
इंडिगो कारमीन, खाद्य ग्रेड — विशिष्टि
(तीसरा पुनरीक्षण)

Indigo Carmine, Food Grade —
Specification
(Third Revision)

ICS 67.220.20

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FOREWORD

This Indian Standard (Third Revision) was adopted by the Bureau of Indian Standards, after the draft finalized by the Food Additives Sectional Committee had been approved by the Food and Agriculture Division Council.

This standard is one of the series of Indian Standards for edible synthetic food colours permitted under the *Food Safety and Standards (Food Products Standards and Food Additives) Regulation, 2011*. This standard was first published in 1960. The first revision of the standard was carried out in 1974 to bring it in line with the FAO/WHO specifications and also taking into account the indigenous data generated. In the second revision published in 1994, the limits for water insoluble matter, combined ether extracts and subsidiary dyes were made more stringent to align them with the International requirements and the requirement for heavy metals was included.

In this revision, the following changes have been made:

- a) The requirement for heavy metals has been removed as the limit of lead (contaminant in food colours) is already covered through the standard; and
- b) The limits for mercury, copper, chromium, cadmium, (dye intermediates) organic compounds other than colouring matters, and unsulphonated primary aromatic amines have been incorporated.

Description

Common Name — Indigo carmine.

Synonyms — Indigotine, FD & C Blue No 2, C 1 Food Blue 1, E E C Serial No. E 132, L Blau 2.

Colour of the 0.1 Percent (m/v) Solution in Distilled Water — Blue.

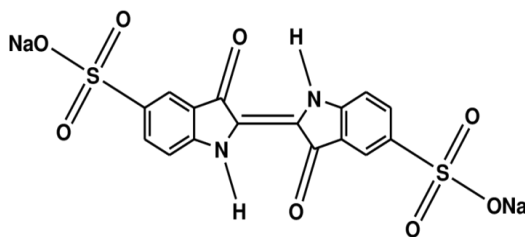
Colour Index Number (1975) — No. 73015.

Class — Indigoid.

Chemical Name — Disodium salt of indigotine 5,5'-disulphonic Acid.

Empirical Formula — $C_{16}H_8N_2Na_2O_8S_2$

Structural Formula



Solubility — Soluble in water (sparingly soluble in ethanol)

Molecular Weight — 466.36 (disodium salt).

In the preparation of this standard, due consideration has been given to the provisions of the *Prevention of Food Adulteration Rules Act, 1977* and the *Legal Metrology (Packaged Commodities) Rules, 2011* and *Food Safety and Standards Act, 2006* and rules framed thereunder. However, this standard is subject to the restrictions imposed under these, wherever applicable.

[*\(Continued on third cover\)*](#)

*Indian Standard***INDIGO CARMINE, FOOD GRADE — SPECIFICATION***(Third Revision)***1 SCOPE**

This standard prescribes the requirements and the methods of sampling and test for indigo carmine, food grade.

2 REFERENCES

The standards given below contain provisions, which through reference in this text, constitute provision of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision and parties to agreements based on this standard is encouraged to investigate the possibility of applying the most recent edition of these standards:

<i>IS No.</i>	<i>Title</i>
IS 1070 : 2023	Reagent grade water — Specification (<i>fourth revision</i>)
IS 1699 : 1995	Methods of sampling and test for food colours (<i>second revision</i>)
IS 2491 : 2013	Food hygiene — General principles — Code of practice (<i>third revision</i>)

3 REQUIREMENTS**3.1 Test for Colour**

In water, neutral or acidic solutions of sunset yellow FCF are yellow-orange where as basic solutions are red-brown. When dissolved in concentrated sulphuric acid, the additive yields an orange solution that turns yellow when diluted with water.

3.2 The product shall be processed, packed, stored and distributed under hygienic conditions (*see* IS 2491).

3.3 The material shall also comply with the requirements given in [Table 1](#).

4 PACKING

The material shall be packed in glass containers, metal containers, polyethylene containers, or cardboard containers suitably lined with polyethylene. Any other suitable containers may also be used, subject to agreement between the purchaser and the vendor.

