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BUREAU OF INDIAN STANDARDS

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भारतीय मानक *मसौदा*

खाद्य रंग तैयारी में कुल रंग सामग्री – परीक्षण पद्धतियाँ

(*आइ एस 6120 का पहला पुनरीक्षण*)

Draft ***Indian Standard***

TOTAL DYE CONTENT IN FOOD COLOUR PREPARATIONS – TEST METHODS

(*First Revision of IS 6120*)

ICS 67.220.20

Food Additives Sectional Committee, FAD 08 **Last date of comments:** 10 February 2025

FOREWORD

(*Adoption clauses will be added later*)

Generally, coal-tar food colours are not marketed as such; instead mixtures of these colours are prepared with some diluents and preservatives so as to develop appealing shades. For proper quality control of these mixtures, commonly known as food colour preparations, it is necessary to: (a) identify constituent dyes, and (b) quantitatively estimate the total dye content. Several methods are available for the identification of dyes but no reproducible method of test is available for determining the total dye content. Based on a series of investigations carried out at the All India Institute of Hygiene and Public Health, Calcutta; Central Food Technological Research Institute, Mysore; and Central Food Laboratory, Calcutta; this standard was published in 1971 and prescribed a chromatographic method for qualitative identification of constituent dyes and their quantitative estimation by spectrophotometric method for adoption by all laboratories.

In this revision, the standard has been brought out in the latest style and format of the Indian Standards, and references to Indian Standards wherever applicable have been updated. It also incorporates two amendments issued to previous version of this standard.

In reporting the result of a test made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2: 2022 'Rules for rounding off numerical values (*second revision*)'.

1 SCOPE

1.1 This standard prescribes a method of identification of constituent dyes and two methods for quantitative estimation of total dye content in food colour preparations.

1.2 The methods given are not applicable for determining dye content in food stuffs.

2 PRINCIPLE

2.1 The component dyes of the food colour preparations should be identified by paper chromatography. Then these components should be estimated by spectrophotometric methods, either:

a) by finding out the absorbance of the individual component dyes at their absorption maxima, after quantitative elution from the paper chromatogram; or

b) by direct estimation of the absorbance, at some selected wavelengths, depending on the nature of the individual component dye.

3 REAGENTS

3.1 Chromatographic Paper — Rectangular sheets (32×19.5 cm) of Whatman No. 1 or equivalent filter paper. Nine slots (5×24 cm) should be cut from the paper, parallel to the long side and at a distance of 2 cm from one of the short edge, so as to have 10 strips, 1.5 cm wide, joined at the top and bottom.

3.2 Chromatographic Solvents

3.2.1 *Solvent No. 1* — 1 ml of ammonium hydroxide (sp gr 0.91) + 99 ml water.

3.2.2 *Solvent No. 2* — isobutanol : ethanol : water (1:2:1).

3.2.3 *Solvent No. 3* — n-butanol : water : glacial acetic acid (20: 12:5).

3.2.4 *Solvent No. 4* — 10 g trisodium citrate + 50 ml ammonium hydroxide (sp gr 0.91) + 50 ml water.

3.2.5 *Solvent No. 5* — isobutanol : ethanol : water (3: 2: 2). To 90 ml of this mixture, add 1 ml of ammonium hydroxide.

NOTE — Food colours fast green FCF, wool green BS and brilliant blue FCF may be separated from other food colours using Solvent No. 4 or 5.

3.3 Solvents for Elution

3.3.1 *Hydrochloric Acid* — in 70 percent ethanol 0.1 N.

3.3.2 *Hydrochloric Acid* — 0.1 N.

3.3.3 *Sodium Hydroxide Solution* — 0.1 N.

3.4 Standard Food Colours — conforming to relevant Indian Standards.

4 APPARATUS

4.1 Chromatographic Tank — All glass chromatographic tank, 50×30×25 cm, suitable for both ascending and descending chromatography.

4.2 Spectrophotometer — A reliable spectrophotometer, fitted with photomultiplier or phototube with amplifier, and glass-cells having 1.00 cm light path.

4.3 Micro-Pipette or Alga-Micro-Syringe

5 PROCEDURE

5.1 Identification

5.1.1 *Spotting* — Prepare aqueous solution (1 mg/ml) of the food colour preparations. Spot accurately measured quantities (20 μ to 40 μ) on the 10 strips of the chromatographic paper (**3.1**), at points 0.5 cm above the line joining the lower ends of the slots, that is, at 2.5 cm from the edge.

