

**SRI LANKA STANDARD 941 : 1991**

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SPECIFICATION FOR  
**FOOD COLOURING MATTER,  
INDIGO CARMINE**

**SRI LANKA STANDARDS INSTITUTION**



SPECIFICATION FOR FOOD COLOURING MATTER, INDIGO CARMINE

SLS 941:1991

Gr. 7

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SRI LANKA STANDARDS INSTITUTION

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This standard does not purport to include all the necessary provisions of a contract.

SRI LANKA STANDARD  
SPECIFICATION FOR FOOD COLOURING MATTER, INDIGO CARMINE

FOREWORD

This Sri Lanka Standard was authorized for adoption and publication by the Council of the Sri Lanka Standards Institution on 1991 - 11 - 14, after the draft, finalized by the Drafting Committee on Food Additives, had been approved by the Agricultural and Food Products Divisional Committee.

This is one of the series of standard specifications for food colours.

This specification is subject to the provisions of the Food Act No. 26 of 1980 and the regulations framed thereunder.

For the purpose of deciding whether a particular requirement of this specification is complied with, the final value, observed or calculated, expressing the result of a test or an analysis, shall be rounded off in accordance with CS 102. The number of significant places retained in the rounded off value shall be the same as that of the specified value in this specification.

In the preparation of this specification the valuable assistance obtained from the relevant publications of the Food and Agriculture Organization (FAO) and the Bureau of Indian Standards is gratefully acknowledged.

1 SCOPE

This specification prescribes the requirements and methods of sampling and test for indigo carmine used as a colouring matter of food stuffs.

2 REFERENCES

- CS 102 Presentation of numerical values.
- SLS 394 Analysis of water soluble coal-tar dyes.
- SLS 467 Labelling of prepackaged foods
  - Part 1 : General guidelines.
  - Part 2 : Guidelines on claims.
- SLS 543 Sampling of food colours.

### 3 DESCRIPTION

3.1 **Common name** : Indigo carmine.

3.2 **Synonyms** : CI Food Blue 1.  
FD & C Blue No. 2.  
Indigotine.

3.3 **Colour index number and EEC number** : 73015, E 132.

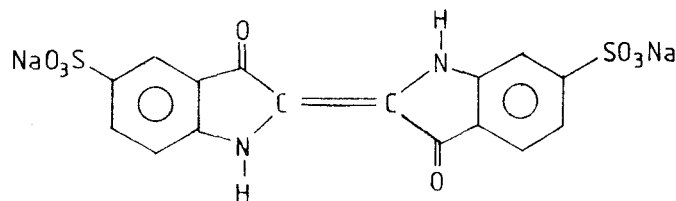
3.4 **Class** : Indigoid

3.5 **Chemical name** : Disodium 3,3'-dioxo-2,2'-bi-indolylidene-5,5'-disulfonate.

3.6 **Empirical formula** :  $C_{16}H_8N_2Na_2O_8S_2$ .

3.7 **Molecular mass** : 466.36

3.8 **Structural formula** :



### 4 REQUIREMENTS

4.1 Indigo carmine shall consist of a mixture of disodium 3,3'-dioxo-2,2'-bi-indolylidene-5,5'-disulfonate and disodium 3,3'-dioxo-2,2'-bi-indolylidene-5,7'-disulfonate (isomer).

It shall not contain any extraneous matter injurious to health.

4.2 Indigo carmine shall be in the form of blue powder or granules.

4.3 Indigo carmine shall conform to the requirements given in Table 1 when tested by the methods prescribed in Column 4 of the table.

