BUREAU OF INDIAN STANDARDS

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Draft Indian Standard

QUINIZARIN, TECHNICAL — SPECIFICATION

(*First Revision* of IS 6265)

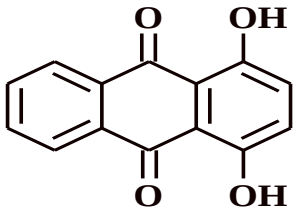
(ICS 71.080.99)

Dye Intermediate Sectional Committee, PCD 26	Last date for Comments is 25th December 2023

FOREWORD

(*Formal clauses to be added later*)

Quinizarin or 1, 4-dihydroxyanthraquinone ($C_{14}H_8O_4$) is a very important dye intermediate used extensively in the manufacture of vat dyes, disperse dyes, etc. It also finds use as an antioxidant in synthetic lubricants. When heated it emits acrid fumes. It is represented by the following structural formula:



QUINIZARIN
Molecular Mass: 240.21
CAS No.: 81-64-1

This standard was originally published in 1971. The committee responsible for the preparation of this standard decided to update it in light of experience gained. In this (*first*) revision, High-performance liquid chromatography method for determination of assay has been incorporated and requirements for characteristics such as matter insoluble in toluene, moisture content and ash content are updated.

The bags in which the material is stored or transported may also be labelled with pictograms, signal word, hazard statement, and precautionary statement as given in Annex C, which are derived from GHS guidelines. At the time of publication, the latest edition of GHS guidelines was referred and are subject to revision and parties to agreement, are encouraged to investigate the possibility of applying the most recent labels as indicated.

For the purpose of deciding whether a particular requirement of this standard is complied with the final value, observed or calculated expressing the result of a test or analysis shall be rounded off in accordance with IS 2 : 2022 'Rules for rounding off numerical values (*second revision*)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

1 SCOPE

This standard prescribes the requirements, the methods of sampling and test for quinizarin, technical.

2 REFERENCES

The following standards contain provisions, which through reference in this text, constitute provisions of this standard. At the time of publication the editions indicated were valid. All standards are subject to revision and parties to agreements based on this standard are encouraged to investigate the possibilities of applying the most recent editions of the standards indicated below.

IS No.	*Title*
1070 : 2023	Reagent grade water — Specification *(fourth revision)*
5299 : 2001	Methods of sampling and tests for dye intermediate (*first revision*)

3 REQUIREMENTS

3.1 Description

The material shall be in the form of red or orange-red crystalline powder, and shall be free from visible impurities. It is soluble in hot water, alcohol, ether and benzene and in potassium hydroxide solution and sulphuric acid.

3.2 The material shall also comply with the requirements given in Table 1, when tested according to the methods prescribed col 4 and 5 of Table 1.

Table 1 Requirements for Quinizarin, Technical
(*Clause* 3.2, 5.3.1, 5.3.2 *and* 6.1)

Sl No.	**Characteristic**	**Requirement**	**Method of Test, Ref to**	
			Annex	Clause of IS 5299
(1)	(2)	(3)	(4)	(5)
i)	Assay by HPLC[1)], percent by area, *Min*	93.0	A	
	Or			
ii)	Assay, percent by weight, *Min*	89	B	
iii)	Moisture content, percent by mass, *Max*	1.0	—	10.3
iv)	Ash, percent by mass, *Max*	0.5	—	12.2
v)	Melting point, °C	Shall melt within the range of 3 °C including	—	9

		191 °C		
vi)	Matter insoluble in toluene, percent by mass, *Max*	1.0	—	11.3

[1)]In case of disputes, determination of assay by HPLC shall be the referee method.

4 PACKING AND MARKING

4.1 Packing

The material shall be suitably packed in polythene bags, or as agreed to between the purchaser and the supplier.

4.2 Marking

4.2.1 Each container shall be securely closed and shall bear legibly and indelibly the following information:

a) Name of the material;
b) Name of the manufacturer and his recognized trade-mark, if any;
c) Batch number;
d) Gross, net and tare mass;
e) Month and year of manufacture;
f) Shelf life of the material; and
g) Any other statutory requirement.