5 MARKING

5.1 Each container shall be legibly and indelibly marked with the following information:

- The words 'Food Colour';
- Common name of the colour;
- Chemical name of the colour;
- Colour index number;
- Names of the major dye intermediates present;
- Source of manufacture;
- Date of manufacture;
- Net quantity, in g or kg;
- Batch or code number; and
- Any other requirements as given under the *Legal metrology (Packaged Commodities) Rules, 2011 and Food Safety and Standards (Labelling and Display) Regulation, 2020* and rules framed thereunder.

5.2 BIS Certification Marking

The product(s) conforming to the requirements of this standard may be certified as per the conformity assessment schemes under the provisions of the *Bureau of Indian Standards Act, 2016* and the Rules and Regulations framed thereunder, and the products may be marked with the Standard Mark.

6 SAMPLING

Representative samples of the material for tests shall be drawn and criteria for ascertaining conformity to the requirements of this standard shall be determined according to the method prescribed in IS 1699.

7 TESTS

Tests shall be carried out as prescribed in co1 (4) of [Table 1](#).

8 Quality of Reagents

Unless specified otherwise, pure chemicals and distilled water (*see* IS 1070) shall be employed in tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the results of analysis.

To access Indian Standards click on the link below:

https://www.services.bis.gov.in/php/BIS_2.0/bisconnect/knowyourstandards/Indian_standards/isdetails/

Table 1 Requirements for Sunset Yellow with Indigo Carmine*(Clauses 3.3 and 7)*

Sl No.	Characteristic	Requirements	Method of Test, Ref to
(1)	(2)	(3)	(4)
i)	Total dye content, corrected for sample dried at 105 °C ± 1 °C for 2 h, percent by mass, <i>Min</i>	85.0	Annex A
ii)	Loss on drying at 135 °C, percent by mass, and Chlorides and sulphates expressed as sodium salt, percent by mass, <i>Max</i>	15.0	IS 1699
iii)	Water-insoluble matter, percent by mass, <i>Max</i>	0.2	Annex B
iv)	Combined ether extracts, percent by percent by mass, <i>Max</i>	0.2	Annex C
v)	Subsidiary dyes, percent by mass, <i>Max</i>	1.0	Annex D
vi)	Isatin sulphonic acid, percentage by mass, <i>Max</i>	0.5	Annex E
vii)	Dye intermediates (organic compounds other than colouring matters), percent by mass, <i>Max</i>	0.5	Annex F
viii)	Unsulphonated primary aromatic amines, percent, <i>Max</i>	0.01	Annex G
ix)	Lead, mg/kg, <i>Max</i>	2.0	IS 1699
x)	Arsenic, mg/kg, <i>Max</i>	3.0	IS 1699
xi)	Mercury, mg/kg, <i>Max</i>	1.0	IS 1699
xii)	Copper, mg/kg, <i>Max</i>	30.0	IS 1699
xiii)	Chromium, mg/kg, <i>Max</i>	50.0	IS 1699
xiv)	Cadmium, mg/kg, <i>Max</i>	1.0	IS 1699

ANNEX A

[Table 1, Sl No. (i)]

DETERMINATION OF TOTAL DYE CONTENT

A-1 GENERAL

Two methods, spectrophotometric method and the titanium trichloride method, are specified. In case of dispute, spectrophotometric method shall be regarded as the reference method.

A-1.1 Spectrophotometric Method

A-1.1.1 Apparatus

Suitable spectrophotometer with properly calibrated scales for both wavelength and optical density. However, a suitable spectrophotometer may also be used after calibration against a spectrophotometer.

A-1.1.2 Reagents

A-1.1.2.1 Ammonium acetate solution — 200 mg of ammonium acetate in one litre of water (re-distilled).

