MINISTRY OF HEALTH OF THE RUSSIAN FEDERATION

GENERAL PHARMACOPOEIA MONOGRAPH

Spectrophotometry in the ultraviolet and visible spectral regions

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The reduction of monochromatic radiation passing through a homogeneous absorbing medium is quantitatively described by the Beer – Lambert – Bouguer law:

$$log_{10}(1/T) = A = \varepsilon \cdot c \cdot b \text{ ,} \quad (1)$$

where: T is the transmittance, which is the ratio of the intensity of light that has passed through a substance and the intensity of incident light; $T = I/I_0$;
I is the intensity of passing monochromatic radiation;
I_0 is the intensity of incident monochromatic radiation;
ε is the molar absorbance;
c is the molar concentration of the substance in the solution;
b is the optical path length or layer thickness (in centimetres).

The $log_{10}(1/T)$ value is called optical density, it is denominated with the letter A and is a measurable variable. In the absence of other physicochemical factors, the measured optical density (A) is proportionate to the concentration of the substance in the solution (c) and the optical path length (b).

The $A_{1\,\text{cm}}^{1\%}$ value is the specific absorbance, i. e. the optical density of a solution of a substance present at a concentration of 10 g/L (1 g/100 mL) in a cuvette with a 1 cm optical path length. The $A_{1\,\text{cm}}^{1\%}$ and ε values relate to each other in the following manner:

$$A_{1\,\text{cm}}^{1\%} = \frac{10 \cdot \varepsilon}{\text{M.M.}}, \quad (2)$$

where: M. м. is the molecular mass of the tested substance.

Optical density measurement. Unless otherwise specified in the Pharmacopoeia Monograph, the optical density measurement should be performed at the specified wavelength, using a cuvette with a 1 cm optical path length, at (20 ± 1) °C, versus the same solvent or the same mixture of solvents in which the substance is dissolved. When the optical density of a solution is measured at a particular wavelength, the optical density of the cuvette with the solvent measured versus air at the same wavelength should not exceed 0.9 and should preferably be not less than 0.2.

The absorbance spectrum should be presented so that the optical density or a function of it is charted along the axis of ordinates and the wavelength or a function of the wavelength is shown along the axis of abscissas.

If the Pharmacopoeia Monograph specifies only one wavelength for absorbance maximum, this means that the obtained maximum value should not differ from the specified one by more than ± 2 nm.

Devices. Spectrophotometers designed for measurement in the ultraviolet and visible spectral regions, consist of an optical system emitting monochromatic radiation in the range from 190 to 800 nm and ensuring its transmission through a sample, as well as an optical density measuring device.

Main parts of these devices include: a source of radiation, a dispersing appliance (prism or grating), a slit for wavelength band separation, cuvettes for samples, a detector for radiated energy, built-in amplifiers, and measuring devices.

Wavelength scale check in the ultraviolet and visible spectral regions. Accuracy of the device's calibration on the wavelength scale in the spectral region is checked by the spectral lines of a hydrogenous (Hβ) or deuterium (Dβ) discharge lamp specified in Table 1, the mercury (Hg) vapour lines of a quartz mercury arc lamp, as well as by the absorbance maxima of a holmium (Ho) perchlorate solution (the ready-for-use reagent for spectrophotometer calibration is 4 % holmium oxide solution in 14.1 % perchloric acid solution). The acceptable deviation is ± 1 nm for the ultraviolet region and ± 3 nm for the visible region.

Table 1 – Absorbance maxima for the wavelength scale check

241.15 nm (Ho)	404.66 nm (Hg)
253.7 nm (Hg)	435.83 nm (Hg)
287.15 nm (Ho)	486.0 nm (Dβ)
302.25 nm (Hg)	486.1 nm (Hβ)
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)

The wavelength scale may also be calibrated using appropriate glass filters that should have fixed absorbance bands in the visible and the ultraviolet regions, as well as standard glasses containing didymium (a mixture of praseodymium and neodymium) and glasses containing holmium.

Optical density scale check. Standard inorganic glass filters or potassium dichromate solution should be used to check the optical density scale at the wavelengths specified in Table 2, which includes the precise specific absorbance value $A^{1\%}_{1cm}$ and acceptable limits for each wavelength.

The potassium dichromate solution for the optical density scale checks at 235 nm, 257 nm, 313 nm, and 350 nm is prepared in the following manner: a weight from 57.0 mg to 63.0 mg (accurately measured weight) of potassium dichromate pre-dried to a constant weight at 130 °C is dissolved in 0.005 M sulphuric acid solution, and the volume of the solution is then brought to 1000 mL with the same solvent. To examine the optical density at 430 nm, a weight from 57.0 mg to 63.0 mg (accurately measured weight) of potassium dichromate is dissolved in 0.005 M sulphuric acid solution, and the volume of the solution is then brought to the mark with the same solvent.

