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Residue Monograph prepared by the meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), 86th Meeting 2018

ERYTHROSINE

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ERYTHROSINE

Prepared at the 86th JECFA and published in FAO JECFA Monograph 22 (2018) superseding specifications prepared at the 41st JECFA (1993), published in FNP 52 Add 2 (1993). 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) and confirmed at the 86th JECFA (2018).

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

DEFINITION Erythrosine consists of the disodium salt of 2-(2,4,5,7-tetraiodo-6-oxido-3-oxoxanthen-9-yl)benzoate monohydrate and subsidiary colouring matters. Sodium chloride and/or sodium sulfate are the principal uncoloured components. Erythrosine is manufactured by iodination of fluorescein, the condensation product of resorcinol and phthalic anhydride.

Erythrosine 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 2-(2,4,5,7-tetraiodo-6-oxido-3-oxoxanthen-9-yl)benzoate monohydrate;

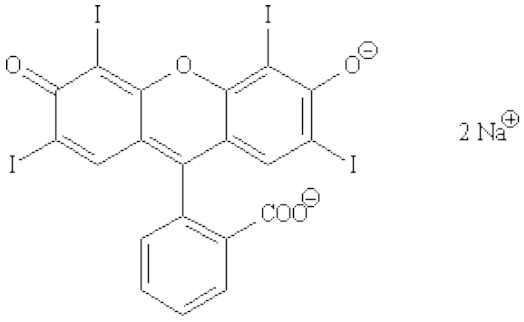
Disodium;2',4',5',7'-tetraiodo-3-oxospiro[2-benzofuran-1,9'-xanthene]-3',6'-diolate;

Disodium 2′,4′,5’,7′-tetraiodofluorescein monohydrate

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

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

Structural formula



Formula weight	879.86
Assay	Not less than 87% total colouring matters
DESCRIPTION	Red powder or granules
FUNCTIONAL USES	Colour
CHARACTERISTICS	
IDENTIFICATION	
Solubility (Vol. 4)	Soluble in water, slightly soluble in ethanol
Spectrophotometry (Vol. 4)	Maximum wavelength approximately 527 nm Determine the UV-visible absorption spectrum of the sample dissolved in water.
PURITY	
Loss on drying, chloride and sulfate as sodium salts (Vol. 4)	Not more than 13% 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”).
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 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”).
Lead (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 4% (except fluorescein) See description under TESTS Note: Do not allow the sample and standard solutions to be exposed to direct sunlight.
Fluorescein	Not more than 20 mg/kg See description under TESTS
Organic compounds other than colouring matters	Triiodoresorcinol: Not more than 0.2% 2-(2,4-dihydroxy-3,5-diiodobenzoyl)benzoic acid: Not more than 0.2% See description under TESTS
Ether extractable matter (Vol. 4)	From a solution of pH not less than 7, not more than 0.2%
Hydrochloric acid-insoluble matter in Erythrosine Lake	Not more than 0.5% See description under TESTS

TESTS

PURITY TESTS

Inorganic iodides	Weigh 1.0 g of the sample into a 100-ml beaker. Add 75 ml distilled water and a magnetic stirrer. Stir to dissolve. Immerse an iodide specific electrode and a reference electrode in the solution and use 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 equivalence point is the volume corresponding to the maximum slope of the curve.

The $\text{percentage of sodium iodide in the sample} = \text{Titre} \times 0.015\%$

where

Titre	ml-equivalent of silver nitrate solution
0.015%	0.001 mol/l x 10^{-3} l/ml x 149.89 g sodium iodide/mol x 1 mol/equivalent x 1/1.0 g (sample weight) x 100.