TABLE 1 - Requirements for indigo carmine

Sl. No. (1)	Characteristic (2)	Requirement (3)	Method of test (4)
(i)	Total dye content, per cent by mass, min.	85	Appendix A
(ii)	Isomer (disodium 3,3'-dioxo-2,2'-bi-indolylidene-5,7'-disulfonate) content, per cent by mass, max.	18	Appendix B
(iii)	Subsidiary dye content (excluding isomer) per cent by mass, max.	01	Appendix B
(iv)	Matter volatile at $135 \pm 1$ °C, per cent by mass and Chlorides and sulfates, as sodium salts, per cent by mass	15 max.	2.1, 2.5 and 2.6 of SLS 394:1976
(v)	Water insoluble matter, per cent by mass, max.		
(vi)	Organic compounds other than dye, per cent by mass, max. a) Isatin-5-sulfonic acid b) 5 - sulfoanthranilic acid c) Anthranilic acid	0.5	Appendix D
(vii)	Ether extractable matter, per cent by mass, max.		
(viii)	Arsenic, mg/kg, max.		
(ix)	Lead, mg/kg, max.	10	2.8 of SLS 394:1976
(x)	Heavy metals, as sulfides	Lighter in colour than the reference standard	

## 5 PACKAGING AND MARKING

### 5.1 Packaging

Indigo carmine shall be packed in suitable containers which shall not affect the nature and composition of the material.

### 5.2 Marking

5.2.1 Each container shall be legibly and indelibly marked or labelled with the following :

- a) Name of the product as "Indigo carmine, Food Grade";
- b) Colour index number " 73015 ";
- c) Brand name or trade name, if any;
- d) Net mass, in grams;
- e) Name and address of the manufacturer and/or distributor (including the country of origin); and
- f) Batch or code number.

5.2.2 Marking and labelling shall be in accordance with SLS 467.

#### NOTE

*Attention is drawn to certification marking facilities offered by the Sri Lanka Standards Institution. See the inside back cover of the standard.*

## 6 SAMPLING

Sampling shall be carried out in accordance with SLS 543. The composite sample for testing shall be prepared as given in the relevant clauses of SLS 543.

## 7 METHODS OF TEST

Tests shall be carried out as prescribed in relevant clauses of SLS 394 and Appendices A to E of this specification.

## 8 CRITERIA FOR CONFORMITY

A lot shall be declared as conforming to the requirements of this specification if the test results obtained on the composite sample satisfy the relevant requirements.



APPENDIX A  
DETERMINATION OF TOTAL DYE CONTENT

**A.1 APPARATUS**

A.1.1 *Spectrophotometer*, having a cell length of 10.0 mm.

A.1.2 *Oven*, maintained at 105 °C.

**A.2 REAGENT**

A.2.1 *Ammonium acetate*, 200 mg/l solution.

**A.3 PROCEDURE**

Weigh, to the nearest 0.01 mg, about 250 mg of the dye sample and dissolve in ammonium acetate solution (A.2) in a 250-ml volumetric flask. Dilute with the same solvent to make the final concentration of approximately 1 mg per 100 ml .

Measure the optical density of diluted solution against ammonium acetate solution as the blank at a wave length of 612 nm.

Simultaneously weigh, to the nearest milligram, about 2 g of the dye sample and dry in the oven (A.1.2) at 105  $\pm$  1 °C for 2 hours. Calculate the loss of mass on drying.

Calculate the dry mass of the sample (m) in the final solution taken for the measurement of the optical density.

**A.4 CALCULATION**

$$\text{Total dye content, per cent by mass} = \frac{A \times 100}{m \times 490}$$

where,

A is the measured optical density; and

m is the dry mass, in mg, of the sample in 100 ml of solution.

APPENDIX B

DETERMINATION OF SUBSIDIARY DYE CONTENT AND ISOMER (DISODIUM 3,3'-DIOXO-2,2'-BI-INDOLYLIDENE-5,7'-DISULFONATE) CONTENT

B.1 APPARATUS

The apparatus listed in 2.4.2 of SLS 394 : 1976

B.2 REAGENTS

B.2.1 *Atmosphere saturating solvent/developing solvent*

Butan - 2 - one : acetone : water (7:3:3)

B.2.2 *Extracting solvent*

Acetone : water (1:1)

B.2.3 *Hydrochloric acid, 0.05 mol/l solution.*

B.3 PROCEDURE

B.3.1 Proceed as given in 2.4.4 (a) and (b) of SLS 394 : 1976.

B.3.2 Proceed as given in 2.4.4 (c), with the following modifications.