A-2 PROCEDURE

A-2.1 Weigh accurately about 250 mg of the dye sample and dissolve with ammonium acetate solution in a 250 ml volumetric flask; dilute this with the same solvent to make a final concentration of 1 mg/100ml (approximately). Find out the optical density of this dilute solution, against ammonium acetate solution as blank, at 610 nm, in a cell with 10.0 mm light path.

A-2.2 Simultaneously weigh accurately about 2 g of the dye-sample and dry this in an air-oven at $105\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ for 2 hours. Calculate the loss of mass on drying; from this data, calculate the dry mass of the sample (M) in the final solution taken for measurement of optical density.

A-3 CALCULATION

Total dye content in the sample, present

$$= \frac{OD \times 100}{M \times 450}$$

where

OD = optical density found;
 M = dry mass, in g, of the sample in 100 ml solution; and
 450 = $E^{1\%}_{1\text{ cm}}$, 610 nm, for indigo carmine in ammonium acetate solution.

A-4 TITANIUM CHLORIDE METHOD

The method given in IS 1699 shall be followed. The percentage of total dye content shall be determined using the following calculation:

1 ml of 0.1 N TiCl_3 = 0.023 32 of indigo carmine.

ANNEX B

[Table 1, Sl No. (iii)]

DETERMINATION OF WATER SOLUBLE MATTER

B-1 PROCEDURE

The method given in IS 1699 shall be followed taking 2 gram of sample instead of 4.5 g (*see* IS 1699).

ANNEX C

[Table 1, Sl No. (iv)]

DETERMINATION OF SUBSIDIARY COLOURING MATTERS

C-1 The method given in IS 1699 shall be followed taking 2 gram of sample instead of 5 g (*see* IS 1699).

ANNEX D

[Table 1, Sl No. (v)]

DETERMINATION OF SUBSIDIARY DYE CONTENT

D-1 PROCEDURE

The method given in IS 1699 using the conditions given below shall be followed:

- a) *Developing solvent*: No. 3
- b) *Height of ascent of solvent front*: approximate 17 cm

NOTES

1 The 5,7 isomer is separated as a wide blue zone just in front of main blue band. Do not include this zone in the subsidiary colouring matter zones which are cut out and measured.

2 The 15 ml sodium hydrogen carbonate solution used in the general procedure replaced by 15 ml 0.05 N hydrochloric acid in order to avoid the decomposition which sulphonated indigo undergoes in alkaline solution.

ANNEX E

[Table 1, Sl No. (vi)]

DETERMINATION OF ISATIN SULPHONIC ACID

E-1 PRINCIPLE

Separate isatin sulphonic acid from indigo carmine by ascending paper chromatography and extract it from the paper. Measure the optical density of the extract at the wavelength of maximum absorbance around 247 nm, from which calculate the content of the intermediate as a percent by mass of the sample.

E-2 APPARATUS

E-2.1 Chromatograph Tank with Ancillary Equipment — as given under IS 1699

E-2.2 Microsyringe — capable of delivering 0.2 ml with a tolerance of ± 0.002 ml.

E-2.3 Spectrophotometer

E-3 REAGENTS

E-3.1 Atmosphere Saturating Solvent — water

E-3.2 Developing Solvent — sodium chloride solution 15 percent.

E-3.3 Extracting Solvent — ammonium sulphate solution 25 percent.

ANNEX F

[Table 1, Sl No. (vii)]

DETERMINATION OF DYE INTERMEDIATES (ORGANIC COMPOUNDS OTHER THAN COLOURING MATTERS)

F-1 GENERAL

Two methods, column chromatographic and ascending paper chromatographic method have been specified. In case of dispute, the column chromatographic method shall be regarded as the reference method.

F-2 METHOD I

F-2.1 Principle

Sunset yellow FCF is prepared by diazotization of aniline-4-sulphonic acid and the diazo so formed is coupled with 2-naphthol-6-sulphonic acid. These intermediates are determined by ascending paper

chromatography.

F-2.2 Apparatus

F-2.2.1 Chromatography Tank and Ancillary Equipment — as given under IS 1699

F-2.2.1 Microsyringe — capable of 0.2 ml with a tolerance of ± 0.0002 ml.

