MINISTRY OF HEALTH OF THE RUSSIAN FEDERATION

GENERAL PHARMACOPOEIA MONOGRAPH

Colour intensity of liquids **GPM**.1.2.1.0006.15

Replaces the State Pharmacopoeia of the Russian Federation XII, Part 1 Monograph, GPM 42-0050-07

Colour intensity is determined by visual examination, using one of the methods described below, by comparison with an appropriate reference standard. This Monograph includes medicinal product quality control methods for the parameters «colour intensity» and «solution colour intensity». Colour intensity is a conditional quantitative characteristic used for slightly coloured liquids.

Colour is an observer's perception or subjective reaction to an objective stimulus that is an energy radiated in the visible spectral region and spanning the wavelength range from 400 nm to 700 nm. The colour intensities of two solutions coincide (for a certain source of light) if their absorbance and reflection spectra are identical and the observer sees no difference between them.

Achromatism or no colour means the absence of absorption in the visible spectral region for the tested solution.

One or two of the methods described in the Monograph should be used for visual examination of the colour of a liquid depending on its intensity in the brown, yellow, and red colour regions.

A colourless liquid is a liquid whose colour does not differ from that of water (or of the respective solvent in the case of solutions) or whose the colour intensity does not exceed that of Reference Standard B_9.

The colour intensity of a liquid is usually compared with that of reference

standards (B, BY, Y, GY, R)$_{1-3}$ using Method 1; Method 2 is utilized when reference standards B$_{4-9}$, (BY, Y, GY, R)$_{4-7}$ are used.

Method 1

Tests are carried out in the same test tubes made of colourless, transparent, neutral glass, with an internal diameter of approximately 12 mm, using equal volumes – 2.0 mL of the tested liquid and water, or the solvent, or the reference standard described in the Monograph. Colour comparison is performed in scattered daylight, by visual examination in the horizontal plane (perpendicular to the test tube axis), against a matted white background.

Method 2

Tests are carried out in the same test tubes made of colourless, transparent, neutral glass, with an internal diameter from 15 mm to 25 mm, using equal, 40 mm thick layers of the tested liquid and water, or the solvent, or the reference standard described in the Monograph. Colour comparison is performed in scattered daylight, by visual examination, from top to bottom along the vertical axis of the test tube, against a matted white background.

Preparation of the stock solutions

Yellow solution. Transfer 46.0 g (accurately measured weight) of iron (III) chloride ($FeCl_3 \times 6\ H_2O$; M. w. 270.30) into a 1000 mL volumetric flask, dissolve in 900 mL of a mixture consisting of 25 mL of concentrated hydrochloric acid and 975 mL of water, bring the volume of the solution in the volumetric flask to the mark with the same mixture, and stir. Determine the content of iron (III) chloride in 1 mL of this solution. The iron (III) chloride solution volume is then diluted with the same mixture so that the content of iron (III) chloride is 45.0 mg per millilitre.

This solution should be stored away from light.

Quantification: Transfer 10.0 mL of the iron (III) chloride solution into a 250 mL conical volumetric flask equipped with a ground-in stopper, add 15 mL of water, 5 mL of concentrated hydrochloric acid, and 4 g of potassium iodide, stir, close the flask with the stopper and leave it to stand for 15 minutes in a dark place;

after that add 100 mL of water. Titrate the released iodine with 0.1 M sodium thiosulphate solution, adding 0.5 mL of 1 % starch solution as an indicator towards the end of titration.

Simultaneously perform a control experiment.

1 mL of 0.1 M sodium thiosulphate solution corresponds to 27.03 mg of iron (III) chloride ($FeCl_3 \times 6\ H_2O$).

Red solution. Transfer 60.0 g (accurately measured weight) of triturated cobalt chloride ($CoCl_2 \times 6\ H_2O$; M. w. 237.93) into a 100 mL volumetric flask, dissolve in 900 mL of a mixture consisting of 25 mL of concentrated hydrochloric acid and 975 mL of water, bring the volume of the solution to the mark with the same mixture, and stir. Determine the content of cobalt chloride in 1 mL of this solution. The cobalt chloride solution volume is then diluted with the same mixture so that the content of cobalt chloride is 59.5 mg per millilitre.

Quantification. Transfer 5.0 mL of the cobalt chloride solution into a 250 mL conical volumetric flask equipped with a ground-in stopper, add 5 mL of 3 % hydrogen peroxide solution and 30 mL of 10 % sodium hydroxide solution. Boil this mixture with a reverse refrigerator for 10 minutes, then cool it down to room temperature, and add 60 mL of 1 M sulphuric acid solution and 2 g of potassium iodide. Close the volumetric flask and dissolve the sediment while stirring carefully. Titrate the released iodine with 0.1 M sodium thiosulphate solution until a pale pink colour is acquired, using 0.5 mL of 1 % starch solution as an indicator towards the end of titration.

Perform a control experiment at the same time.

1 mL of 0.1 M sodium thiosulphate solution corresponds to 23.79 mg of cobalt chloride ($CoC1_2 \times 6\ H_2O$).

Blue solution. Transfer 63.0 g (accurately measured weight) of copper (II) sulphate ($CuS0_4 \times 5\ H_2O$; M. w, 249.68) into a 1000 mL volumetric flask, dissolve in 900 mL of a mixture consisting of 25 mL of concentrated hydrochloric acid and 975 mL of water, in a 1000 mL volumetric flask, then bring the volume of the solution in the volumetric flask to the mark with the same mixture and stir.

