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BUREAU OF INDIAN STANDARDS

AGENDA

Textiles Speciality Chemicals and Dyestuffs Sectional Committee, TXD 07 **22nd Meeting**

Date/ Time	Venue
12 November 2024, 1100 h (Tuesday)	Through videoconferencing

CHAIRPERSON: Dr. Sujata Saxena**,** Principal Scientist, ICAR – Central Institute for Research on Cotton Technology, Mumbai (CIRCOT)

MEMBER SECRETARY Shri Himanshu Shukla**,** Bureau of Indian Standards**,** New Delhi

Item 0 WELCOME & INTRODUCTORY REMARKS

Item 1 CONFIRMATION OF THE MINUTES OF THE PREVIOUS MEETING

1.1 The minutes of the 21st meeting of the committee held on 11 June, 2024 through videoconferencing were circulated vide BISDG letter no. TXD 07/A2.21 dated 14 June, 2024. Comments were received from Shri Rahul Bhajekar, GOTS, and are given in **Annex 1 (Page-05) 04).**

1.1.1 The committee may **CONSIDER** and **CONFIRM** the minutes.

Item 2 SCOPE AND COMPOSITION OF TXD 07

2.1 The present scope and composition of the committee is given in **Annex 2 (Pages- 6 to 7).**

2.1.1 The committee may **REVIEW**.

Item 3 ISSUES ARISING OUT OF PREVIOUS MEETING OF TXD 07

3.1 Summary of actions taken on the various decisions of the 21st meeting is given in **Annex 3 (Page – 8 to 9).**

3.1.1 The committee may **NOTE**.

Item 4 INTERNATIONAL ACTIVITY

4.1 A meeting of ISO TC 38/WG/31 'Non-fibrous bio-based material for textiles' was held on 10 July 2024 through videoconferencing. New work item proposed by BIS on ISO/CD 22195-9 Textiles — Determination of index ingredient from coloured textile — Part 9: Gallnut was approved for DIS registration after incorporating the decisions arrived in the meeting.

The extract of the minutes of meeting is given as below:

a) All editorial comments that are identical to those presented in the previously published ISO 22195 standard development process will follow the previous processing.

b) It was decided to consider replacing the chromatogram in Figure A.2, in which gallic acid was not clearly separated from adjacent peaks, with a new chromatogram from other analysis results, if possible. PL will prepare a revised document and propose it as soon as possible.

c) When preparing a revised document, it will proceed in accordance with previously published standards, and accordingly, if there are no special technical comments after DIS registration, it may immediately enter the 'publication' stage as in the case of part 7 and part 8.

The working draft as submitted to ISO TC 38/WG 31 for CD registration is given in **Annex 4** **(Pages 09 to 20)**. The Agenda and Minutes of the last meeting of ISO TC 38/WG/31 'Non-fibrous bio-based material for textiles' held on 10 July 2024 is given in **Annex 5** (*Enclosed separately*).

4.1.1 The committee may **DELIBERATE** and **DECIDE.**

Item 5 R&D PROJECT UNDER TXD 07

5.1 An R&D project was awarded by BIS to Dr. Sujata Saxena, ICAR-CIRCOT on 'Development and validation of test method for determination of index ingredient from plant-based indigo and textiles dyed with plant-based indigo by using Carbon-14 (C-14) analysis'. The date of award of project was 07 June 2024.

As per the R&D guideline, second instalment to the extent of 50 percent of the approved estimated cost would be released on the submission of progress report along with the report on utilization of the 75 percent of the fund and acceptance of the same by the Sectional Committee.

The progress report, statement of expenditure and fund utilization certificate as received from Dr. Sujata Saxena, ICAR-CIRCOT, Mumbai is given in **Annex 6** **(Pages 22 to 27)**.

5.1.1 The committee may **CONSIDER** and **DECIDE**.

Item 6 REVIEW OF STANDARDS

6.1 As decided by the committee in the 21st meeting, following standards were circulated to all committee members for their review and sharing their comments/suggestion:

a) IS 15565 : 2005 Textiles — Method of test — Estimation of free benzidine in dyes
b) IS 4360 : 2020 Method for determination of strength of fast bases
c) IS 4459 : 2020 Method for determination of strength of direct dyestuffs by dyeing test
d) IS 7845 : 2020 Textile Dyestuffs - Method for Evaluating Strength of Reactive Dyes (Trichloropyrimidyl Type) by Dyeing Test (First Revision)

e) IS 17430 : 2020 Textile Dyestuffs — Natural Dye from Red Flowers of Canna indica (Indian Shot, Sarvajjaya) — Identification
f) IS 17431 : 2020 Textile Dyestuffs — Natural Dye from Red Flowers of Impateins balsamina (Balsam, Gulmehndi) — Identification
g) IS 17432 : 2020 Textile Dyestuffs — Natural Dye from Tectona grandis (Sagaun) Leaves — Identification
h) IS 17433 : 2020 Textile Dyestuffs — Natural Dye from Terminalia arjuna (Arjun) Bark — Identification

Comments have been received from Dr. Padma S Vanakar, Somaiya Vidyavihar University, Mumbai, Shri Gaurav Gupta, Office of the Textile Commissioner, Mumbai and given in **Annex 7 (Pages 28 to 91).**

6.1.1 The committee may **DELIBERATE** and **DECIDE.**

7 SUSTAINABILITY IN INDIAN STANDARDS

7.1 To address sustainability in Indian standards, the competent authority of BIS has established sector-wise Consultative Groups on Sustainability, comprising a pool of domain experts across the following sectors:

i) Civil engineering sector
ii) Chemical sector
iii) Electrotechnical sector
iv) Food and Agriculture sector
v) Petroleum and Coal sector
vi) Transport engineering sector
vii) Textile sector

