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INDIGOTINE

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INDIGOTINE

Prepared at the 86th JECFA and published in JECFA Monograph 22 (2018) superseding specifications 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) and confirmed at the 86th JECFA (2018).

SYNONYMS

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

DEFINITION

Indigotine consists of a mixture of disodium 3,3'-dioxo-[delta2,2'-biindoline]-5,5'-disulfonate and disodium 3,3'-dioxo-[delta2,2'-biindoline]-5,7'-disulfonate and subsidiary colouring matters. Sodium chloride and/or sodium sulfate are the principal uncoloured components. Indigotine is manufactured by heating indigo in the presence of sulfuric acid. The indigo (or indigo paste) is manufactured by the fusion of N-phenylglycine (prepared from aniline and formaldehyde) in a molten mixture of sodamide and sodium and potassium hydroxides under ammonia pressure. It is isolated and subjected to purification procedures prior to sulfonation.

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

Chemical names

Disodium 3,3'-dioxo-[delta2,2'-biindoline]-5,5'-disulfonate

Disodium (2E)-3-oxo-2-(3-oxo-5-sulfonato-2,3-dihydro-1H-indol-2-ylidene)-2,3-dihydro-1H-indole-5-sulfonate

Disodium;(2E)-3-oxo-2-(3-oxo-5-sulfonato-1H-indol-2-ylidene)-1H-indole-5-sulfonate

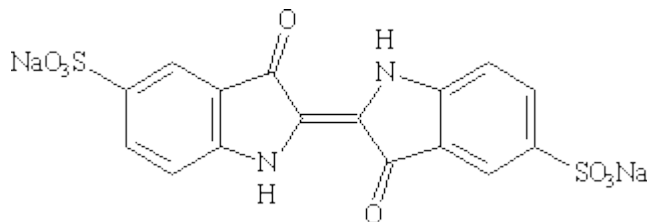
C.A.S. number

860-22-0 (5,5' isomer)

Chemical formula

C₁₆H₈N₂Na₂O₈S₂

Structural formula



Formula weight	466.36
Assay	Not less than 85% total colouring matters Not more than 18% of disodium 3,3'-dioxo-[delta2,2'-biindoline]-5,7'-disulfonate
DESCRIPTION	Blue powder or granules
FUNCTIONAL USES	Colour
CHARACTERISTICS	
IDENTIFICATION	
<u>Solubility</u> (Vol. 4)	Soluble in water, sparingly soluble in ethanol
<u>Spectrophotometry</u> (Vol. 4)	Maximum wavelength approximately 610 nm Determine the UV-visible absorption spectrum of the sample dissolved in water.
PURITY	
<u>Loss on drying, chloride and sulfate as sodium salts</u> (Vol. 4)	Not more than 15% Determine chloride as sodium chloride, sulfate as sodium sulfate, and loss on drying (135°, 6 h) as described in Volume 4 (under "Specific Methods, Food Colours").
<u>Water insoluble matter</u> (Vol. 4)	Not more than 0.2%
<u>Lead</u> (Vol. 4)	Not more than 2 mg/kg Determine using a method 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 (under “General Methods, Metallic Impurities”).

Subsidiary colouring matters

Not more than 18% disodium 3,3'-dioxo-[delta2,2'-biindoline]-5,7'-disulfonate (isomeric subsidiary colouring matter)

Not more than 1% other subsidiary colouring matters

See description under TESTS

Organic compounds other than colouring matters

Not more than 0.5% of sum of isatin-5-sulfonic acid, 5-sulfoanthranilic acid, and anthranilic acid

See description under TESTS

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

TESTS

PURITY TESTS

Subsidiary colouring matters

Determine subsidiary colouring matters content by reversed-phase HPLC (Vol. 4) using the following conditions:

- Column: C18 (250 mm x 4 mm i.d., 5 µm particle size)
- Eluent A: 0.2 M ammonium acetate in water
- Eluent B: acetonitrile
- Injection volume: 20 µl
- Column temperature: ambient
- Detector: UV-visible/diode array at 610 nm
- Flow rate: 1.0 ml/min

Gradient:

Elution time (min)	Eluent A (%)	Eluent B (%)
0	100	0
20.0	40	60

30.0	40	60
32.0	100	0
40.0	100	0

Reagents: HPLC grade

Standards:

- Indigotine disodium salt (5,7' isomer) (isomeric subsidiary colour) (C.A.S. 27414-68-2) – Angene Chemical, Cat. No. AGN-PC-0R372R or equivalent
- Sodium indigo sulfonate monosodium salt (monosulfonated subsidiary colour) (C.A.S. 27414-69-3) – Atomax Chemicals Co., Ltd., Cat No. AM27414693 or equivalent
- Trisodium indigo-5,5',7'-trisulfonate (trisulfonated subsidiary colour), potassium salt (C.A.S. 67627-18-3) – Sigma-Aldrich, Cat No. 234087 or equivalent
- Indigotine (C.A.S. No. 860-22-0) – TCI, Cat. No. F0148 or equivalent (use if subsidiary colouring matter standards are not available)

Prepare standard solutions as required.

Sample preparation:

Weigh accurately 100 ± 2 mg sample and dissolve in 100 ml of water. Dilute the solution, if required, to separate subsidiary colours from the primary colour component in order to improve their resolution. Analyze immediately after preparation.

Calculations:

Construct the relevant standard curves. Integrate all peaks of the chromatogram obtained at 610 nm. If Indigotine is used as a standard, calculate the ratio of the sum of all peaks not corresponding to Indigotine to the sum of all peaks.

Organic compounds other than colouring matters

Determine organic compounds other than colouring matters content by reversed-phase HPLC (Vol. 4) using the following conditions:

- Column: Luna C18 (250 mm x 4.6 mm i.d., 5 μ m particle size) or equivalent
- Eluent A: 0.1% trifluoroacetic acid in water
- Eluent B: acetonitrile
- Injection volume: 20 μ l
- Column temperature: 25°
- Detector: UV-visible/diode array at 244 nm
- Flow rate: 1.0 ml/min

Gradient:

Elution time (min)	Eluent A (%)	Eluent B (%)
0	100	0
15	80	20
20	0	100
22	0	100
22.1	100	0
32	100	0

Reagents: HPLC grade

Standards:

- Isatin-5-sulfonic acid sodium salt dihydrate (C.A.S. 207399-16-4) – Sigma-Aldrich Cat. No. 58245 or equivalent
- 5-Sulfoanthranilic acid (2-amino-5-sulfobenzoic acid) (C.A.S. 3577-63-7) – TCI Cat No. S0802 or equivalent
- Anthranilic acid (C.A.S. 118-92-3) – Sigma-Aldrich Cat No. A89855 or equivalent

Prepare standard solutions as required.

Sample preparation:

Weigh accurately 100 ± 2 mg sample and dissolve in 100 ml of water. Analyse immediately after preparation.

Calculations:

Construct the relevant standard curves. Integrate the chromatogram peaks obtained at 244 nm.

METHOD OF ASSAY

Determine total colouring matters content by spectrophotometry using Procedure 1 in Volume 4 (under “Specific Methods, Food Colours”) and an appropriate solvent. Analyse immediately after preparation.

Using water as the solvent:

absorptivity (a) = $48.0 \text{ l}/(\text{g} \times \text{cm})$
wavelength of maximum absorbance = 610 nm.

Determine isomer content by HPLC using the test for subsidiary colouring matters.