Subsidiary colouring matters

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

- Column: C8 (250 mm x 4.6 mm i.d., 5 µm particle size)
- Eluent A: 0.1 M ammonium acetate in water
- Eluent B: methanol
- Injection volume: 20 µl
- Column temperature: ambient
- Detector: UV-visible/diode array at 514 nm
- Flow rate: 1.0 ml/min

Gradient:

Elution time (min)	Eluent A (%)	Eluent B (%)
0	55	45
20	34	66
21.1	0	100
25.5	0	100
26.0	55	45
40.0	55	45

Reagents: HPLC grade

Standards:

- 2′,4′,5′-Triiodofluorescein (C.A.S. 56254-06-9) – synthesized material (see Appendix)

- 2′,4′,7′-Triiodofluorescein (C.A.S. 83498-90-2) – synthesized material (see Appendix)
- 4′,5′-Diiodofluorescein, disodium salt (C.A.S. 33239-19-9) – Alfa Aesar, Cat. No. A15626 or equivalent
- 2′-Monoiodofluorescein, disodium salt (C.A.S. 52010-85-2) – synthesized material (see Appendix)
- 4′-Monoiodofluorescein, disodium salt (C.A.S. 52010-86-3) – synthesized material (see Appendix)
- Erythrosine (C.A.S. 16423-68-0) – TCI, >95.0% disodium 2′,4′,5’,7′-tetraiodofluorescein, Cat. No. F0139 or equivalent (use if subsidiary colouring matter standards are not available)

Prepare standard solutions as required.

Sample preparation:

Weigh accurately 200±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.

Calculations:

Construct the relevant standard curves. Integrate all peaks of the chromatogram obtained at 514 nm. If Erythrosine is used as a standard, calculate the ratio of the sum of all peaks not corresponding to Erythrosine to the sum of all peaks.

Fluorescein

Determine fluorescein by the test for subsidiary colouring matters content except use the following conditions:

- Injection volume: 50 µl
- Detector: UV-visible/diode array at 492 nm

Standard: Fluorescein, disodium salt (C.A.S. 518-47-8) – TCI, Cat. No. F0096 or equivalent

Sample preparation:

Weigh accurately 2.00±0.05 g sample and dissolve in 10 ml of water.

Organic compounds other than colouring matters

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

- Column: C18 (150 mm x 2.1 mm i.d., 5 µm particle size)
- Eluent A: 0.05 M sodium dihydrogen phosphate in 95/5 water/methanol, pH 4.0
- Eluent B: methanol
- Injection volume: 5 µl
- Column temperature: 27°
- Detector: UV-visible/diode array at 223 nm

- Flow rate: 0.5 ml/min

Gradient:

Elution time (min)	Eluent A (%)	Eluent B (%)
0	95	5
3	95	5
5	80	20
13	35	65
15	0	100
25	0	100
27	95	5
37	95	5

Reagents: HPLC grade

Standards:
- 2,4,6-Triiodoresorcinol (C.A.S. 19403-92-0) – Alfa Chemistry, Cat. No. ACM19403920 or equivalent
- 2-(2,4-Dihydroxy-3,5-diiodobenzoyl)benzoic acid (C.A.S. 3480-21-5) – Wako, Cat. No. 043-32981 or equivalent

Prepare standard solutions as required. Dissolve the standards in methanol. Use an amber glass volumetric flask for 2,4,6-triiodoresorcinol and prepare the standard and calibration solutions immediately before use.

Sample preparation:

Weigh accurately 100±5 mg sample and dissolve in 10 ml of methanol.

Calculations:

Construct the relevant standard curves. Integrate all peaks of the chromatogram obtained at 223 nm.

Hydrochloric acid-insoluble matter in

Reagents
- Concentrated hydrochloric acid
- Hydrochloric acid, 0.5% v/v

Erythrosine Lake

- Dilute ammonium hydroxide solution (dilute 10 ml of 14.5 M ammonium hydroxide 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 ammonium hydroxide solution until the washings are colourless. Dry the crucible to constant weight at 135º. Express the residue as a percentage of the weight taken.