First and second meeting of consultative group was held on 22 May 2024 and 11September 2024. Followings were the salient outcomes of meetings:

i) To develop Code of Practice for 'Recycling of Pre-consumer and Post-consumer Waste' including stages involved in textile waste processing like collection, sorting, mechanical/chemical recycling, purification/ cleaning to remove contaminants, fibre and/or yarn production, fabric production.

ii) To formulate the standards for the product/include the varieties manufactured using recycled materials for the Denims, Polyester and Nylon Tyre Cords, Agrotextile Products, HDPE/PP Woven Bags, Recycled Viscose and Polyester etc.

iii) To formulate a common guideline covering sustainable practices such as minimizing environmental impact, including waste reduction and efficient resource use, ensuring transparency and traceability throughout the supply chain by referring to the national/international guidelines e.g. GOTS and OEKO-TEX, ZDHC, Blue Sign etc.

7.1.1 The committee may **NOTE** and **DECIDE.**

Item 8 DATE AND PLACE OF NEXT MEETING

Item 9 ANY OTHER BUSINESS

ANNEX 1
(*Item* 1.1)

COMMENTS ON MINUTES OF 21ST MEETING OF TXD 07

Commentator: *Shri Rahul Bhajekar, GOTS*
Comment:

Thank you for the very quick circulation of the draft minutes. I do have one comment to make.

In the record of Item 5, I feel it is important that we additionally minute that there were members who were unsure of the scope and title of the proposed work item / standard.

ANNEX 2

(*Item* 2.1)

Scope & Composition of Textiles Speciality Chemicals and Dyestuffs Sectional Committee, TXD 07

Scope: To formulate Indian Standards on identification, terminology, packaging methods of test and specifications for textile speciality chemicals and dyestuffs on textile materials and in substance.

Meeting(s) held	**Date & Place**
19th Meeting	20 September, 2023, Videoconferencing
20th Meeting	17 November 2023, Videoconferencing
21st Meeting	11 June, 2024, Videoconferencing

SL. NO.	ORGANIZATION REPRESENTED	NAME OF THE REPRESENTATIVE PRINCIPLE/ALTENRATE	MEETINGS HELD/ATTENDED
1.	**Dr. Sujata Saxena (Chairperson)**	**Principal Scientist, ICAR – Central Institute for Research on Cotton Technology, Mumbai (CIRCOT)**	3/3
2.	Ahmedabad Textile Industry's Research Association, Ahmedabad	Smt. Deepali Plawat (Fahimunnisa Khatib)	2/3
3.	Ama Herbals, Lucknow	Shri Y. A. Shah	3/3
4.	Atul Limited (Colors Business), Valsad	Shri Rajaram Jamdade (Shri Arindam Chakraborty)	3/3
5.	Bio Dyes, Goa	Dr. Bosco Henriques	3/3
6.	Central Coir Research Institute, Alappuzha	Dr. Shanmugasundaram O L (Dr. S. Radhakrishnan)	1/3
7.	Central Institute for Research on Cotton Technology, Mumbai	Dr. A. S. M. Raja	3/3
8.	Dye Chem International, Kolkata	Shri Sushil Jain (Smt. Ranjeeta Rai)	1/3
9.	Global Organic Textile Standard, (GOTS)	Shri Rahul Bhajekar (Ms. Prachi Gupta)	3/3
10.	Indian Jute Industries Research Association, Kolkata	Dr. Amit Paul (Ms. Ipsita Roy)	0/0

11.	Northern India Textile Research Association, Ghaziabad	Dr. M S Parmar (Dr. Nidhi Sisodia)	3/3
12.	Office of the Textile Commissioner, Mumbai	Shri Gaurav Gupta (Shri Rajesh Mahajan)	2/3
13.	Resil Chemicals Pvt Ltd, Bangalore	Shri Ganesh Srinivasan (Shri P.T. Senthilkumar)	0/1
14.	S G S India Pvt. Ltd., Gurugram	Shri Karthikeyan K. (Shri Gaurav Saraswat)	2/3
15.	Somaiya Vidyavihar University, Mumbai	Dr. Padma S. Vankar	1/1
16.	Shree Pushkar Chemicals & Fertilizers Ltd, Mumbai	Dr. N. N. Mahapatra	3/3
17.	Textiles Committee, Mumbai	Shri Kartikeya Dhanda (Smt. Shilpi Chauhan)	0/3
18.	The Bombay Textile Research Association Mumbai	Shri M. P. Sathianarayanan (Smt. Karishma Hemani)	3/3
19.	The South India Textile Research Association,	Dr. Prakash Vasudevan (Shri S. Sivakumar)	2/3
20.	The Synthetic and Art Silk Mills Research Association, Mumbai	Smt. (Dr.) Manisha Mathur (Smt. Ashwini Sudam)	3/3
21.	U P Textile Technology Institute, Kanpur	Dr. Arun Patra (Dr. Shubhankar Maity)	3/3
22.	Wool Research Association, Thane	Dr. Mrinal Choudhari	1/3
23.	In personal capacity	Dr. A. K. Samanta	3/3

ANNEX 3
(*Item* 3.1)