4.2.2 *BIS Certification Marking*

The product(s) conforming to the requirements of this standard may be certified as per the conformity assessment schemes under the provisions of the *Bureau of Indian Standards Act, 2016* and the Rules and Regulations framed thereunder, and the products may be marked with the Standard Mark.'

5 SAMPLING

5.1 The method of drawing representative samples of the material shall be as prescribed in 4 of IS 5299, using an appropriate sampling implement.

5.2 Number of Tests

5.2.1 Test for assay shall be conducted on each of the individual samples.

5.2.2 Tests for the remaining characteristics, namely moisture content, ash and matter insoluble shall be conducted on the composite sample.

5.3 Criteria for Conformity

5.3.1 *For Individual Samples*

The lot shall be declared as conforming to the requirement of assay if each of the individual test results satisfies the relevant requirement given in Table 1.

5.3.2 *For Composite Sample*

For declaring the conformity of the lot to the requirements of characteristics tested on the composite sample (*see* **5.2.2**) the test results for each of the characteristics shall satisfy the relevant requirement given in Table 1.

6 TEST METHODS

6.1 Tests shall be carried out as prescribed in col 4 and col 5 of Table 1.

6.2 Quality of Reagents

Unless specified otherwise, pure chemicals and distilled water (*see* IS 1070) shall be used in tests.

NOTE — 'Pure Chemicals' shall mean chemicals that do not contain impurities which affect the results of analysis.

ANNEX A
[*Table* 1, *Sl No.* (i)]
DETERMINATION OF QUINIZARIN CONTENT (ASSAY) BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

A-1 GENERAL

High-performance liquid chromatography or high-pressure liquid chromatography (HPLC) is a Chromatographic method that is used to separate a mixture of compounds in analytical chemistry and biochemistry so as to identify, quantify or purify the individual components of the mixture.

A-2 APPARATUS

A-2.1 High-performance Liquid Chromatography — quaternary gradient liquid chromatography system with UV-visible detector capable of being operated under conditions suitable for resolving the individual constituents into distinct peak may be used.

A-2.1.1 *Column* **—** C18 column of 100 Å with length 250 m, internal diameter 4.6 mm and particle size 5 µm or equivalent.

A-2.2 Volumetric Flask — class A grade.

A-3 REAGENTS

A-3.1 Quinizarin — known purity.

A-3.2 Acetonitrile — HPLC grade.

A-3.3 Tetrahydrofuran (THF) — HPLC grade.

A-3.4 Water — HPLC grade.

B-4 SAMPLE PREPARATION

Weigh accurately 20 mg sample into 50 ml volumetric flask. Add 2 ml tetrahydrofuran (THF) to it and dissolve. Make the volume up to the mark with acetonitrile.

A-5 BUFFER PREPARATION

Take 1ml of orthophosphoric acid in l liter volumetric flask and make it up to the mark with HPLC grade water.

A-6 FLOW RATE — 1.0 ml/min.

A-7 MOBILE PHASE — Acetonitrile: Buffer 80 : 20 (*v/v*)

A-8 COLUMN OVEN TEMPERATURE — Ambient Temperature.