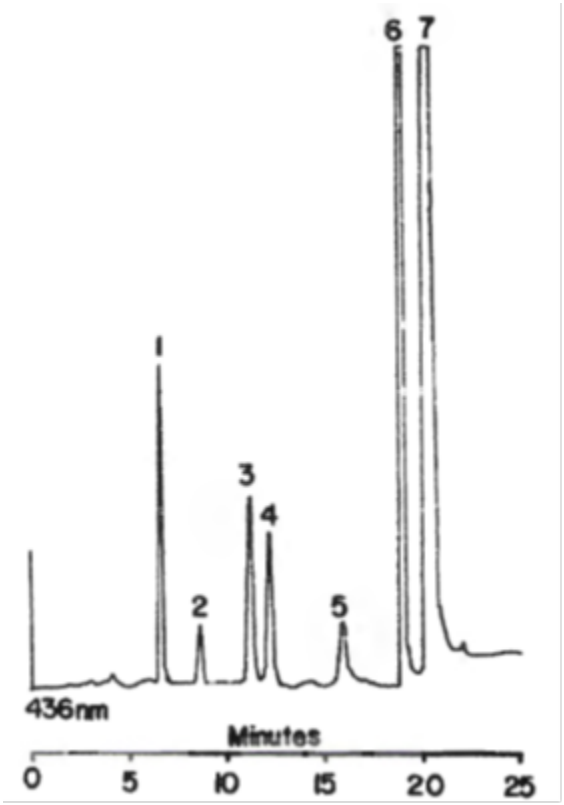
METHOD OF ASSAY

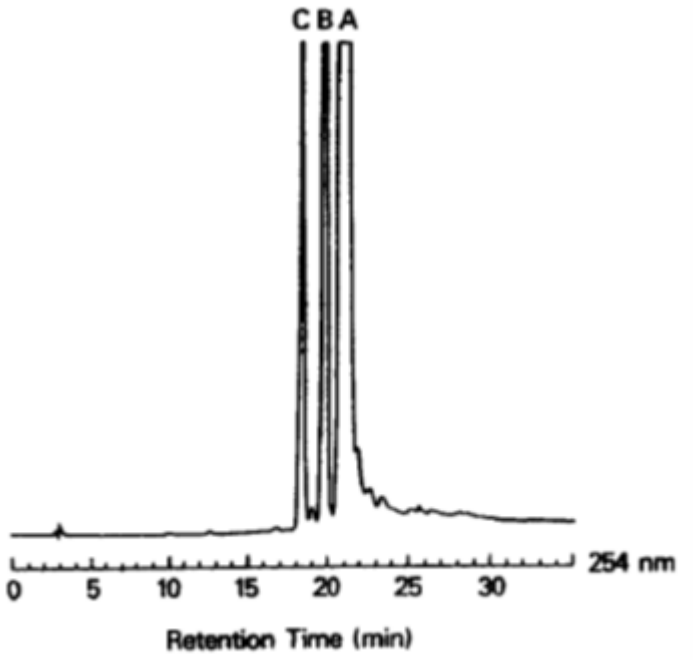
Determine total colouring matters content by spectrophotometry using Procedure 1 in Volume 4 (under “Specific Methods, Food Colours”) and an appropriate solvent.

Using water as the solvent:
absorptivity $(a) = 110\ l/(g \times cm)$
wavelength of maximum absorbance = 527 nm.

Appendix. Typical chromatograms of Erythrosine

B A





1 – FluoresceinA – 2′,4′,5’,7′-tetraiodofluorescein
2 – 4’-IodofluoresceinB – 2′,4′,7′-triiodofluorescein

3 – 2'-IodofluoresceinC – 2′,4′,5′-triiodofluorescein
4 – 4',5'-Diiodofluorescein
5 – 2',5'-Diiodofluorescein
6 – 2',7'-Diiodofluorescein and 2',4',5'-Triiodofluorescein
7 – 2',4',7'-Triiodofluorescein and 2′,4′,5',7′-tetraiodofluorescein

[References] (A) Calvey, R. J., and Goldberg, A. J., High performance liquid chromatographic determination of subsidiary colors in FD&C Red No. 3, Journal of Association of Official Analytical Chemists, vol. 65, pp. 1080-1085, 1982.
(B) Weisz, A., Andrzejewski, D., Highet, R. J., and Ito, Y., Preparative separation of components of the color additive FD&C Red No. 3 (Erythrosine) by pH-zone-refining counter-current chromatography, Journal of Chromatography A, vol. 658, pp. 505-510, 1994.