SUMMARY OF ACTIONS TAKEN ON THE MINUTES OF THE LAST MEETING

Item No.	Decision	Action taken
2.1	Changes in the composition/nominations	Updated composition is given in **Annex 2.**
4.1	**INTERNATIONAL ACTIVITIES** In the last meeting, the comments received on the new work item on 'ISO/NP 22195-9 Textiles — Determination of index ingredient from coloured textile — Part 9: Gallnut' were referred to ISO/TC/38/WG 31 members for critically examining and incorporating in the draft suitably.	Comments on the draft were discussed in the Meeting of ISO/TC/38/WG 31 held on 10 July 2024 through videoconferencing. Coming for discussion under Agenda item 4.1.
5.1	**NEW WORK ITEM PROPOSAL** For the new work item proposal on ''Eco-Friendly and Aqueous Based Bio Degradable Liquid Metallic Paste', in last meeting, followings were decided, a) Shri Sushil Jain, Dyechem International, Kolkata will prepare the working draft on the aforementioned subject in consultation with Dr. A. K. Samanta and Dr. Padma S. Vankar covering, but not limited to the following aspects: i) Physical and Chemical Properties ii) Guidelines for handling and storage iii) Ecological requirements iv) Performance parameters v) Applications (limited to textiles and related products) b) Similar standards published by the committee, TXD 07 on the specifications of dyestuffs/auxiliaries will also be referred to during the preparation of working draft on the subject and the draft will cover only the textile and related applications in line with the scope of the committee, TXD 07. c) The draft will cover only the textile and related applications in line with the scope of the committee, TXD 07.	Inputs awaited.
6.1	**REVIEW OF PUBLISHED STANDARDS** In the last meeting, it was decided to circulate the standards due for review to all committee members for reviewed thoroughly by the experts of relevant field in	Standards were circulated to all committee members for their review. Coming for discussion under Agenda item **6.1**.

	today's context, to suggest suitable modification/changes and decided to circulate the standards after filling their review proforma.	
8.1	The committee also considered the ongoing subject under the domain of TXD 07 on 'Performance Requirement of Natural Dyed Textile Material' outlining the quality parameters of textile materials dyed with natural dyes, as discussed during the 19th Meeting of TXD 07. The committee decided that Shri Y. A. Shah, Ama Herbals, Lucknow in consultation with Dr. A.K. Samanta and Dr. Padma S. Vankar will prepare the working draft on the aforementioned subject, which shall be placed in the next technical committee meeting for discussion and decision.	Inputs awaited.

ANNEX 4
(*Item* 4.1)

DRAFT ON 'TEXTILES — DETERMINATION OF INDEX INGREDIENT FROM COLORED TEXTILE' : GALLNUT (PART 9)

Reference number of working document: **ISO/TC 38 N 0000**

Date: 2023-XX-XX

Reference number of document: **ISO/NWIP 22195-9 Gallnut (E)**

Committee identification: ISO/TC 38

Secretariat: SAC/JISC

Textiles — Determination of index ingredient from colored textile -

Part 9: Gallnut

Warning

This document is not an ISO International Standard. It is distributed for review and comment. It is subject to change without notice and may not be referred to as an International Standard.

Recipients of this draft are invited to submit, with their comments, notification of any relevant patent rights of which they are aware and to provide supporting documentation.

Document type: International standard
Document subtype: N/A
Document stage: 30.20 - CD ballot initiated
Document language: E

Contents

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing international standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare international standards. Draft international standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an international standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO should not be held responsible for identifying any or all such patent rights.

ISO WD was prepared by Technical Committee ISO/TC 38, *Textiles*.

Introduction

There is no doubt that dyeing plays the most important role in expressing the color of clothes. Until the invention of synthetic dyes capable of expressing diverse colors today, humankind used materials obtained from nature to dye fabric. Typically, colorants were obtained from plants or various materials were extracted from minerals or insects. When dyeing fabrics using materials derived from these natures, it became necessary to identify which substances the colorant was derived from. In other words, there has been a demand to confirm whether a fabric dyed with a natural substance is dyed using a natural substance. In addition, it is developed test method to meet the requirement to ascertain what sort of natural substances were identified as such.

 Textiles — Determination of index ingredient from colored textile — Part 9: Gallnut

1 Scope

This International Standard specifies a test method which identifies the index ingredient chemical included in colored fabric dyed with Gallnut as Gallic Acid .

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696: 1987, *Water for analytical laboratory use – Specification and test methods*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at http://www.iso.org/obp
- IEC Electropedia: available at http://www.electropedia.org/

3.1

Gallnut

A nut-like gall on plants ~~Fruit~~ of Quercus infectoria is called gallnut. Gallnut contains gallic acid which gives color to textile

3.2 Coloured Textile

Expressing of colors to textiles by dyeing, printing or coating.

3.3

Natural colourant

Materials obtained from plants, wood, rocks, soil, insects or any other thing existing on earth without any chemical reaction adopted before coloring of textiles

4 Principle

The identification of natural colorant is very important in the scientific examination of the coloring sources of textiles, colored print, paintings, illuminated manuscripts and other works where natural colorants are used. Natural colorants are usually composed of several phyto chemicals. Each colorant has distinctive chemical constituent which act as a chromophore and imparts specific color . Colorants from natural sources can be identified by chemical structure of chromophoric chemical . This chromophoric chemical can be extracted from the dyed textile and identified by chromatography.

Note: On the other hand, if the index component Gallic Acid is detected through this test method, it cannot be said that it is necessarily stained with Gallic Acid alone. However, based on this principle, applying this test method to unknown colored fabrics or textiles is useful to provide a minimum amount of information that can be used to confirm whether the fabric is colored using Gallic Acid dye.