- i) Do not include the strip of the 5 7' isomer separated as a wide blue zone just in front of the main blue band (See B.4.2).
- ii) Use 15 ml of concentration 0.05 mol/l solution of hydrochloric acid (B.2.3) instead of 15 ml of 0.05 N sodium hydrogen carbonate.

B.4 DETERMINATION AND CALCULATION

B.4.1 *Subsidiary dye content*

**Preparation of the standard solution**

Prepare 0.01 per cent solution from the 1.0 per cent solution of the dye sample. Apply 0.10 ml of this solution on a 180- mm x 7- mm rectangle of a chromatographic paper as uniformly as possible, holding the nozzle of the micro-syringe steadily in contact with the paper. Dry the paper at 50 °C to 60 °C for 10 minutes to 15 minutes. Cut the band from the paper as a strip. Cut an equivalent strip from a plain but marked paper. Proceed as given in 2.4.4 (c) of SLS 394 : 1976 and determine the net optical density ( $A_S$ ) of the standard solution.

$$\text{Subsidiary dyes, per cent by mass} = \frac{a + b + c + \dots + n}{A_S} \times L \times \frac{D}{100}$$

where,

a,b,c and etc are the net optical densities of the subsidiary dyes;  
 D is the total dye content of the sample (see Appendix A);  
 A<sub>S</sub> is the optical density of the standard solution; and  
 L is the limit of subsidiary dye content.

#### B.4.2 Isomer content

Using the isomer band obtained in B.3.2, proceed as given in 2.4.4(c) of SLS 394 : 1976 and measure the optical density at its  $\lambda_{\text{max}}$ .

Measure the optical density of the corresponding blank at the same wave length.

Use 0.1 ml of 0.20 per cent solution of the dye sample applied on a 180- mm x 7-mm rectangle and proceed as given in 2.4.4 (c) of SLS 394 : 1976. Measure the optical density of this standard solution.

$$\text{Isomer (disodium 3,3'-dioxo-2,2'-bi-indolylidene-5-7'-disulfonate) content, per cent by mass} = \frac{A}{A_S} \times \frac{20}{100} \times \frac{D}{100}$$

where,

A is the optical density of the isomer;  
 A<sub>S</sub> is the optical density of the standard solution; and  
 D is the total dye content of the sample (see Appendix A).

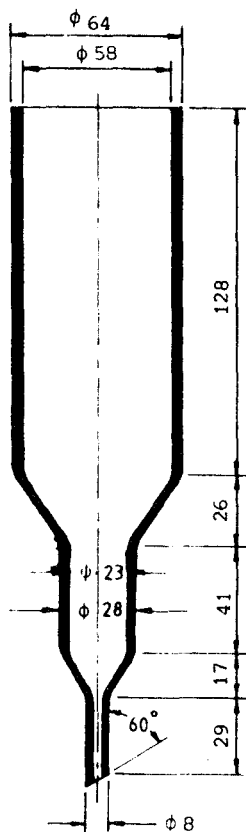
### APPENDIX C DETERMINATION OF WATER INSOLUBLE MATTER

Weigh, to the nearest milligram, about 2 g of the dye sample and proceed as given in 2.2 of SLS 394 : 1976.

APPENDIX D  
DETERMINATION OF ORGANIC COMPOUNDS  
OTHER THAN DYE

D.1 APPARATUS

D.1.1 *Chromatographic tube*, as given in Figure 1.



*Dimensions in millimetres*

FIGURE 1 - Chromatographic tube

D.1.2 *Spectrophotometer*, suitable for ultraviolet range.

## D.2 REAGENTS

The reagents used shall be of analytical grade.

D.2.1 *Ammonium sulfate*, 250 g/l solution

D.2.2 *Ammonium sulfate*

D.2.3 *Isatin - 5 - sulfonic acid*

D.2.4 *5 - sulfoanthranilic acid*

D.2.5 *Anthranilic acid*

## D.3 PROCEDURE

### D.3.1 Preparation of the column

Prepare a slurry using about 75 g of Whatman powdered cellulose or an equivalent and 500 ml of the ammonium sulfate solution (D.2.1). Wash the tube with 200 ml of the ammonium sulfate solution (D.2.1). Place a small disc of stainless steel gauze in the constriction above the tip of the tube. Pour sufficient amount of slurry into the tube until the tube is filled up to a level of 50 mm from the rim. Tap the tube occasionally to ensure a well packed column. Wash the column with 200 ml of the eluant.