F-2.2.3 Ultraviolet Lamp — with a wavelength of 365.5 nm.

F-2.2.4 Filter Paper — Whatman No. 1 or equivalent.

F-2.3 Reagents

F-2.3.1 Ammonium Hydroxide Solution — specific gravity, 0.923.

F-2.3.2 Developing Solvent — sodium chloride solution, 10 percent.

F-2.3.3 2-Naphthol-6-Sulphonic Acid

F-2.3.4 Aniline-4-Sulphonic Acid

F-2.3.5 Sodium Nitrite — crystals

F-2.3.6 Hydrochloric Acid — 1 N

F-2.3.7 Chromotropic Acid Solution — 0.05 percent

F-2.3.8 Sodium Acetate Solution — 40 percent

F-2.4 Procedure**F-2.4.1 Preparation of solutions**

F-2.4.1.1 Prepare 2 percent (*m/v*) solution of the dye in a mixture of nine parts of water and one part of the ammonium hydroxide.

F-2.4.1.2 Reference substances

- a) Prepare 0.01 percent on 100 percent basis, (*m/v*) solution of 2-naphthol-6-sulphonic acid in nine parts of water and one part of ammonium hydroxide; and
- b) Prepare 0.01 percent, on 100 percent basis, (*m/v*) solution of aniline-4-sulphonic acid in a mixture of nine parts of water and one part of ammonium hydroxide.

F-2.4.2 Test Substances**F-2.4.2.1 Dye solution**

Ten microlitres of the solution (**F-2.4.4**) shall be equivalent to 200 µg of the sample.

F-2.4.2.2 Reference substances

- a) 2-naphthol-6-sulphonic acid [**F-2.4.1.2 (a)**] — 5 µl shall be equivalent to 0.5 µg corresponding to 0.25 percent in the dye sample. 10 µg shall be equivalent to 1 µg

corresponding to 0.5 percent in the dye sample; and

- b) Aniline-4-sulphonic acid [**F-2.4.1.2 (b)**] — 5 µl shall be equivalent to 0.5 µg corresponding to 0.25 percent in the dye sample. 10 µl shall be equivalent to one µg corresponding to 0.5 percent in the dye sample.

F-2.4.3 Mark out a sheet of chromatographic paper as shown in Fig. 3 of IS 1699 and apply 10 microlitres of the dye solution as uniformly as possible with the help of microsyringe. Also apply reference substances (**F-2.4.1.2**). Mount the sheet together with plane sheet to act a blank in the frame (C). Pour sufficient developing solvent into the tray (D) to bring the surface of the solvent about 1 cm below the base line of the chromatogram sheet. Put the frame (C) in the same position and replace the cover. Take out the filter paper after 30 cm run. Dry in an oven at 70 °C to 75 °C.

F-2.4.4 Detection and Semi-Quantitative Estimation

Inspect the chromatogram under the ultraviolet light. Compare visually the intensity of the fluorescence of the sample with the 2-naphthol-6-sulphonic acid and report the value nearest to the intensity of the reference substance.

Diazotize the chromatogram for 10 min under nitrous acid vapours. (Introduce the paper into a rectangular glass jar in which a beaker containing crystals of sodium nitrate has been kept. Pour the hydrochloric acid into the beaker with the help of the pipette through the hole of the cover). Take out the paper and keep in air for 10 min. Spray the chromatogram with the chromotropic acid solution in the sodium acetate solution. Compare the intensity of the fluorescence of the sample with the reference, substance and report the content of aniline-4-sulphonic acid.

F-3 METHOD II

The column chromatographic method given under IS 1699 shall be followed.

ANNEX G

[Table 1, Sl No. (viii)]

DETERMINATION OF UNSULPHONATED PRIMARY AROMATIC AMINES**G-1 GENERAL**

Unsulphonated primary aromatic amines are extracted into toluene from an alkaline solution of the sample, re-extracted into acid, and then determined spectrophotometrically after diazotization and coupling. They are expressed as aniline unless they are known to be some other amine.