Determine the content of copper (II) sulphate in 1 mL of this solution. The copper (II) sulphate solution volume is then diluted with the same mixture so that the content of copper (II) sulphate is 62.4 mg per millilitre.

Quantification. Transfer 10.0 mL of the copper (II) sulphate solution into a 250 mL conical volumetric flask equipped with a ground-in glass stopper, add 50 mL of water, 12 mL of 2 M acetic acid solution, and 3 g of potassium iodide, and stir this mixture. Titrate the released iodine with 0.1 M sodium thiosulphate solution until a pale brown colour is acquired, using 0.5 mL of 1 % starch solution as an indicator towards the end of titration.

Perform a control experiment at the same time.

1 mL of 0.1 M sodium thiosulphate solution corresponds to 24.97 mg of copper (II) sulphate ($CuSO_4 \times 5H_2O$).

Preparation of the standard solutions

The standard solutions are obtained by mixing the stock solutions of iron (III) chloride, cobalt chloride, and copper (II) sulphate with 1 % hydrochloric acid solution, measuring them with a calibrated pipette or burette within the accuracy of up to 0.02 mL (Table 1).

Table 1 – Standard solutions

Standard solutions	Yellow stock solution, mL	Red stock solution, mL	Blue stock solution, mL	1 % hydrochloric acid solution, mL
B (brown)	30.0	30.0	24.0	16.0
BY (brownish yellow)	24.0	10.0	4.0	62.0
Y (yellow)	24.0	6.0	0	70.0
GY (greenish yellow)	96.0	2.0	2.0	0
R (red)	10.0	20.0	0	70.0

The prepared stock and standard solutions should be transferred into dry stoppered flasks and stored at (20±3) °C in a place protected from direct sunlight.

The shelf-life of the stock and standard solutions is 1 year.

Before use, one should make sure that the stored stock and standard solutions contain no feculence, sediment, or flocculi. If any of these are present, such solution should be replaced with a freshly prepared one.

Preparation of the reference standards

The reference standards are prepared from five standard solutions by diluting them with 1 % hydrochloric acid solution.

Measurement of the stock and standard solutions for scale preparation is done using a calibration pipette or burette within the accuracy of 0.02 mL.

The colour intensity reference standards for liquids used in Method I should be stored in ampoules made of colourless, transparent, neutral glass, with an external diameter of 12 mm, away from light, for 1 year.

The colour intensity reference standards for liquids used in Method II should be prepared from respective standard solutions immediately before use.

The quantities of the components used for the preparation of colour intensity reference standards are presented in Tables 2 - 6.

Table 2 – Brown colour reference standards (Scale B)

Scale B reference standards	**Standard solution B, mL**	**1 % hydrochloric acid solution, mL**
B_1	75.0	25.0
B_2	50.0	50.0
B_3	37.5	62.5
B_4	25.0	75.0
B_5	12.5	87.5
B_6	5.0	95.0
B_7	2.5	97.5
B_8	1.5	98.5
B_9	1.0	99.0

Table 3 - Brown-yellow colour reference standards (Scale BY)

Scale BY reference standards	**Standard solution BY, mL**	**1 % hydrochloric acid solution, mL**
BY_1	100.0	0.0
BY_2	75.0	25.0
BY_3	50.0	50.0
BY_4	25.0	75.0
BY_5	12.5	87.5
BY_6	5.0	95.0
BY_7	2.5	97.5

Table 4 – Yellow colour reference standards (Scale Y)

Scale Y reference standards	Standard solution Y, mL	1 % hydrochloric acid solution, mL
Y_1	100.0	0.0
Y_2	75.0	25.0
Y_3	50.0	50.0
Y_4	25.0	75.0
Y_5	12.5	87.5
Y_6	5.0	95.0
Y_7	2.5	97.5

Table 5 - Greenish-yellow colour reference standards (Scale GY)

Scale GY reference standards	Standard solution GY, mL	1 % hydrochloric acid solution, mL
GY_1	25.0	75.0
GY_2	15.0	85.0
GY_3	8.5	91.5
GY_4	5.0	95.0
GY_5	3.0	97.0
GY_6	1.5	98.5
GY_7	0.75	99.25

Table 6 - Red colour reference standards (Scale R)

Scale R reference standards	Standard solution R, mL	1 % hydrochloric acid solution, mL
R_1	100.0	0.0
R_2	75.0	25.0
R_3	50.0	50.0
R_4	37.5	62.5
R_5	25.0	75.0
R_6	12.5	87.5
R_7	5.0	95.00

The colour intensity of the tested solution should not exceed the colour intensity of the respective reference standard. The colour of the tested sample should be as much the same as the colour of the respective reference standard as possible.

When comparing the colour of the tested solution versus a reference standard, the letter of the scale should be specified along with the reference standard used. For instance, the colour intensity of the solution should not exceed that of Reference Standard B_7.

Other reference standards may be used if necessary; they should be prepared by mixing stock solutions of different colour scales; their exact volumes should be specified that should be used to ensure the right colour – close to the colour of the tested solution, if this is required by the Pharmacopoeia Monograph.

Spectrophotometry may be used the evaluate the colour of a liquid, if this is envisaged by the Pharmacopoeia Monograph; the following should be specified: the wavelength at which the absorbance maximum is observed in the visible spectral region, the pathlength of the cuvette, and the optical density value with the acceptable deviation.