### D.3.2 Determination

Weigh, to the nearest milligram, about 0.2 g of the dye sample in a beaker. Dissolve in 20 ml of water. Add approximately 5 g of powdered cellulose and 50 g of ammonium sulfate. Transfer the mixture to the column, rinse the beaker with the ammonium sulfate solution (D.2.1) and add the washings to the tube. Allow the column to drain until flow ceases, or nearly so. Add the ammonium sulfate solution to the column at a rate equivalent to the rate of flow through the column. Collect the effluent in 100-ml fractions. Collect twelve fractions. Reserve the column and the contents.

Shake each fraction and obtain the ultraviolet absorption spectrum from 220 nm to 400 nm. If the spectrum of the twelfth fraction shows the presence of any compound, continue collecting fractions until all compounds present are eluted.

Compare the absorption spectra of the eluted material with the spectra of solutions of pure compounds (D.2.3, D.2.4 and D.2.5) in the same solvent for identification and quantification.

#### NOTE

*If more than one compound is present in significant quantities in any fraction, the amounts of these compounds should be determined by the procedure customarily used in spectrophotometric analysis of mixtures of absorbing materials.*

### D.3.3 Correction for background absorption

Determine the absorption of the fractions collected from the column immediately before and after each fraction containing the compounds. Subtract one-half of the sum of these two determinations from the observed absorbance of the fractions containing the compounds. This should be taken as the absorbance due to the compound present.

### APPENDIX E DETERMINATION OF ETHER EXTRACTABLE MATTER

Weigh, to the nearest milligram, about 2 g of the dye sample and proceed as given in 2.3 of SLS 394 : 1976.

## **SLS CERTIFICATION MARK**

*The Sri Lanka Standards Institution is the owner of the registered certification mark shown below. Beneath the mark, the number of the Sri Lanka Standard relevant to the product is indicated. This mark may be used only by those who have obtained permits under the SLS certification marks scheme. The presence of this mark on or in relation to a product conveys the assurance that they have been produced to comply with the requirements of the relevant Sri Lanka Standard under a well designed system of quality control inspection and testing operated by the manufacturer and supervised by the SLSI which includes surveillance inspection of the factory, testing of both factory and market samples.*

*Further particulars of the terms and conditions of the permit may be obtained from the Sri Lanka Standards Institution, 17, Victoria Place, Elvitigala Mawatha, Colombo 08.*



## **SRI LANKA STANDARDS INSTITUTION**

The Sri Lanka Standards Institution (SLSI) is the National Standards Organization of Sri Lanka established under the Sri Lanka Standards Institution Act No. 6 of 1984 which repealed and replaced the Bureau of Ceylon Standards Act No. 38 of 1964. The Institution functions under the Ministry of Science & Technology.

The principal objects of the Institution as set out in the Act are to prepare standards and promote their adoption, to provide facilities for examination and testing of products, to operate a Certification Marks Scheme, to certify the quality of products meant for local consumption or exports and to promote standardization and quality control by educational, consultancy and research activity.

The Institution is financed by Government grants, and by the income from the sale of its publications and other services offered for Industry and Business Sector. Financial and administrative control is vested in a Council appointed in accordance with the provisions of the Act.

The development and formulation of National Standards is carried out by Technical Experts and representatives of other interest groups, assisted by the permanent officers of the Institution. These Technical Committees are appointed under the purview of the Sectoral Committees which in turn are appointed by the Council. The Sectoral Committees give the final Technical approval for the Draft National Standards prior to the approval by the Council of the SLSI.

All members of the Technical and Sectoral Committees render their services in an honorary capacity. In this process the Institution endeavours to ensure adequate representation of all view points.

In the International field the Institution represents Sri Lanka in the International Organization for Standardization (ISO), and participates in such fields of standardization as are of special interest to Sri Lanka.