A-9 INJECTION VOLUME — 20 µl

A-10 RUN TIME — 20 min

A-11 WAVELENGTH — 254 nm

A-12 PEAK TIME — Quinizarin: 7 min

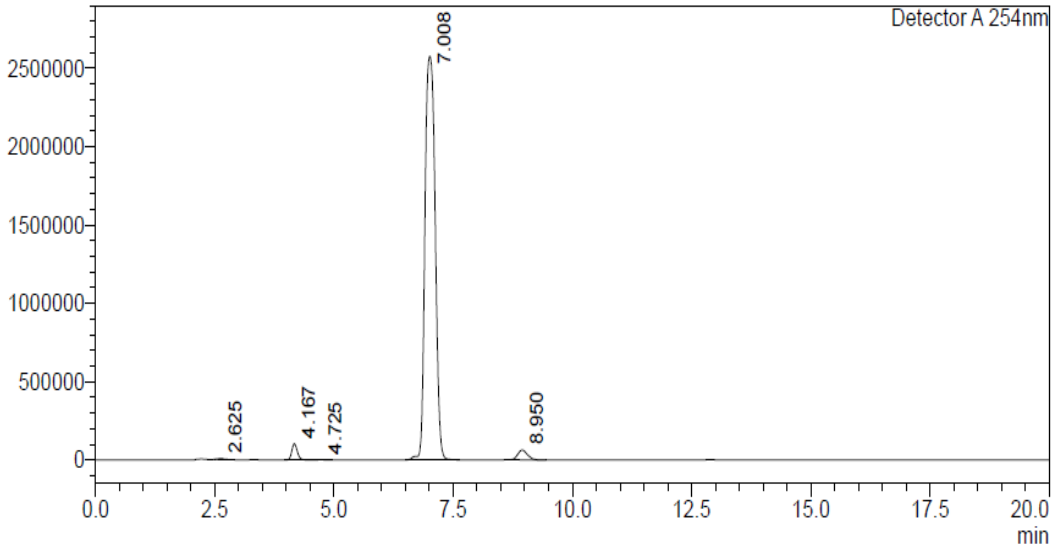


FIG. 1 TYPICAL CHROMATOGRAPH

TABLE 2 TYPICAL PEAK TABLE

Components	Retention time	Area	Area percent
	2.63	61249	0.15
	4.17	835846	2.08
	4.73	15833	0.04
Quinizarin	7.01	38468773	95.52
	8.95	889347	2.21
		40271048	100.00

A-13 CALCULATION

Calculate the peak area of individual constituent pertaining to quinizarin on the chromatogram of the material. The concentration of the constituent may be obtained on the basis of peak area on chromatogram obtained with known amount of pure quinizarin.

$$\text{Quinizarin, percent by area} = \frac{\text{Quinizarin Peak area in the sample}}{\text{Sum Areas of all peaks in the chromatogram}} \times 100$$

ANNEX B
[*Table* 1, *Sl No.* (ii)]
DETERMINATION OF QUINIZARIN CONTENT (ASSAY)

B-1 REAGENTS

B-1.1 Polycaprolactum — Polycaprolactum for column chromatography.

NOTE — Any other equally effective column material may also be used.

B-1.2 Toluene — Dried for 24 h over fused calcium chloride, filtered and distilled. The portion distilling between 109 ºC and 111°C is collected for use.

B-1.3 Quinizarine (Chromatographically pure) — Five grams of commercial sample of quinizarine is treated with 200 ml of hot boiling water, filtered on sintered-glass crucible (G4) washed with hot water and dried. This treatment removes water-soluble impurities present in the sample. Two grams of the dried sample is dissolved in about 300 ml of toluene by refluxing for about 30 minutes. The solution of quinizarine is poured in the chromatographic column prepared as in **B-2.1** and allowed to get chromatographed. When all the solution has passed into the adsorbent column, first wash the sides of the tube with small portions of toluene. When the cotton plug at the top is free of colour of quinizarine, fill the whole tube with toluene. The chromatogram will develop into two distinct bands (a) lower yellow one is quinizarine, and (b) upper pink one is an impurity. Collect the quinizarine band in a suitable conical flask. Filter the collected solution through a filter paper to remove any extraneous matter. Concentrate the solution, cool and allow to crystallize. Filter off the crystallized quinizarine and wash with a small portion of toluene. Dry at 100 °C to 110 °C. Finally, keep under vacuum at 100 °C to 110 °C for 1h to 2 h to drive off any solvent. In order to check the purity of chromatographed sample, dissolve about 10 mg, accurately weighed, in 10 ml of toluene and pass the cooled solution through a chromatographic column set up as described in **B-2.2** eluting with toluene as necessary. Collect the band carefully (only one band shall appear) and determine its optical density at 475 mμ. Make up a solution of purified crystals using the same proportion of crystals to solvent. Determine its optical density in the same way. A difference in the two figures for optical density amounting to more than 0.003 indicates the presence of impurity. The chromatographic purification shall then be repeated until this check test is satisfied.

B-2 APPARATUS

B-2.1 Polycaprolactum Column — A glass tube of one meter length and having internal diameter 2.5 cm joined with a stop-cock at the lower end, is set up vertically so that the percolation passing through the tube can be collected conveniently in a conical flask. Place a cotton-wool plug in the tube and press it to the bottom of the tube by means of a glass ‘rod flattened at the end. Place a disc of filter paper, cut to the approximate internal diameter of the chromatographic tube, on the top of the cotton-wool.