NOTE — This method is not sufficiently sensitive for determining aniline at low mg/kg levels or below.

G-2 APPARATUS**G-2.1 Visible Range Spectrophotometer**

G-2.2 Spectrophotometer Cells — 40 mm path length

G-3 REAGENTS**G-3.1 Toluene**

G-3.2 Hydrochloric Acid — 1 N

G-3.3 Hydrochloric Acid — 3 N

G-3.4 Potassium Bromide — 50 percent solution

G-3.5 Sodium Carbonate Solution — 2 N

G-3.6 Sodium Hydroxide — 1 N

G-3.7 Sodium Hydroxide — 0.1 N

G-3.8 R Salt (2-naphthol-3, 6-disulphonic acid, disodium salt) Solution — 0.05 N

G-3.9 Sodium Nitrite Solution — 0.5 N

G-3.10 Aniline**G-4 PROCEDURE****G-4.1 Preparation of a Standard Aniline Solution**

Weigh 0.100 g of re-distilled aniline into a small beaker and transfer to a 100 ml volumetric flask, rinsing the beaker several times with water. Add 30 ml of 3N hydrochloric acid and dilute to the mark with water at room temperature. Dilute 10.0 ml of this solution to 100 ml with water and mix well; 1 ml of this solution is equivalent to 0.000 1 g of aniline. Prepare the standard aniline solution freshly when required.

G-4.2 Preparation of a Calibration Graph

Measure the following volumes of the standard aniline solution into a series of 100 ml volumetric flasks: 5 ml, 10 ml, 15 ml, 20 ml, and 25 ml. Dilute to 100 ml with 1 N hydrochloric acid and mix well. Pipette 10 ml of each solution into clean, dry test tubes; cool them for 10 min by immersion in a beaker of ice water. To each tube add 1 ml of the potassium bromide solution and 0.05 ml of the sodium nitrite solution. Mix and allow the tubes to stand for 10 min in the ice water bath while the aniline is diazotized. Into each of five 25 ml volumetric flasks, measure 1 ml of the R salt solution and 10 ml of the sodium carbonate solution. Pour each diazotized aniline solution into a separate flask containing R salt solution and sodium carbonate solution; rinse each test tube with a few drops of water. Dilute to the mark with water, stopper the flasks, mix the contents well and allow them to stand for 15 min in the dark. Measure the absorbance of each coupled solution at 510 nm using 40 mm cells. As a reference solution, use a mixture of 10.0 ml of N hydrochloric acid, 10.0 ml of the sodium carbonate solution, and 2.0 ml of the R salt solution, diluted to 25.0 ml with water. Plot a graph relating absorbance to weight of aniline in each 100 ml of aniline solution.

G-4.3 Preparation and Evaluation of a Test Solution

Weigh, to the nearest 0.01 g, about 2.0 g of the colouring matter sample (M) into a separatory funnel containing 100 ml of water, rinse down the sides of the funnel with a further 50 ml of water, swirling to dissolve the sample, and add 5 ml of 1 N sodium hydroxide. Extract with two 50 ml portions of toluene and wash the combined toluene extracts with 10 ml portions of 0.1 N sodium hydroxide to remove traces of colour. Extract the washed toluene with three 10 ml portions of 3 N hydrochloric acid and dilute the combined extract to 100 ml with water. Mix well. Call this solution T. Pipette 10.0 ml of solution T into a clean, dry test tube, cool for 10 min by immersion in a beaker of ice/water, add 1 ml of the potassium bromide solution and proceed as described above for the preparation of the calibration graph, starting with the addition of 0.05 ml of the sodium nitrite solution. Measure the absorbance of the coupled test solution at 510 nm using a 40 mm cell. Use a reference solution

prepared from 10.0 ml of Solution T, 10 ml of the sodium carbonate solution, and 2.0 ml of the R salt solution diluted to 25.0 ml with water. From the calibration graph, read the weight of aniline (M_A) corresponding to the observed absorbance of the test solution.