5 Reagents

Unless otherwise specified, use only reagents of recognized analytical grade.

5.1 Water, distilled water or grade 3 water complying with ISO 3696.

5.2 Methanol HPLC Grade

5.3 Gallic acid 99%w/w

5.4 Methanol AR Grade

6 Apparatus

6.1 Analytical balance, resolution at 0.001 g

6.2 Ultrasonic water bath, to be set up at (30 ± 2) °C

6.3 Boro silicate glass container, 50 ml ~~(4 Nos)~~

6.4 Membrane filter, with 0.45 µm pore size.

6.5 Pipette 20ml

6.6 ~~Glass~~ Disposable syringe 2ml

6.7 High Performance Liquid Chromatograph (HPLC) with PDA or HPLC with Variable Wavelength UV detector (VWD)

7 Procedure

7.1 Standard preparation Gallic Acid is prepared in methanol containing 1000 mg/l.

7.2 Preparation of specimen

Cut the sample into pieces of approximately (5 x 5) mm. Prepare approximately 2 g of the cut sample, weigh it to nearest 0.01 g, and then place it into the borosilicate glass container.

Pipette 20 ml of Methanol each into the other glass container and it poured to cut sample containing glass container. Place the sample contained glass container into an ultrasonic bath at (30 ± 2) °C for (20 ± 1) min. Afterwards, let the extract cool down to room temperature.

Filter about 1 ml of the extracted solution into a HPLC vial using disposable syringe equipped with a membrane filer.

7.3 Analysis

The detection and qualification of Gallic Acid is conducted using HPLC with PDA detector. The recommendable chromatographic conditions are given in **Annex A**.

7.4 Qualification of Gallic Acid

Comparison between analyses of standard and sample through **7.3** could show the existence of Gallic Acid in sample.

NOTE Detection of Gallic Acid could be variable due to conditions of colored sample. In this case amount of specimen and extraction solution can be modified and concentration of extracted solution could be adopted. In this case, the modified sample preparation conditions should be described in test result too.

8 Test report

The test report shall include the following

information

a) A reference to this part of ISO/NWIP 222195-

9 Gallnut

b) identification of the sample

c) Conditions of chromatographic analysis

d) Any deviation from the specified procedure in this International Standard.

Annex A
(informative)

Example of test result

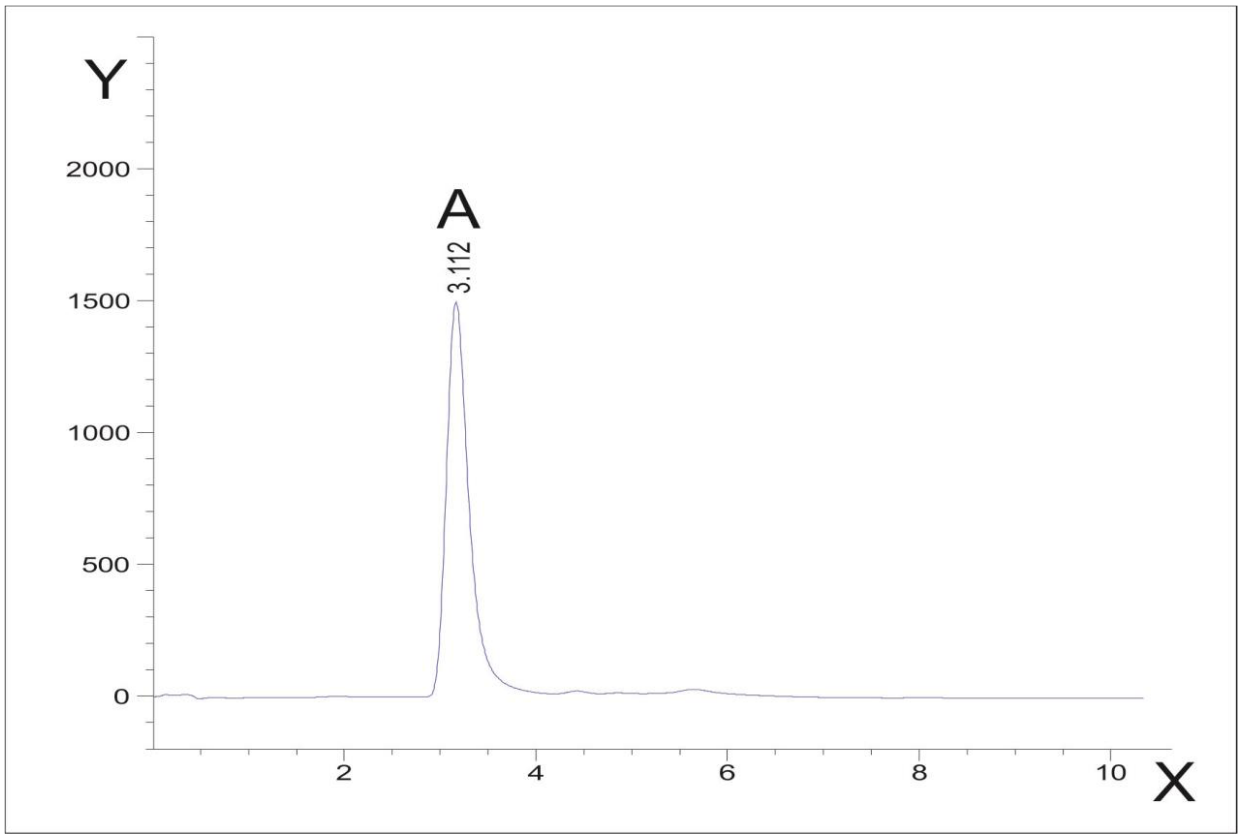
A.1 Analysis of Gallic Acid colorant

Prepare 1mg/ml of solution of Gallic Acid colorants according to 7.1

A.1.1 Chromatographic conditions for the HPLC-PDA

The HPLC-PDA analysis is done ~~adopted~~ ~~to find out~~ at 272 nm lambda max ~~the specified wavelength in 300nm~~ and its chromatographic conditions are as follows,