Table 2 - Specific absorbance of standards at different wavelengths

Wavelength (in nanometres)	Specific absorbance $A_{1\,cm}^{1\%}$	Acceptable limits for $A_{1\,cm}^{1\%}$
235	124.5	From 122.9 to 126.2
257	144.5	From 142.8 to 146.2
313	48.6	From 47.0 to 50.3
350	107.3	From 105.6 to 109.0
430	15.9	From 15.7 to 16.1

Scattered light limit. Scattered light may be detected at a given wavelength using appropriate filters or solutions: for instance, the optical density of a 12 g/L potassium chloride solution in a cuvette with a 1 cm optical path length increases dramatically between 220 nm and 200 nm, and should be more than 2 at 198 nm when water is used as a reference solution.

Resolution (for quantitative analysis). If required by the Pharmacopoeia Monograph, the resolution of the spectrophotometer should be determined in the following manner. The spectrum of 0.02 % (volume / volume) toluene solution in hexane is recorded. The minimum acceptable ratio of the optical density at absorbance maximum at 269 nm to the optical density at absorbance minimum at 266 nm should be specified in the Pharmacopoeia Monograph.

Spectral slit width (for quantitative analysis). Use of a spectrophotometer with an adjustable spectral slit width at a selected wavelength may entail inaccuracies associated with the width of this slit. To rule them out, the slit width should be small compared with the absorbance band half-width (width at half the optical density) and at the same time be as high as possible as to allow obtaining a high intensity of falling monochromatic radiation (I_o). Therefore, the slit width should be such that its further reduction cannot change the measured optical density value.

Cuvettes. The acceptable optical path length deviations of cuvettes used should not exceed ± 0.005 cm. Cuvettes intended for the tested solution and the reference solution should have the same transmittance (or optical density) when filled with the same solvent. Otherwise, this difference should be taken into account.

Requirements for solvents. For determinations carried out in the ultraviolet and visible spectral regions, a sample of the analyzed substance is dissolved in an appropriate solvent, which should be optically transparent in the wavelength range used. Many solvents are appropriate for these wavelength regions, including water, alcohols, chloroform, inferior carbohydrates, ethers, and diluted solutions of potent acids and alkalies.

Identification

Absorption spectrophotometry in the ultraviolet and visible spectral regions is used to identify medicinal products in the following ways:

- comparison of the absorbance spectra of the tested solution and the standard sample solution; the positions of the maxima, minima, shoulders, and isosbestic points should coincide in the particular spectral region;

- localization of the maxima, minima, shoulders, and isosbestic points in the absorbance spectrum of the tested solution; the disagreement between the determined and specified wavelengths at absorbance maxima and minima should not exceed ± 2 nm in most cases.

Other application options are possible as well, provided that they are specified in the Pharmacopoeia Monographs.

Assay

Concentrations of substances are determined with the spectrophotometry method based on the Beer – Lambert – Bouguer law, as described by the following equation:

$$C = \frac{A}{A_{1\,\text{cm}}^{1\%} \cdot b}, \quad \text{…….(3)}$$

where: C is the concentration of the substance (in g/100 mL);

A is the optical density of the tested solution;

$A_{1\,\text{cm}}^{1\%}$ is the specific absorbance of the substance;

b is the optical path length or layer thickness (in centimetres).

In some cases, even the use of monochromatic radiation may entail deviations from the Beer – Lambert – Bouguer law, which are due to the processes

of dissociation, association, and complex formation. Therefore, the relationship between the optical density of the solution and the concentration in the analytical region should be examined for linearity beforehand. In the presence of a deviation from linear relationship, the dependence found experimentally rather than the equation (3) should be employed.

In the spectrophotometry method, the concentration is usually determined using a standard sample. The concentration calculation is based on the following equation:

$$\frac{C}{C_0} = \frac{A}{A_0} \quad , \qquad (4)$$

where: C and C_0 are the concentrations of the tested solution and the standard sample solution, respectively;

A and A_0 are the optical densities of the tested solution and the standard sample solution, respectively.

The concentration of the tested solution should be close to the concentration of the standard solution.

First the optical density of the standard sample solution should be determined, which should be prepared as specified in the Pharmacopoeia Monograph, and then the optical density of the tested solution is measured. The second measurement should be performed immediately after the first one, using the same cuvette and under the same experimental conditions.

The standard sample-based method is the more accurate and reliable one. The possibility of using the specific absorbance value should be justified in each individual case. In most cases, the specific absorbance method is applicable when acceptable deviations in the content of the analyzed substance are not less than ± 10 % from the nominal content.

