

- **LEUCO BASE**, *Color Determination*, Appendix III C

Sample solution: 130 µg/mL

Acceptance criteria: NMT 5.0%

- **SUBSIDIARY COLORS**, *Thin-Layer Chromatography*, Appendix II A

Adsorbent: Silica Gel G

Developing solvent system: Acetonitrile, isoamyl alcohol, 2-butanone, water, and ammonium hydroxide [10:10:3:2:1]

Sample solution: Transfer 1 g of sample into a 100-mL volumetric flask. Fill the flask about 3/4 full with water, place it in the dark for 1 h, dilute to volume with water, and mix well.

Application volume: 0.1 mL

Analysis: Prepare a 20- × 20-cm glass plate coated with a 0.25-mm layer of Adsorbent. Spot the Sample solution 3 cm from the bottom edge. Allow the plate to dry for about 20 min in the dark, then develop with the Developing solvent system in an unlined tank equilibrated for at least 20 min before the plate is inserted. Allow the solvent front to reach within about 3 cm of the top of the plate. Dry the developed plate in the dark. When the plate has dried, scrape off all the colored bands above the Fast Green, which remains close to the origin, into a 30-mL beaker. Extract the subsidiary colors with three 6-mL portions of 95% ethanol, or until no color remains on the gel by visual inspection. Record the volume of ethanol used and the spectrum of the solution between 400 and 700 nm. Calculate the percent of subsidiary colors by the formula:

$$\text{Result} = (A \times V \times 100) / (a \times W \times b)$$

- A = absorbance at the wavelength maximum
 V = volume (mL) of the ethanol extract
 a = absorptivity (0.126 L/(mg · cm))
 W = weight (mg) of the sample taken to prepare the Sample solution
 b = cell pathlength (cm)

Acceptance criteria

Isomeric inner salt of N-ethyl-N-[4-[[4-[ethyl[(3-sulfophenyl)methyl] amino]phenyl](4-hydroxy-2-sulfophenyl)-methylene]-2,5-cyclohexadien-1-ylidene]-4-sulfobenzene methanaminium hydroxide, Disodium salt
 and

N-ethyl-N-[4-[[4-[ethyl[(4-sulfophenyl)methyl]amino]phenyl](4-hydroxy-2-sulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-4-sulfobenzenemethanaminium hydroxide
 and

N-ethyl-N-[4-[[4-[ethyl[(2-sulfophenyl)methyl] amino] phenyl](4-hydroxy-2-sulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-3-sulfobenzenemethanaminium hydroxide:
 NMT 6.0%, combined

- **WATER-INSOLUBLE MATTER**, *Color Determination*, Appendix III C

Acceptance criteria: NMT 0.2%

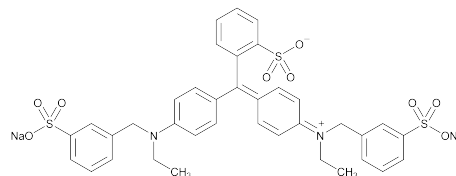
FD&C Blue No. 1¹

First Published: Prior to FCC 6

Brilliant Blue FCF²

CI 42090²

Class: Triphenylmethane



C₃₇H₃₄N₂O₉S₃Na₂

INS: 133

UNII: H3R47K3TBD [fd&c blue no. 1]

Formula wt 792.86

CAS: [3844-45-9]

DESCRIPTION

FD&C Blue No. 1 is principally the disodium salt of ethyl[4-[p-[ethyl(m-sulfobenzyl)amino]-α-(o-sulfophenyl)benzylidene]-2,5-cyclohexadien-1-ylidene](m-sulfobenzyl)ammonium hydroxide inner salt, with smaller amounts of the isomeric disodium salts of ethyl[4-[p-[ethyl(p-sulfobenzyl)amino]-α-(o-sulfophenyl)benzylidene]-2,5-cyclohexadien-1-ylidene](p-sulfobenzyl)ammonium hydroxide inner salt and ethyl[4-[p-[ethyl(o-sulfobenzyl)amino]-α-(o-sulfophenyl)benzylidene]-2,5-cyclohexadien-1-ylidene](o-sulfobenzyl)ammonium hydroxide inner salt.

Function: Color

Packaging and Storage: Store in well-closed containers. [NOTE—FDA-certifiable color additives are batch certified by the United States Food and Drug Administration using analytical chemistry methods developed for this purpose by the FDA. The color additive regulations are described in Title 21, Parts 70 to 82, of the United States Code of Federal Regulations (21 CFR Parts 70 to 82). The batch certification process is described in 21 CFR Part 80. Current certification analytical methods are available from the Office of Cosmetics and Colors, Colors Certification Branch (HFS-107), U.S. Food and Drug Administration, 5100 Paint Branch Parkway, College Park, Maryland 20740.]

IDENTIFICATION

- **VISIBLE ABSORPTION SPECTRUM**

Acceptance criteria: A sample dissolved in 0.04 N aqueous ammonium acetate gives a spectrum exhibiting a wavelength maximum at 630 nm, with an absorptivity of 0.164 L/(mg · cm).