- Mobile phase:Methanol and Water (ratio 80:20)
- Column :C18 column 4.6x250mm (column coupled with Temperature control module)
- Detection :Lambda max 272 nm
- Injection : 10 $\mu\ell$
- Flow Rate :1ml/min



X: min
Y: mAU
A: peak of Gallic Acid

Figure A.1 Chromatogram of Gallic Acid colorant by HPLC-PDA

A.2Analysis of colored fabric dyed with Gall nut for Gallic Acid colorant

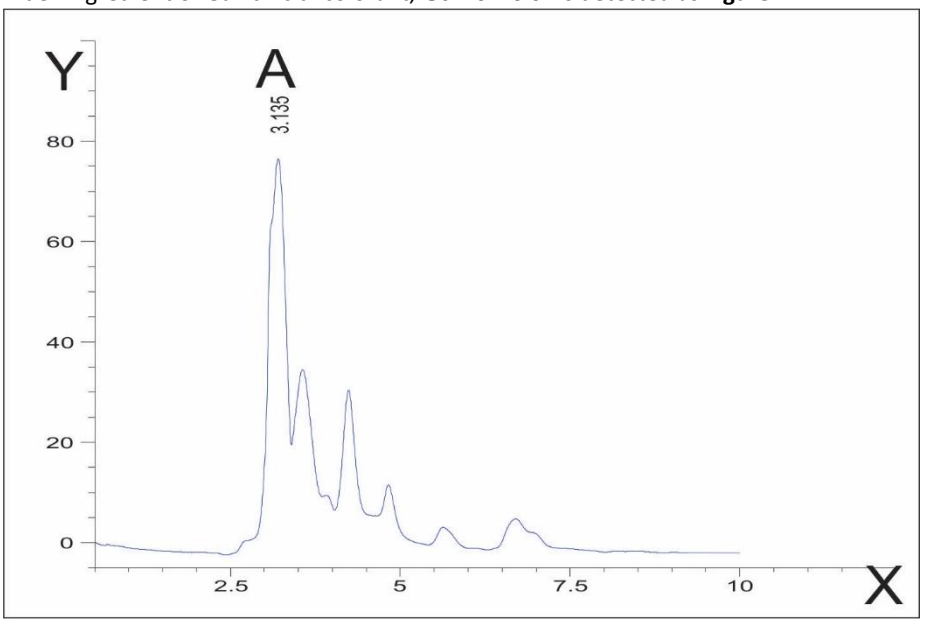
A.2.1 Chromatographic conditions for the HPLC

As the instrumental equipment of the laboratories may vary, no generally applicable parameters can be provided for chromatographic analyses.

- Mobile phase: Methanol and Water (ratio 80:20)
- Column :C18 column 4.6x250mm (column coupled with Temperature control module)
- Detection. : Lamda max 272 nm
- Injection : 10 $\mu\ell$
- Flow Rate :1ml/min

A.2.2 Determination example of index ingredient of Gallic Acid colourant from coloured fabric

Take colored fabric dyed with Gallic Acid and prepare test solution in according to **7.2**. The chromatogram is found out through **7.3** and the index ingredient of Gallic Acid colorant, Gallic Acid is detected as **Figure A.2**.



X: min
Y: mAU
A: peak of Gallic Acid

Figure A.2 Chromatogram of colored fabric extraction by HPLC-PDA

ANNEX 5

(*Clause* 4.1)

AGENDA AND MINUTES OF LAST MEETING OF ISO TC 38/WG 31 'NON-FIBROUS BIO-BASED MATERIAL FOR TEXTILES '

Attached separately

ANNEX 6

(*Clause* 5.1)

PROGRESS REPORT, FUND UTILIZATION CERTIFICATION, STATEMENT OF EXPEDITURE FOR R&D PROJECT

A) DETAILED PROGRESS REPORT

(Project Sponsored by Bureau of Indian Standards)

Project Title	: Development and validation of test method for determination of index ingredient from plant-based indigo and textiles dyed with plant-based indigo by using Carbon-14 (C-14) analysis Code TXD 0125
Project work order	: SCMD/R&D Projects dated 15th July 2024
Total project cost	: 9.9 Lakh
Project Start Date	:07th June 2024
Principal Investigator	: Dr. Sujata Saxena
Project duration	: 06 months
Date and amount of 1" instalment release	: 07th June 2024, ₹ 2.673 Lakhs

Project Investigators

1.	Dr. Sujata Saxena	ICAR-CIRCOT, Mumbai	Principal Investigator
2.	Dr. Bosco Henriques	M/s Bio Dye India, Pvt. Ltd., Mumbai	Co-Investigator
3.	Dr. A. Arputharaj	ICAR-CIRCOT, Mumbai	Co-Investigator

Objectives of the Study:

