Indigotine[1]

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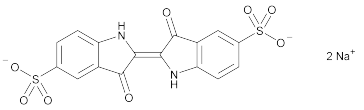
CI Food Blue 1
Indigotine Disulfonate
Indigo Carmine
CI 73015
Class: Indigoid



$C_{16}H_8N_2O_8S_2Na_2$ Formula wt 466.36
INS: 132 CAS: [860-22-0]
UNII: L06K8R7DQK [fd&c blue no. 2]

DESCRIPTION

Indigotine occurs as a blue-brown to red-brown powder or as granules. It is principally the disodium salt of 2-(1,3-dihydro-3-oxo-5-sulfo-2*H*-indol-2-ylidene)-2,3-dihydro-3-oxo-1*H*-indole-5-sulfonic acid. It dissolves in water to give a solution that is blue at neutrality, blue-violet in acid, and green to yellow-green in base. When dissolved in concentrated sulfuric acid, it yields a blue-violet solution that turns blue when diluted with water. It is insoluble in ethanol.

Function: Color

Packaging and Storage: Store in well-closed containers.

IDENTIFICATION

- **PROCEDURE**

Sample solution: 20 µg/mL, freshly prepared

Analysis: Adjust the pH of three aliquots of the *Sample solution* to pH 1, pH 7, and pH 13. Measure the absorbance intensities (A) and wavelength maxima of these solutions with a suitable UV-visible spectrophotometer.

Acceptance criteria

pH 1: A = 0.81 at 610 nm
pH 7: A = 0.82 at 610 nm
pH 13: A = 0.2 at 610 nm and A = 0.31 at 442 nm

ASSAY

- **TOTAL COLOR,** *Color Determination, Methods I and II,* Appendix IIIC**:** Both methods must be used.

Method I (Spectrophotometric)

Sample: 175 to 225 mg

Analysis: Transfer the *Sample* into a 1-L volumetric flask; dissolve in and dilute to volume with water. Determine as directed at 610 nm using 0.0478 L/(mg · cm) for the absorptivity (*a*) for Indigotine.

Method II ($TiCl_3$ Titration)

Sample: 0.3 g

Analysis: Determine as directed using 4.29 as the stoichiometric factor (F_S) for Indigotine.

Acceptance criteria: The average of results obtained from *Methods I and II* is NLT 85.0% total coloring matters.

IMPURITIES

Inorganic Impurities

- **ARSENIC,** *Arsenic Limit Test,* Appendix IIIB

Sample solution: Prepare as directed for organic compounds.

Acceptance criteria: NMT 3 mg/kg

- **LEAD,** *Lead Limit Test,* Appendix IIIB

Sample solution: Prepare as directed for organic compounds.

Control: 10 µg Pb (10 mL of *Diluted Standard Lead Solution*)

Acceptance criteria: NMT 10 mg/kg

- **MERCURY,** *Color Determination,* Appendix IIIC

Acceptance criteria: NMT 1 mg/kg

Organic Impurities

- **UNCOMBINED INTERMEDIATES AND PRODUCTS OF SIDE REACTIONS,** *Color Determination, Method I,* Appendix IIIC

Analysis: Calculate the concentration of isatin-5-sulfonic acid using an absorptivity of 0.089 L/(mg · cm) at 245 nm.

Acceptance criteria

Isatin-5-sulfonic acid: NMT 0.4%
5-Sulfoanthranilic acid: NMT 0.2%

SPECIFIC TESTS

- **COMBINED TESTS**

Tests

- LOSS ON DRYING (VOLATILE MATTER), *Color Determination,* Appendix IIIC
- CHLORIDE, *Sodium Chloride, Color Determination,* Appendix IIIC
- SULFATES (AS SODIUM SALTS), *Sodium Sulfate, Color Determination,* Appendix IIIC

Acceptance criteria: NMT 15.0% in combination

- **ETHER EXTRACTS,** *Color Determination,* Appendix IIIC

Acceptance criteria: NMT 0.2%

- **SUBSIDIARY AND ISOMERIC COLORS,** *Column Chromatography,* Appendix IIA

Mobile phase and stationary phase: Dissolve 20 g of hydroxylamine hydrochloride in 500 mL of water, place the solution into a 2-L separatory funnel, and add 450 mL of butanol, 450 mL of chloroform, 300 mL of water, and 100 mL of hydrochloric acid. Agitate the mixture well, periodically venting the funnel. After settling, separate and store the bottom layer (organic), which is the *Mobile phase*, and the top layer (aqueous), which is the *Stationary phase*.