ASSAY

- **TOTAL COLOR**

Acceptance criteria: NLT 85.0%

¹ To be used or sold in the United States, this color additive must be batch certified by the U.S. Food and Drug Administration. The monograph title is the name of the color additive only after batch certification has been completed.

² Generic designations; not synonyms for certified batches of color additive.

IMPURITIES**Inorganic Impurities**

- **ARSENIC (AS AS)**
Acceptance criteria: NMT 3 mg/kg
- **CHROMIUM (AS CR)**
Acceptance criteria: NMT 0.005%
- **LEAD (AS PB)**
Acceptance criteria: NMT 10 mg/kg
- **MANGANESE (AS MN)**
Acceptance criteria: NMT 0.01%

Organic Impurities**• UNCOMBINED INTERMEDIATES AND PRODUCTS OF SIDE REACTIONS****Acceptance criteria**

- o*-, *m*-, and *p*-Sulfobenzaldehydes: NMT 1.5%, combined
- N*-Ethyl-*N*-(*m*-sulfobenzyl)sulfanilic acid: NMT 0.3%

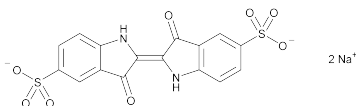
SPECIFIC TESTS

- **ETHER EXTRACTS³ (COMBINED)**
Acceptance criteria: NMT 0.4%
- **LEUCO BASE**
Acceptance criteria: NMT 5%
- **SUBSIDIARY COLORS**
Acceptance criteria: NMT 6%
- **VOLATILE MATTER (AT 135°) AND CHLORIDES AND SULFATES (AS SODIUM SALTS)**
Acceptance criteria: NMT 15.0% in combination
- **WATER-INSOLUBLE MATTER**
Acceptance criteria: NMT 0.2%

FD&C Blue No. 2¹

First Published: Prior to FCC 6

Indigotine²
Indigo Carmine²
CI 73015²
Class: Indigoid



Formula wt 466.36
CAS: [860-22-0]

INS: 132
UNII: L06K8R7DQK [fd&c blue no. 2]

DESCRIPTION

FD&C Blue No. 2 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, with smaller amounts of the

disodium salt of 2-(1,3-dihydro-3-oxo-7-sulfo-2*H*-indol-2-ylidene)-2,3-dihydro-3-oxo-1*H*-indole-5-sulfonic acid and the sodium salt of 2-(1,3-dihydro-3-oxo-2*H*-indol-2-ylidene)-2,3-dihydro-3-oxo-1*H*-indole-5-sulfonic acid.

Function: Color**Packaging and Storage:** Store in well-closed containers.

[NOTE—FDA-certifiable color additives are batch certified by the United States Food and Drug Administration using analytical chemistry methods developed for this purpose by the FDA. The color additive regulations are described in Title 21, Parts 70 to 82, of the United States Code of Federal Regulations (21 CFR Parts 70 to 82). The batch certification process is described in 21 CFR Part 80. Current certification analytical methods are available from the Office of Cosmetics and Colors, Colors Certification Branch (HFS-107), U.S. Food and Drug Administration, 5100 Paint Branch Parkway, College Park, Maryland 20740.]

IDENTIFICATION**• VISIBLE ABSORPTION SPECTRUM**

Acceptance criteria: A sample dissolved in 0.04 N aqueous ammonium acetate gives a spectrum exhibiting a wavelength maximum at 610 nm, with an absorptivity of 0.0478 L/(mg · cm).

ASSAY

- **TOTAL COLOR**
Acceptance criteria: NLT 85%

IMPURITIES**Inorganic Impurities**

- **ARSENIC (AS AS)**
Acceptance criteria: NMT 3 mg/kg
- **LEAD (AS PB)**
Acceptance criteria: NMT 10 mg/kg
- **MERCURY (AS HG)**
Acceptance criteria: NMT 1 mg/kg

Organic Impurities**• DECOMPOSITION PRODUCTS**

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

SPECIFIC TESTS

- **ETHER EXTRACTS³ (COMBINED)**
Acceptance criteria: NMT 0.4%
- **SUBSIDIARY AND ISOMERIC COLORS**
Acceptance criteria
2-(1,3-Dihydro-3-oxo-7-sulfo-2*H*-indol-2-ylidene)-2,3-dihydro-3-oxo-1*H*-indole-5-sulfonic acid, Disodium salt: NMT 18%
2-(1,3-Dihydro-3-oxo-2*H*-indol-2-ylidene)-2,3-dihydro-3-oxo-1*H*-indole-5-sulfonic acid, Sodium salt: NMT 2%
- **VOLATILE MATTER (AT 135°) AND CHLORIDES AND SULFATES (AS SODIUM SALTS)**
Acceptance criteria: NMT 15% in combination
- **WATER INSOLUBLE MATTER**
Acceptance criteria: NMT 0.4%

³ Not required for certification in the United States.

¹ To be used or sold in the United States, this color additive must be batch certified by the U.S. Food and Drug Administration. The monograph title is the name of the color additive only after batch certification has been completed.

² Generic designations; not synonyms for certified batches of color additive.

³ Not required for certification in the United States.