B-2.1.1 Prepare a slurry of about 25 g of the polycaprolactum in toluene and pour it into the tube. Wash down the sides of the tube and allow the adsorbent to set for one hour. Place, first, a disc of filter paper and then a cotton-wool plug at the top of the column. Care should be taken to see that the polycaprolactum column always remains soaked with the solvent so that at least a 2 cm layer of the solvent remains on top of the polycaprolactum. On no account shall the column be left to dry. In the event bf this happening, the adsorbent in the tube shall be re-slurried and re-packed.

B-2.2 Set up another column taking a tube 40 cm long and 1.3 cm in diameter and packing 3 g of polycaprolactum in the same manner.

B-3 STANDARD CALIBRATION GRAPH

Prepare a number of standard solutions of chromatographically pure quinizarine, as obtained in **B-1.3**, in toluene, varying in concentrations from 0.6 mg/100 ml to 2.0 mg/100 ml, with a difference of 0.2 mg/100 ml between successive concentration. Take readings for optical density or percentage transmittance for the above mentioned concentrations at a wavelength of 475 mμ (*see* Notes) using the specially matched cells specified for the particular spectrophotometer used. The temperature of the solutions immediately before and after measurement shall be (27 ± 2) °C. Where the instrument is calibrated in percentage transmittance only, the optical density can be read out from standard tables. Plot the calibration curve of concentration (in mg/100 ml) against optical density. This is the standard calibration graph.

NOTES

1 The readings for optical density or percentage transmittance are taken at the predetermined wavelength at which there is maximum absorption.

2 The standard calibration curve and the spectrophotometer shall be checked for accuracy from time to time.

B-4 PROCEDURE

B-4.1 Weigh accurately 100 mg to 120 mg of the sample in a 100 ml clean, dry conical flask. Add about 75 ml of toluene and reflux for 30 min Cool and transfer the solution to a 100 ml, volumetric flask. Wash the conical flask with toluene and then fully transfer the material to the volumetric flask. Dilute the solution to the 100 ml mark (I). Pipette out 10 ml from this solution (I) and transfer to another 100 ml volumetric flask and further dilute to the 100 ml mark with toluene (II). With the help of l0 ml burette, transfer 10 ml of this latter diluted solution (II) accurately to the chromatographic column (prepared as in **B-2.1**). The solution starts passing down the column. When it is just fully adsorbed at the top of the column, wash the sides carefully with small quantities of toluene.

Finally fill the upper part of the column with toluene and allow the chromatogram to develop, keeping a good ahead of solvent above the column throughout. Start collecting the main yellow band of quinizarine in a l00-ml dry volumetric flask. When the entire band of quinizarine is thus eluted, dilute to 100 ml exactly with toluene (III). Use this solution to take readings from the optical instrument.

B-4.2 Adjust the wavelength of maximum absorption (λ_{max}) at 475 mμ and then adjust the instrument to 100 percent transmittance with toluene blank. Now replace the blank with the solution (III) of the sample and read the percentage transmittance. Refer to the standard tables for conversion of percent transmittance to optical density. Read out the concentration (C) against the optical density from the standard calibration graph (**B-3**) in terms of milligrams per 100 ml of the final diluted solution (III).

B-5 CALCULATION

The amount of quinizarine in the sample is C × 100 mg.

$$\text{Assay, percent by weight} = \frac{10\,000C}{W}$$

where

C = quantity of quinizarine in milligrams contained in 100 ml of the final dilute solution (III) as read from the calibration graph; and

W = weight, in milligrams of the sample taken for the test.

ANNEX C
(*Foreword*)

Pictograms, signal word, hazard statement and precautionary statement

Pictogram(s)	**:**	
Signal Word	**:**	Warning
Hazard Statement	**:**	Very toxic to aquatic life with long lasting-effects.
Precautionary Statement	**:**	Avoid release to the environment. Collect spillage. Dispose of contents / container in accordance with local / regional / national / international regulations.