1. To collect authentic and commercial samples of natural indigo and high purity synthetic indigo

2. To develop procedure for purification of indigo samples and check for purity by CHNO analysis (outsourcing)

3. To check the C14 content of pure indigo samples at identified Indian laboratory

4. To develop test method for differentiation of natural and synthetic indigo by C14 analysis

Status of work done

Activity 1 (Month 1-2): Review of literature, desktop study, Identifying natural indigo vendors and institutions with required testing infrastructure equipped with CHNO/C-14 test facilities in the country

a) Review of literature and desktop study: COMPLETED

A systematic review of the literature to collect information about solubility of indigo in various solvents; methods for purification of indigo; spectroscopic and chromatographic methods for

identification and quantification of indigo; Carbon, Hydrogen, Oxygen, Nitrogen (CHON) composition of indigo has been completed from varied sources listed below:

- National/ International Standards on the subject and related subjects
- National and International journals, research and review papers on the subject

b) Identifying natural indigo vendors, and specialized testing facilities: COMPLETED

Five natural Indigo vendors in the country were identified

- **Institutions for CHNO analysis**

i) Sophisticated Analytical Instrumentation Facility (SAIF), IIT, Bombay was contacted and they were willing to do analysis on payment basis

- **C14 analysis facilities**

Following five institutions in the country likely to have C14 analysis facility were identified and these were contacted through email/ phone call:

i) Institute of Physics. Bhubaneshwar
ii) CSIR-National Geophysical Research Institute (NGRI), Hyderabad
iii) Inter University accelerator Centre (IUAC), N. Delhi
iv) Radiocarbon dating laboratory, Planetary and Geosciences division, Physical Research
v) Y Laboratory (PRL), Ahmedabad Birbal Sahni Institute of Paleobotany, Lucknow

It was found that the Lucknow institute does not have the complete facility. No response was received from PRL, Ahmedabad and Institute at Bhubaneshwar. NGRI had an old instrument which required minimum 8-10 g purified sample for a run hence was not suitable for our purpose. IUAC had AMS facility which requires only a few milligram sample hence was suitable for our work

Feasibility of using C14/C12 ratios through Isotope Ratio Mass Spectroscopy (IRMS) similar to its usage to knowI5N/14N ratio in ISO 20921: 2019 was also explored but an inquiry with the relevant labs revealed that the equipment is not suitable to know C14/C12 ratios

Activity 2 (Month 2-3): Collecting Indigo dye samples and standardization of laboratory methods for characterization and purification of indigo dye samples- COMPLETED

Commercial natural indigo dye samples were obtained from the following Natural dye vendors:

i) Sam Vegetable Colours, Moradabad, UP

ii) Sodhani Biotech, Jaipur, Raj.
iii) Indigo Design, Rudrapur, Uttarakhand

Besides, authentic natural indigo samples were also obtained. Some quantity of 95% pure synthetic indigo sample was available with us and the same was used. A few labware items needed for purification of indigo samples were procured. Methods for characterization and purification of indigo were standardized. Ethyl acetate, DMF and DMSO solvents were tried for indigo dissolution and its spectroscopic quantification.

Activity 3 (Month 3-4): Characterization and purification of the collected natural indigo samples: COMPLETED

Commercial and authentic natural indigo samples were analyzed for ash and acid insoluble ash content and also for indigo content by spectrophotometric method. All natural indigo samples had high ash contents and differed in indigo content. These were purified first by chemical method and then by sublimation. As very high purity is needed for AMS analysis, synthetic indigo sample has also been purified in the similar manner.

Activity 4 (Month 4-5): CHNO Analysis of purified indigo to confirm purity: ONGOING

Payment of prescribed charges for analyzing CHNO content of six purified indigo dye samples has been made to SAIF, IIT, Bombay and samples have been submitted on 17th October to check their purity status.

Activity 5 (Month 5 &6): AMS analysis of purified indigo samples: TO BE TAKEN UP

Once the purity of the purified indigo dye samples is established by CHNO analysis, these samples will be analyzed by AMS to know their C14/C12 ratios which will be used to differentiate between the natural and synthetic indigo. Regarding AMS analysis, proposal for use of the facility at the identified Indian institution, IUAC, N. Delhi has been submitted on 14th October, 2024. As per the usual procedure, proposals will be presented during December 15-18, 2024 for approval and after the approval, time slot will be allocated. As that would cause delays, efforts are being made to expedite the process and BIS intervention may be required in the matter.

Future plan of work:

1 CHNO analysis results will be analyzed to get information about the purity of indigo dye samples purified in the laboratory which would help in fine-tuning the purification process. More dye samples will be purified and tested for purity by CHNO analysis.
2 Indigo samples, of confirmed purity will be analyzed by AMS at the identified institution, IUAC, Delhi (sub-contractor) to determine C to 12C ratio. First the process protocol will be developed by analyzing natural and synthetic indigo and their blends. The data would be analyzed and used to determine natural indigo content of commercial purified samples of natural indigo.
3 All data obtained will be compiled and analyzed, methods will be documented and final project report will be submitted.