5.1.2 *Preparation of Chromatographic Tank*

5.1.2.1 Set up the all-glass chromatographic tank (**4.1**) at a place, free from any vibration. Hang from one of the troughs (at the top) a filter paper dummy, 35 x 20 cm at the inner side; cut some serrations along the full length at the bottom of the dummy paper to allow easy and uniform dripping of the solvent. Keep the trough always filled with solvent during chromatography by adding solvent through the corresponding hole in the lid. Fix a glass rod, with a bent hook at the bottom, with a rubber plastic stopper, through a hole of the cover near the centre and at a distance of about 3 cm from the plane of the dummy paper; attach the glass rod in. such a way so that it is possible to push it up and down, without causing any vibration to the tank.

5.1.3 *Chromatography*

5.1.3.1 Pour about 750 ml of the solvent to be used **(3.2)** inside the tank. Fill the trough with dummy paper also with the same solvent. The solvent would start to soak the dummy paper and descend. Attach after an hour the spotted chromatographic paper at the top to a flat metal strip, 200×15×1.5 mm (approximately), with a central hole for the hook. Suspend this from the hook of the glass rod, inside the tank. Allow the chromatographic paper to get saturated in the closed chamber for two and half hours. Push the paper down with the help of the suspending glass rod, so that about 5 mm of the lower edge of the chromatographic paper dips in the solvent below.

The solvent would gradually ascend the paper. When the solvent reaches about 1 cm below the line joining the upper end of the slots, remove the paper carefully, mark the solvent line immediately with a pencil and allow to dry in the air.

5.1.3.2 Identify the different spots in the developed chromatogram from R_f values of standard dyes determined under identical conditions.

5.2 Quantitative Estimation

5.2.1 *Determination of Pure Dye Content of Standard Food Colours* — The pure dye content of each food colour, having purity according to relevant Indian Standard specification shall be estimated by titanium trichloride reduction method, prescribed in Indian Standards for each individual food colour.

5.2.2 *Determination of Absorption Spectrum of Standard Food Colours* — Weigh accurately about 100 mg/100 ml of each of the standard food colours separately. Dissolve in redistilled water. Make from these stock solutions, solutions of the dyes 1 mg/100 ml, approximately in water. Also make the solutions in 0.1 N hydrochloric acid, 0.1 N hydrochloric acid in 70 percent alcohol and 0.1 N sodium hydroxide. Find out absorption spectra of these solutions in the range 420 to 650 nm, using cells of 1.00 cm light path. From these absorption spectra, calculate extinction coefficient ($E^{1\%}_{1\ cm}$) at absorption maxima on the basis of pure dye contents (**5.2.1**).

5.2.3 *Separation and Elution Method*

5.2.3.1 From the chromatogram of the food colour preparation (**5.1.3.1**) cut the separated bands of individual colours carefully and elute with 0.1 N hydrochloric acid in 70 percent ethanol or with other suitable eluting solutions (**3.3**). Make up the elute to 25 ml. Find out optical density of these elutes at respective wavelengths of absorption maxima (**5.2.2**), using cells of 1.00 cm light path. Use the extracts of equivalent portion from the blank part of the chromatogram in the same solvent as 'blank' in the optical density determination.

5.2.3.2 Calculate from these optical densities, the amounts of individual component colours present in the food colour preparation using the extinction coefficients ($E^{1\%}_{1\ cm}$) of the respective standard colour (**5.2.2**) given below. Compute amounts together to find out the total dye content food colour preparations:

Amount of a dry component in a food colour preparation (g / 100 g of food colour preparation) $= \frac{OD}{E^{1\%}\ 1\ cm} \times \frac{100}{C}$

where

OD = the observed optical density at absorption maxima of the individual component, separated and eluted;

$E^{1\%}_{1cm}$ = extinction coefficient of the standard sample of the same dye content, in the same solvent; and

C = equivalent concentration of the food-colour preparation per 100 ml of the final solution.

NOTE 1 — Following three major factors shall be taken into consideration for calculation of 'C':

a) Concentration of original food-colour solution for chromatography, which should be approximately 1 mg/ml (**5.1.1**).

b) Amount of dye solution spotted, which should be 20 µ to 40 µ (**5.1.1**).

c) Final volume of the elute (which should be about 25 ml, but may have to be varied according to the intensity of colour), to be used for measuring OD (**5.2.3.1**).

NOTE 2 — This method shall not be applicable for determining indigotine, which might be present in some food colour preparations. For its determination direct spectrophotometric method (**5.2.4**) should be used.

5.2.4 *Direct Spectrophotometric Method*

Some food colours, like indigotine and erythrosine, are unstable in paper chromatogram and should be directly estimated by suitable optical methods. Moreover, as the elution method (**5.2.3**) requires several manipulative steps, there might be some difference in the results of duplicate estimations. By direct spectrophotometric method, this can be avoided.

