ERYTHROSINE

Prepared at the 41st JECFA (1993), published in FNP 52 Add 2 (1993) superseding specifications prepared at the 37th JECFA (1990), published in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI of 0-0.1 mg/kg bw was established at the 37th JECFA (1991)

SYNONYMS CI Food Red 14, FD&C Red No. 3; C.I. (1975) No. 45430 INS No. 127

DEFINITION Consists essentially of disodium salt of 9-(o-carboxyphenyl)-6-hydroxy-2,4,5,7-tetraiodo-3-isoxanthone monohydrate and subsidiary colouring matters together with water, sodium chloride and/or sodium sulfate as the principal uncoloured components.

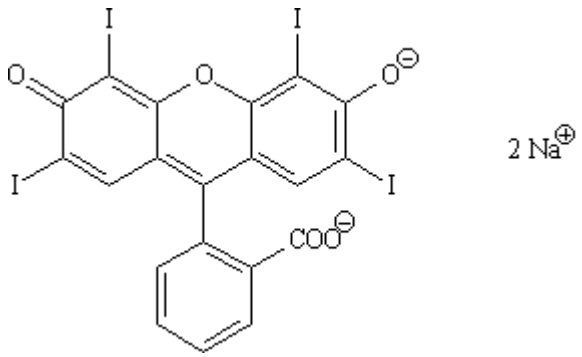
May be converted to the corresponding aluminium lake in which case only the *General Specifications for Aluminium Lakes of Colouring Matters* shall apply.

Chemical names Disodium salt of 9-(o-carboxyphenyl)-6-hydroxy-2,4,5,7-tetraiodo-3-isoxanthone monohydrate

C.A.S. number 16423-68-0

Chemical formula $C_{20}H_6I_4Na_2O_5 \cdot H_2O$

Structural formula



Formula weight 897.88

Assay Not less than 87% total colouring matters

DESCRIPTION Red powder or granules

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Soluble in water and in ethanol

Identification of colouring matters (Vol. 4) Passes test

PURITY

Loss on drying at 135° (Vol. 4)	Not more than 13% together with chloride and sulfate calculated as sodium salts
Inorganic iodides	Not more than 0.1% calculated as sodium iodide See description under TESTS
Water insoluble matter (Vol. 4)	Not more than 0.2%
Zinc (Vol. 4)	Not more than 50 mg/kg
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 4% (except fluorescein) Use the following conditions: Developing solvent: No. 5 Height of ascent of solvent front: 17 cm Note: Take special care not to allow the chromatograms to be exposed to direct sunlight.
Fluorescein	Not more than 20 mg/kg See description under TESTS
Organic compounds other than colouring matters (Vol. 4)	Tri-iodoresorcinol: Not more than 0.2% 2-(2,4-dihydroxy-3,5-di-iodobenzoyl) benzoic acid: Not more than 0.2% Proceed as directed under *Column chromatography*, using, for example, the following absorptivities: 2(2,4-dihydroxy-3,5-di-iodobenzoyl)benzoic acid: 0.047 mg $L^{-1}cm^{-1}$ at 348 nm (alkaline) Tri-iodoresorcinol: 0.079 mg $L^{-1}cm^{-1}$ at 223 nm (acidic).
Ether extractable matter (Vol. 4)	From a solution of pH not less than 7, not more than 0.2%

TESTS

PURITY TESTS

Inorganic iodides	Weigh 1.0 g of the sample into a 100-ml beaker. Add 75 ml distilled water and the magnetic follower. Stir to dissolve. Immerse a iodide specific electrode and a reference electrode in the solution and set a suitable millivoltmeter to read the potential of the system in millivolts. Add 0.001 M silver nitrate solution from a burette initially in 0.5 ml aliquots, reducing these to 0.1 ml as the end-point approaches as indicated by an

increasing change in potential for each addition. After allowing time for the reading to stabilize, record the millivolt readings after each addition. Continue the titration until further additions make little change in the potential.

Plot the millivolt readings against the volume of silver nitrate solution added. The equivalent point is the volume corresponding to the maximum slope of the curve. The percentage of sodium iodide in sample is: Titre x 0.015%

Fluorescein

Principle
The fluorescein is separated from the sample by TLC and compared with a standard chromatogram prepared from fluorescein at the concentration corresponding to the limit figure.

Solvent
Methanol+water+ammonia (s.g. 0.890) (500 ml+400 ml+100 ml)

Sample
Weigh 1.0 g of the sample, dissolve in about 50 ml solvent and dilute to 100 ml in a volumetric flask.

Standard
Weigh an amount of fluorescein, previously purified by recrystallisation from ethanol, equal to 1 g x the colouring matter content of the sample as determined under Assay. Dissolve in water (or in water with 10 ml ammonia s.g. 0.890 if fluorescein-free acid is being used) and dilute to 100 ml. Make further sequential dilutions as follows:
- 1 ml to 100 ml with water
- 1 ml to 100 ml with water
- 20 ml to 100 ml with solvent

Chromatography solvent
n-Butanol+water+ammonia (s.g. 0.890)+ethanol (100 ml+44 ml+1 ml+ 22.5 ml)

Procedure
Spot 25 µl of the sample and standard solutions side by side on a cellulose plate. Develop for 16 h in the chromatography solvent. Allow the plate to dry. View under a UV light source and compare the fluorescence of the standard with the fluorescence of the corresponding area on the chromatogram of the sample. The intensity of the latter shall not be greater than that of the former.

Note**:** Take special care not to allow the chromatograms to be exposed to direct sunlight.

METHOD OF ASSAY

Dissolve about 1 g of the sample, accurately weighed, in 250 ml of water, transfer to a clean 500-ml beaker, add 8.0 ml of 1.5 N nitric acid and stir well. Filter through a sintered glass crucible (porosity 3, diameter 5 cm) which has been weighed containing a small glass stirring rod. Wash thoroughly with 0.5% nitric acid until the filtrate gives no turbidity with silver nitrate TS, and then wash with 30 ml water. Dry to constant weight at 135±5º, carefully breaking up the precipitate by means of the glass rod.

Cool in a desiccator and weigh.

$$Total\ colouring\ matters = \frac{weight\ of\ residue\ x\ 107.4}{weight\ of\ sample}\ \%$$

Determination of Hydrochloric Acid-insoluble Matter in Erythrosine Lake

Reagents
- Concentrated hydrochloric acid
- Hydrochloric acid 0.5% v/v
- Dilute ammonia solution (dilute 10 ml ammonia, s.g. 0.890 to 100 ml with water).

Procedure
Accurately weigh approximately 5 g of the lake into a 500-ml beaker. Add 250 ml water and 60 ml concentrated hydrochloric acid. Boil to dissolve the alumina while the Erythrosine converts to its "free acid" form, which is insoluble in acid. Filter through a tared No. 4 sintered glass crucible. Wash the crucible with a small amount of hot 0.5% hydrochloric acid and then with some hot distilled water. Remove the acid filtrate from the filter flask, replace the crucible and wash with hot dilute ammonia solution until the washings are colourless. Dry the crucible to constant weight at 135º. Express the residue as a percentage of the weight taken.