B) FUND UTILIZATION CERTIFICATE

आज़ादी का अमृत महोत्सव

An ISO 9001:2015 Certified Institute and NABL accredited Lab

कृषि एवं किसान कल्याण मंत्रालय (कृषि अनुसंधान एवं शिक्षा विभाग), भारत सरकार

भाकृअनुप - केन्द्रीय कपास प्रौद्योगिकी अनुसंधान संस्थान

(भारतीय कृषि अनुसंधान परिषद)

Ministry of Agriculture and Farmers Welfare (Department of Agricultural Research and Education), GOVERNMENT OF INDIA

ICAR - CENTRAL INSTITUTE FOR RESEARCH ON COTTON TECHNOLOGY

(Indian Council of Agricultural Research)

No. 6/ICAR-CIRCOT/A&A/INDIGO-C-14 /2024-25 04th Nov, 2024

GFR 12 - A

[(See Rule 238 (1)]

FORM OF UTILIZATION CERTIFICATE

FOR AUTONOMOUS BODIES OF THE GRANTEE ORGANIZATION

UTILIZATION CERTIFICATE FOR THE YEAR 2024-25 (01.04.24 to 31.10.24) in respect of recurring/non-recurring

GRANTS-IN-AID/SALARIES/CREATION OF CAPITAL ASSETS

1. Name of the Scheme: BIS Project titled " DEVELOPMENT AND VALIDATION OF TEST METHOD FOR DETERMINATION OF INDEX INGREDIENT FROM PLANT-BASED INDIGO AND TEXTILES DYED WITH PLANT-BASED INDIGO BY USING CARBON-14 (C-14) **". (TXD 0125)**

2. Whether recurring or non-recurring grants: Recurring

3. Grants position at the beginning of the financial year.

(i) Cash at Bank NIL

(ii) Unadjusted advances NIL

(iii) Total NIL

4. Details of grants received, expenditure incurred and closing balances: (Actuals)

Unspent Balances of Grants received years [figure as at Sl. No. 3 (iii)]	Interest Earned thereon	Interest deposited back to the Govern-ment	Grant received during the year 2024-25			Total Available funds (1 +2-3+4)	Expenditure incurred	Closing Balances (5-6)
1	2	3	4			5	6	7
			Sanction No.	Date (ii)	Amount (iii)			
0	0	0	SCMD /R&D/ Project	07.06.2024	267300	267300	199507	67793

Component wise utilization of grants:

Grand-in-aid-General	Grand-in-aid-Salary	Grand-in-aid-creation of capital assets	Total
40991	158516	0.00	199507

Details of grants position **Up to 31.10.24.**

(i) Cash in Hand/Bank Rs. 67793/-

(ii) Unadjusted Advances Nil

(iii) Total Rs. 67793/-

पोस्ट बैग न. 16640, एडनवाला रोड, माटुंगा, मुंबई - 400 019

Post Bag No. 16640, Adenwala Road, Matunga, Mumbai - 400 019

टेलीफोन / Telephone: 022-2412 7273/76, 2418427475, 24157238

फैक्स / Fax: 022-2413 0835 / 2415 7239

Certified that I have satisfied myself that the conditions on which grants were sanctioned have been duly fulfilled/are being fulfilled and that I have exercised following checks to see that the money has been actually utilized for the purpose for which it was sanctioned:

(i) The main accounts and other subsidiary accounts and registers (including assets registers) are maintained as prescribed in the relevant Act/Rules/Standing instructions (mention the Act/Rules) and have been duly audited by designated auditors. The figures depicted above tally with the audited figures mentioned in financial statements/accounts.

(ii) There exist internal controls for safeguarding public funds/assets, watching outcomes and achievements of physical targets against the financial inputs, ensuring quality in asset creation etc. & the periodic evaluation of internal controls is exercised to ensure their effectiveness.

(iii) To the best of our knowledge and belief, no transactions have been entered that are in violation of relevant Act/Rules/standing instructions and scheme guidelines.

(iv) The responsibilities among the key functionaries for execution of the scheme have been assigned in clear terms and are not general in nature.

(v) The benefits were extended to the intended beneficiaries and only such areas/districts were covered where the scheme was intended to operate.

(vi) The expenditure on various components of the scheme was in the proportions authorized as per the scheme guidelines and terms and conditions of the grants-in-aid.

(vii) It has been ensured that the physical and financial performance under **Project titled "DEVELOPMENT AND VALIDATION OF TEST METHOD FOR DETERMINATION OF INDEX INGREDIENT FROM PLANT-BASED INDIGO AND TEXTILES DYED WITH PLANT-BASED INDIGO BY USING CARBON-14 (C-14) " for BUREAU OF INDIAN STANDARDS** has been according to the requirements, as prescribed in the guidelines issued by Govt. of India and the performance/targets achieved statement for the year to which the utilization of the fund resulted in outcomes given at Annexure — I duly enclosed.

Date: 04th Nov, 2024.

Place: ICAR-CIRCOT, Matunga, Mumbai

S.Saxena

Dr. (Smt.) S. Saxena
PI

Principal Scientist
(Head CBP Division)

Saurabh 4-11-24

Sumit Saurabh
Fin. & A/c's Officer,

(Head of the Finance)

S.K. Shukla
Director

(Head of the Organization)

डॉ. एस. के. शुक्ल / Dr. S. K. SHUKLA
निदेशक / DIRECTOR
भाकृअनुप-केन्द्रीय कपास प्रौद्योगिकी अनुसंधान संस्थान
ICAR- Central Institute for Research on Cotton Technology
एडनवाला रोड, माटुंगा, मुंबई - 400 019.
Adenwala Road, Matunga, Mumbai - 400 019.

ICAR-Central Institute for Research on Cotton Technology (ICAR), Mumbai

Expenditure statement from 01-04-2024 to 17-10-2025

Name of PI : Dr. Smt. S. Saxena, Principal Scientist, Head- CBP Division

Project title : "DEVELOPMENT AND VALIDATION OF TEST METHOD FOR DETERMINATION OF INDEX INGREDIENT FROM PLANT-BASED INDIGO AND TEXTILES DYED WITH PLANT-BASED INDIGO BY USING CARBON-14 (C-14) FOR BUREAU OF INDIAN STANDARDS"

Scheme code : TXD 0125

(Amount in Rs.)