Multi-component spectrophotometry analysis

Multi-component spectrophotometry analysis (analysis of mixtures) is utilized for simultaneously assaying several components of medicinal products, provided that each of them complies with the Beer – Lambert – Bouguer law.

Assays in the multi-component spectrophotometry analysis are usually based on the use of the following equation:

$$A_i = \sum_{j=1}^{m} E_{ij} \cdot c_j, \qquad i = 1, \ldots n, \qquad (5)$$

where: A_i is the optical density of the tested solution at wavelength i;

E_{ij} are the absorbance values (depending on the concentration expression method) of the sample's component j at wavelength i;

c_j is the concentration of the sample's component j.

The respective analytical procedures and calculation equations are specified in respective Pharmacopoeia Monographs.

Derivative spectrophotometry

In derivative spectrophotometry, the original absorbance spectra (zero-order) are transformed into spectra of derivatives of the first, second, and higher orders.

The spectrum of the first derivative is a diagram that shows the dependence between the absorbance curve gradient (rate of change in optical density with wavelength, $dA/d\lambda$) and the wavelength.

The spectrum of the second derivative is a diagram that shows the dependence between the absorbance spectrum curve ($d^2A/d\lambda^2$) and the wavelength. The second derivative is in the following relationship with the concentration at any wavelength:

$$\frac{d^2A}{d\lambda^2} = \frac{d^2A^{1\%}_{1cm}}{d\lambda^2} \cdot c \cdot l, \qquad (6)$$

where: A is the optical density at wavelength λ;

$A^{1\%}_{1cm}$ is the specific absorbance at wavelength λ;

c is the concentration of the substance in the solution (in grammes per 100 mL);

l is the optical path length (in centimetres).

Derivative spectrophotometry may be used both for identification of substances and for their assaying in multi-component mixtures, as well as whenever there is a background absorption resulting from a presence of non-regulated substances.

Instruments. Spectrophotometers used for this analysis should meet the aforementioned requirements and be equipped with an analogue resistance – capacity differentiating module or a digital differentiator, or with other means of obtaining derivative spectra, as required by the Instructions for Use of the instrument. Some of the methods used to obtain second-order derivative spectra result in displacement of wavelengths compared with the original spectrum, which should be taken into account where appropriate.

Resolution capacity. If required by the Pharmacopoeia Monographs, record the spectrum of the second derivative for a 0.2 g/L toluene solution in methanol, using methanol as a reference solution. The spectrum should contain a small negative extremum located between two large negative extremums at 261 nm and 268 nm, as shown in Figure 1. Unless otherwise specified in the Pharmacopoeia Monographs, the A/B ratio should be not less than 0.2.

Procedure description. The analytical procedure is the same as the one used in regular spectrophotometry, but derivatives are used instead of optical densities. A solution of the tested sample is prepared, and then the instrument is set in accordance with the manufacturer's Instructions for Use and the quantity of the substance being determined is calculated as specified in the Pharmacopoeia Monograph.

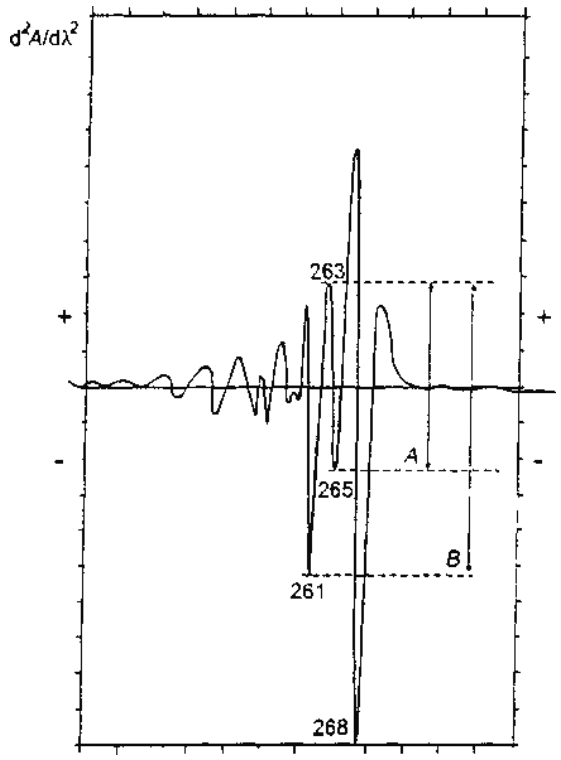

Figure 1 – Spectrum of the second derivative of the toluene solution (0.2 g/L) in methanol