G-5 CALCULATION

Percent unsulphonated primary aromatic amine (as

aniline)

$$= \frac{100 \times M_A}{M}$$

where

M_A = mass of aniline from calibration graph;
and

M = dry mass, in g, of the colouring matter sample.

ANNEX H

(Foreword)

COMMITTEE COMPOSITION

Food Additives Sectional Committee, FAD 08

<i>Organization</i>	<i>Representative(s)</i>
CSIR - Indian Institute of Toxicology Research, Lucknow	DR YOGESHWAR SHUKLA (<i>Chairperson</i>)
All India Food Processors Association, New Delhi	MS SHREYA PANDEY SHRI KRISHNA KUMAR JOSHI (<i>Alternate</i>)
Association of Food Scientists and Technologists India, Mumbai	DR VIKAS SINGH CHAUHAN DR NANDINI P. SHETTY (<i>Alternate</i>)
Bose Institute, Kolkata	PROF GAOURISHNKAR
Confederation of Indian Food Trade and Industry, New Delhi	DR JASVIR SINGH MS PRIYANKA SHARMA (<i>Alternate</i>)
Confederation of Indian Industry, New Delhi	MS NEHA AGGARAWAL MS MAMTA ARORA BUDHIRAJA (<i>Alternate</i>)
Consumer Education and Research Centre, Ahmedabad	MS ANINDITA MEHTA MS DOLLY A. JANI (<i>Alternate</i>)
Consumer Guidance Society of India, Mumbai	DR SITARAM DIXIT DR M. S. KAMATH (<i>Alternate</i>)
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Defence Food Research Laboratory, Mysuru	DR G. K. SHARMA DR D. D. WADIKAR (<i>Alternate</i>)
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Envirocare Labs Private Limited, Thane	DR PRITI AMRITKAR DR NILESH AMRITKAR (<i>Alternate</i>)
Food Ingredients Manufacturers & Suppliers of India Association	SHRI FIROZ H. NAQVI
Grasim Industry, Mumbai	SHRI PANKAJ KUMAR GUPTA
ICMR - National Institute of Nutrition, Hyderabad	DR J. PADMAJA
Indian Institute of Chemical Technology, Hyderabad	DR ASHOK KUMAR TIWARI DR T. KUMARAGURU (<i>Alternate</i>)
Indian Pharmacopoeia Commission, Ghaziabad	MS SHRUTI RASTOGI SHRI JAI SHIV CHAUHAN (<i>Alternate</i>)
Indian Salt Manufacturers Association, Ahmedabad	SHRI B. C. RAWAL SHRI P. R. DHRUVE (<i>Alternate</i>)

<i>Organization</i>	<i>Representative(s)</i>
Office of the Salt Commissioner, Jaipur	SHRI M. A. ANSARI SHRI B. S. NAGAR (<i>Alternate</i>)
Protein Foods and Nutrition Development Association of India, Mumbai	SHRI BHUPINDER SINGH DR JAGADISH PAI (<i>Alternate</i>)
Roha Dyechem, Mumbai	SHRI ZAINULABIDIN DHANSE
VR Food Tech Pvt Ltd, Mumbai	DR ASHLESH PARCHURE DR N. RAM (<i>Alternate</i>)
BIS Directorate General	SHRIMATI SUNEETI TOTEJA, SCIENTIST 'E'/DIRECTOR AND HEAD (FOOD AND AGRICULTURE) [REPRESENTING DIRECTOR GENERAL (<i>Ex-officio</i>)]

Member Secretary
SHRI KULDEEP MITTAL
SCIENTIST 'B'/ASSISTANT DIRECTOR
(FOOD AND AGRICULTURE), BIS

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The composition of the Committee responsible for the formulation of this standard is given in [Annex H](#).

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 2022 'Rules for rounding off numerical values (*second revision*)'. This number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

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This Indian Standard has been developed from Doc No.:FAD 08 (22586).

Amendments Issued Since Publication

Amend No.	Date of Issue	Text Affected

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