Column: Slurry 12 g of Celite (Johns Manville No. 595, or equivalent) with 7 mL of *Stationary phase* and pour the slurry into a glass column (40- × 2.5-cm (id)).

Sample solution: 1 mg/mL in *Stationary phase*. [NOTE—Warm on a steam bath, if necessary, to dissolve the sample.]

[1] To be used or sold for use to color food that is marketed in the United States, this color additive must be from a batch that has been certified by the U.S. Food and Drug Administration (FDA). If it is not from an FDA-certified batch, it is not a permitted color additive for food use in the United States, even if it is compositionally equivalent. The name FD&C Blue No. 2 can be applied only to FDA-certified batches of this color additive. Indigotine is a common name given to the uncertified colorant. See the monograph entitled *FD&C Blue No. 2* for directions for producing an FDA-certified batch.

Analysis: Mix 5 mL of *Sample solution* with 10 g of Celite and pour the mixture into the column over the slurry, ensuring that the sample is quantitatively transferred to the column. Elute the column with *Mobile phase*. Collect the monosulfonated derivative, the first band eluting, in a 25-mL graduated cylinder, and note the volume. Collect the next band, the isomeric (unsulfonated) derivative, in a similar manner. Mix each aliquot collected with an equal volume of hexane, and transfer to a separatory funnel. Extract this mixture with three 15-mL aliquots of water and combine the extracts. Calculate the percent of the monosulfonated derivative (a = 0.0513 L/(mg · cm) at 615 nm) and the isomeric derivative (a = 0.0478 L/(mg · cm) at 610 nm) by the formula:

$$\text{Result} = (A \times V)/(a \times W \times 10)$$

A = absorbance at the wavelength maximum
V = volume (mL) of extract
a = absorptivity (L/(mg · cm))
W = weight (mg) of the sample taken to prepare the *Sample solution*

Acceptance criteria

2-(1,3-Dihydro-3-oxo-7-sulfo-2*H*-indole-2-ylidene)-2,3-dihydro-3-oxo-1*H*-indole-5-sulfonic acid, Disodium salt: NMT 18.0%

2-(1,3-Dihydro-3-oxo-2*H*-indole-2-ylidene)-2,3-dihydro-3-oxo-1*H*-indole-5-sulfonic acid, Sodium salt: NMT 2.0%

- **WATER-INSOLUBLE MATTER,** *Color Determination,* Appendix IIIC
  Acceptance criteria: NMT 0.4%

Indole

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C_8H_7N Formula wt 117.15
FEMA: 2593

UNII: 8724FJW4M5 [indole]

DESCRIPTION

Indole occurs as a white, lustrous, flaky, crystalline solid.
Odor: Unpleasant odor in high concentration, free of fecal quality; floral on dilution
Solubility: Soluble in alcohol, most fixed oils, propylene glycol; insoluble or practically insoluble in glycerin
Boiling Point: ~253° to 254°
Solubility in Alcohol, Appendix VI**:** One g dissolves in 3 mL of 70% alcohol.
Function: Flavoring agent

IDENTIFICATION

- **INFRARED SPECTRA,** *Spectrophotometric Identification Tests,* Appendix IIIC
  Sample preparation: Mineral oil mull
  Acceptance criteria: The spectrum of the sample exhibits relative maxima at the same wavelengths as those of the spectrum below.

ASSAY

- **PROCEDURE:** Proceed as directed under *M-1a,* Appendix XI.
  Acceptance criteria: NLT 99.0% of C_8H_7N

OTHER REQUIREMENTS

- **SOLIDIFICATION POINT,** Appendix IIB**:** Dry over H_2SO_4.
  Acceptance criteria: NLT 51°