5.2.4.1 *Principle* – From the absorption spectra of the standard food colours (**5.2.2**), ratios of *OD* (optical density) of a particular dye at wavelength maxima and minima of other dyes, to the *OD* at its wavelength maxima are calculated. For example, for tatrazine, it shall be necessary to find out:

$$\frac{E_{486}}{E_{430}}, \frac{E_{505}}{E_{430}}, \frac{E_{516}}{E_{430}}, \frac{E_{520}}{E_{430}}, \frac{E_{560}}{E_{430}}, \frac{E_{610}}{E_{430}}$$

Where

E_{430} = OD for tatrazine at the wavelength of maximum absorption, and E_{485}, E_{505}, E_{505}, E_{516}, E_{520}, E_{560}, E_{610}, are respectively OD's of tatrazine, at wavelength maxima of sunset yellow, Ponceau 4 R, amaranth, carmoisine sunset yellow (minima) and indigotine.

5.2.4.2 *Procedure* — Dissolve accurately weighed quantity of the food colour preparation in water and then appropriately dilute with water or 0.1 N hydrochloric acid or 0.1 N sodium hydroxide, to give a final concentration of about 1 mg/100 ml. Determine the optical densities of this final diluted solution at the wavelength maxima of the component dyes in this food colour preparation, as revealed by chromatography (**5.1**). In case of the mixture of Ponceau 4R with carmoisine and amaranth, OD values are found out, in 0.1 N sodium hydroxide at appropriate fixation points [**5.2.4.3** (c)].

For fast green FCF, wool green BS and brilliant blue FCF use water containing 200 mg of ammonium acetate per litre as diluent and estimate the content directly in neutral medium, using the same as blank.

5.2.4.3 *Calculation*

a) In case of mixtures, where one of the components has got an absorption maxima at a wavelength, where other components have little or no absorption, value shall be directly calculated, after necessary correction, as in the case of mixture of tatrazine and indigotine, a mixture of sunset yellow and carmoisine or similar other mixtures.

b) In case, where each of the components of a mixture, has got some optical absorption at the wavelength maxima of other components, value shall be calculated using the following formula:

$$x+b_1y+c_1z = OD_1$$

$$a_1x+y+c_2z = OD_2$$

$$a_2x+b_2y+z = OD_3$$

where

x, y, z are the corrected OD of the three components at their wavelength maxima, OD_1, OD_2, OD_3, are the observed OD's at the three wavelength maxima; and a_1, a_2, b_1,b_2 and C_1, C_2 are ratios of OD at the wavelength maxima of the other components to the OD of the particular component at its wavelength maxima (**5.2.4.1**). Calculate from x, y and z, the concentration of the respective colour components.

c) In case of mixtures of Ponceau 4R with carmoisine and amaranth, calculate according to the following equation:

$$\frac{E\lambda_1 - (E\lambda_2 - E\lambda_3)\frac{\lambda_3 - \lambda_1}{\lambda_3 - \lambda_2}}{\mathrm{E}\lambda_3 - x} = k$$

Where

λ_1 = wavelength of absorption maximum for carmoisine or amaranth;

λ_2 and λ_3 = wavelengths on either side of λ_1 where OD for the particular dye are equal;

$E\lambda_1$, $E\lambda_2$ and $E\lambda_3$ = observed OD's in 0.1 N sodium hydroxide at λ_1, λ_2 and λ_3;

k = ratio of OD of the particular pure dye at λ_1, and λ_2; and

x = unknown which may be solved from the above equation.

When x is known, the true OD at maxima for the particular dye, amaranth or carmoisine present in the mixture can be calculated from the numerator of the above equation, namely:

$$\mathrm{E\ (Corr)} = \mathrm{E}\lambda_1 - (\mathrm{E}\lambda_2 - \mathrm{E}\lambda_3)\frac{\lambda 3-\lambda \iota}{\lambda 3-\lambda 2} - x$$

From the corrected OD, the amount of amaranth or carmoisine present in the mixture may be calculated from extinction coefficient, $E^{1\%}_{1cm}$. Subtracting the contribution of amaranth or carmoisine from the observed OD, the amount of Ponceau 4R may be found out.

NOTES

1. Only three readings are necessary for all the calculation.
2. Values for fixation point for carmoisine and amaranth in 0.1 N sodium hydroxide were found to be:

Value	*Carmoisine*	*Amaranth*
λ_1	500 nm	490 nm
λ_2	490 nm	485 nm
λ_3	517.5 nm	495 nm
k	1.040	1.009

5.2.5 The test report shall indicate which of the two methods (**5.2.3** or **5.2.4**) has been employed for quantitative determination of dyes in food colour preparations.