spectral slit-width (for quantitative analysis)** To avoid errors due to spectral slit-width, when using an instrument on which the slit-width is variable at the selected wavelength, the slit-width must be small compared with the half-width of the absorption band but it must be as large as possible to obtain a high value of  $I_0$ . Therefore, a slit-width is chosen such that further reduction does not result in a change in absorbance reading.

**Cells** The tolerance on the path length of the cells used is  $\pm 0.005$  cm. When filled with the same solvent, the cells intended to contain the solution to be examined and the compensation liquid must have the same transmittance. If this is not the case, an appropriate correction must be applied.

The cells must be cleaned and handled with care.

## DERIVATIVE SPECTROPHOTOMETRY

Derivative spectrophotometry involves the transformation of absorption spectra (zero-order) into first-, second- or higher-order-derivative spectra.

A *first-order-derivative spectrum* is a plot of the gradient of the absorption curve (rate of change of the absorbance with wavelength,  $dA/d\lambda$ ) against wavelength.

A *second-order-derivative spectrum* is a plot of the curvature of the absorption spectrum against wavelength ( $d^2A/d\lambda^2$ ). The second-order-derivative spectrum at any wavelength  $\lambda$  is related to concentration by the following equation:

$$\frac{d^2A}{d\lambda^2} = \frac{d^2A_{1\text{ cm}}^{1\text{ per cent}}}{d\lambda^2} \times \frac{c'b}{10} = \frac{d^2A\varepsilon}{d\lambda^2} \times \frac{cb}{10}$$

$c'$  = concentration of the absorbing solute, in grams per litre.

**Apparatus** Use a spectrophotometer complying with the requirements prescribed above and equipped with an analogue resistance-capacitance differentiation module or a digital differentiator or other means of producing derivative spectra. Some methods of producing second-order-derivative spectra produce a wavelength shift relative to the zero-order spectrum and this is to be taken into account where applicable.

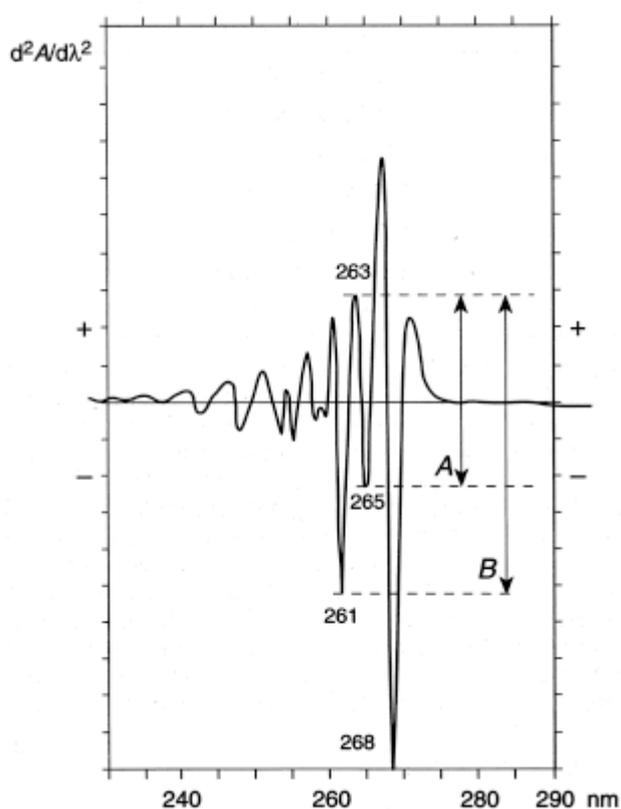


Figure 2.2.25.-1

**Resolution power** When prescribed in a monograph, record the second-order-derivative spectrum of a 0.02 per cent V/V solution of *toluene R* in *methanol R*, using *methanol R* as the

compensation liquid. The spectrum shows a small negative extremum located between 2 large negative extrema at 261 nm and 268 nm, respectively, as shown in Figure 2.2.25.-1. Unless otherwise prescribed in the monograph, the ratio  $A/B$  (see Figure 2.2.25.-1) is not less than 0.2.

**Procedure** Prepare the solution of the substance to be examined, adjust the various instrument settings according to the manufacturer's instructions, and calculate the amount of the substance to be determined as prescribed in the monograph.

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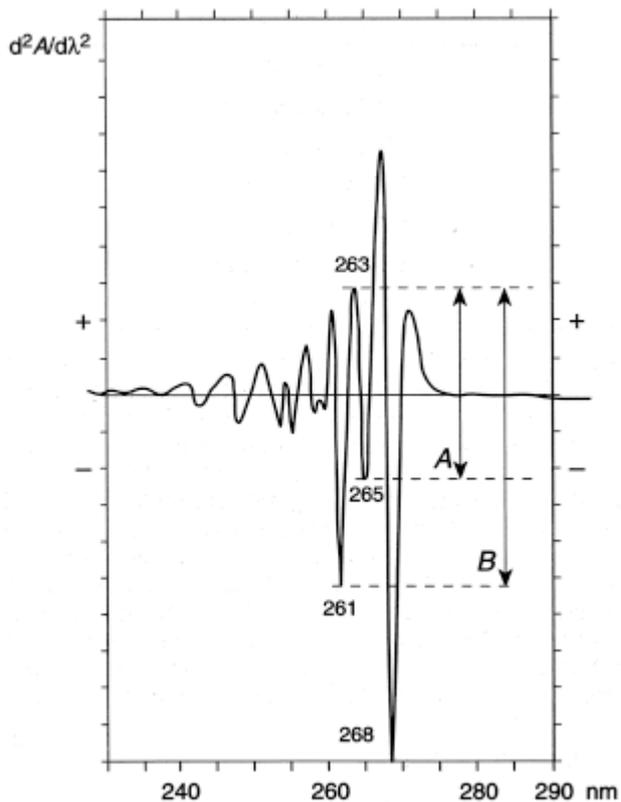


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