INDIGOTINE

Prepared at the 28th JECFA (1984), published in FNP 31/1 (1984) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI of 0-5 mg/kg bw was established at the 18th JECFA (1974)

SYNONYMS CI Food Blue 1, FD&C Blue No. 2, Indigo Carmine, CI (1975) No. 73015, INS No. 132

DEFINITION Consists essentially of a mixture of disodium 3,3' -dioxo-[delta$^{2,2'}$-biindoline]-5,5'-disulfonate, and disodium 3,3'-dioxo-[delta$^{2,2'}$-biindoline]-5,7'-disulfonate and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components.

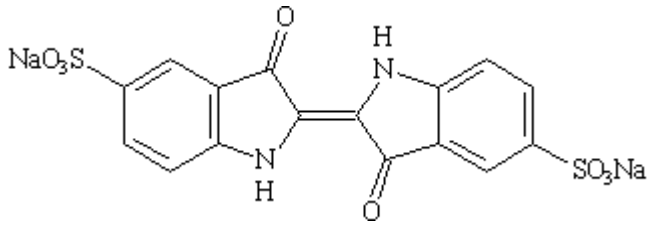
May be converted to the corresponding aluminium lake in which case only the *General Specifications for Aluminium Lakes of Colouring Matters* apply.

Chemical names: Disodium 3,3'-dioxo-[delta$^{2,2'}$-biindoline]-5,5'-disulfonate

C.A.S. number: 860-22-0 (5,5' isomer)

Chemical formula: $C_{16}H_8N_2Na_2O_8S_2$

Structural formula



Formula weight: 466.36

Assay: Not less than 85% total colouring matters.
Not more than 18% of disodium 3,3'-dioxo-[delta$^{2,2'}$-biindoline]-5,7'-disulfonate

DESCRIPTION Blue powder or granules

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Soluble in water; sparingly soluble in ethanol

Identification of colouring matters (Vol. 4): Passes test

PURITY

Loss on drying at 135°	Not more than 15% together with chloride and sulfate calculated as sodium salts
Water insoluble matter (Vol. 4)	Not more than 0.2%
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."
Subsidiary colouring matters (Vol. 4)	Not more than 1% (except disodium 3,3'-dioxo-[delta$^{2,2'}$-biindoline]- 5,7'-disulfonate) Use the following conditions: Developing solvent: No. 3 Height of ascent of solvent front: approximately 17 cm Note 1. The 5,7' isomer is separated as a wide blue zone just in front of the main blue band. Do not include this zone in the subsidiary colouring matter zones which are cut out and measured. Note 2. The 15 ml sodium hydrogen carbonate solution used in the general procedure is replaced by 15 ml 0.05 N hydrochloric acid in order to avoid the decomposition which the sulfonated indigo undergoes in alkaline solution.
Organic compounds other than colouring matters (Vol. 4)	Not more than 0.5% of sum of isatin-5-sulfonic acid, 5-sulfoanthranilic acid and anthranilic acid Use *liquid chromatography* under the following conditions: HPLC elution gradient: 2 to 100% gradient followed by elution at 100%
Unsulfonated primary aromatic amines (Vol. 4)	Not more than 0.01% calculated as aniline
Ether extractable matter (Vol. 4)	Not more than 0.2% Weigh accurately about 2 g sample instead of the 5 g stated in the general methods
METHOD OF ASSAY	Proceed as directed under *Total Content by Titration with Titanous Chloride* in Volume 4*,* using the following: Weight of sample: 1.0-1.1 g Buffer: 15 g sodium hydrogen tartrate Weight (D) of colouring matters equivalent to 1.00 ml of 0.1 N $TiCl_3$: 0.02332 g Isomer content by paper chromatography Refer to the conditions for the determination of subsidiary colouring matters (above). Cut the isomer band from the chromatogram in the manner detailed for the subsidiary bands, extract into solvent and measure the absorbance at its Smax. Measure the absorbance of the corresponding blank at the same wavelength. As a standard use 0.1 ml of an 0.20% solution of the sample applied to the 18 cm x 0.7 cm rectangle.

Isomer expressed as a percentage of the sample =
[A/As] x 20% x [D/100]

where A and As are the net absorbances of the isomer and standard, respectively, and D is the total colouring matters content of the sample.

Isomer content by HPLC

The 5,7' isomer separates under the HPLC conditions detailed above for the separation of subsidiary colouring matters, and the amount present can be quantified.