Sr.No.	Budget Heads	Sanctioned Budget	Opening Balance as on 01.04.2024	Release Fund 2024-25 (07.06.24)	Total Funds	Total Expenditure 2024-25	Funds Refunded during 2024-25	Closing Balance as on 17.10.24
1	Manpower Cost (YP-II-Salary for 6 Months)	250000.00	0.00	67500.00	67500.00	158516.00	0.00	-91016.00
2	Consumables (Chemicals, Samples Testing, Glassware, Stationery, Information Search (from databasses)	200000.00	0.00	54000.00	54000.00	15309.00	0.00	38691.00
3	Equipment	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	Travel (For collection & analysis of samples)	150000.00	0.00	40500.00	40500.00	0.00	0.00	40500.00
5	Any other (Olutsourceing CHNO and C-14 analysis)	300000.00	0.00	81000.00	81000.00	21240.00	0.00	59760.00
6	Overhead Expenses	90000.00	0.00	24300.00	24300.00	4442.00	0.00	19858.00
		0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Grand Total (1+2+3+4)	990000.00	0.00	267300.00	267300.00	199507.00	0.00	67793.00

Finance & A/c's Officer

सुमित सौरभ/Sumit Saurabh
वित्त एवं लेखा अधिकारी / Finance & Account Officer
वित्त विभाग प्रमुख /Head of Finance
भाकृअनुप-केन्द्रीय कपास प्रौद्योगिकी अनुसंधान संस्थान (मुंबई)
ICAR-Central Institute for Research on Cotton Technology (Mumbai)

डॉ. एस. के. शुक्ल / Dr. S. K. SHUKLA
निदेशक / DIRECTOR
भाकृअनुप-केन्द्रीय कपास प्रौद्योगिकी अनुसंधान संस्थान
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एडनवाला रोड, माटुंगा, मुंबई - 400 019.
Adenwala Road, Matunga, Mumbai - 400 019.

S. Saxena
PI

ANNEX 7
(*Clause* 5.1)

COMMENTS ON REVIEW OF PUBLISHED STANDARDS

Commentator: *Dr. Padma S. Vankar*
Comment:

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft was finalized by Textile Specialty Chemicals and Dyestuffs Sectional Committee and had been approved by the Textile Division Council.

The revival of natural dyes has happened for two reasons:

a) Due to the harmful effect of synthetic dyes, safer natural dyes are being preferred globally, and
b) The ban on the use of azo dyes which release carcinogenic amines has also brought back natural dyes into the limelight. Natural dyes are being used extensively for the dyeing of cotton, linen, silk and wool substrates.

Canna indica (Indian shot, *Sarvajjaya* in Hindi) is a perennial garden plant cultivated in gardens for its ornamental flowers and edible starchy rhizome. The false petals of the flowers are red, yellow or spotted (red on yellow or vice versa). The red flowered variety produces red dye which has recently been used for dyeing textiles.

In order to achieve optimum quality with minimum lot-to-lot shade variation, and little differences in colour when the same dyestuff is obtained from different manufacturers, it is essential to standardize this natural dyestuff. This will improve the cost benefit ratio and help the user adjust their dyeing or printing recipes appropriately.

It is for the first time that the standard for the natural dyestuff *Canna indica* (red) flower dye has been written, for the benefit of buyers, sellers and users of this natural dyestuff. Standardization and identification methods will bring all the major parameters under scan and match. The main intention of this specification is to identify the natural dyestuff.

The composition of the Committee responsible for the formulation of this standard is given in Annex E.

In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 ‘Rules for rounding off numerical values (*revised*)’.

Indian Standard

TEXTILE DYESTUFFS — NATURAL DYE FROM RED FLOWERS OF *CANNA INDICA* (INDIAN SHOT, *SARVAJJAYA*) — IDENTIFICATION

1 SCOPE

This standard prescribes test methods and identification requirements of powder / aqueous extract of *Canna indica* (*Sarvajjaya*) red flower dye, and of textiles colored with this dye. The detection and identification of the natural colorant from red flowers of *Canna indica* is conducted using chromatographic techniques i.e., Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) as given in Annex B and C and by UV-Visible spectroscopic technique as given in Annex D. The method for extraction of the dye from fabric dyed with *Canna indica* red flower extracts is given in Annex E.

2 REFERENCES

The standards listed in Annex A 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 subjected to revision, and parties to agreements based on this standard are encourage to investigate the possibility of applying the most recent editions of the standards indicated in Annex A.

3 TERMS AND DEFINITIONS

For the purpose of this standard, the following terms and definitions apply:

3.1 *Canna indica* Red Flower Dye — *Canna indica* is the botanical name of the perennial plant Indian shot or *Sarvajjaya* (Hindi) widely grown for its attractive ornamental flowers. It belongs to family Cannaceae. *Canna indica* sps. can be used for the treatment of industrial waste waters through constructed wetlands. *Canna indica* produces red dye from the red variety of its flowers, which has been recently used for dyeing textiles.

3.2 Natural Colorant — Colorants from natural origin, particularly materials obtained from plant parts, namely - stem, root and leaves, extracted without any chemical reaction primarily used in textiles for dyeing or printing.

4 PRINCIPLE

4.1 Natural dyestuffs are usually never a single compound; they are composed of several compounds many of which are structurally similar. Their individual character will determine how they bind to the substrate, and the ratios bound to the substrate may be different from their ratios in the extract. These individual components can be analyzed by spectroscopic and chromatographic techniques which can determine the authenticity of the natural colorant.

4.2 The coloration in the red false petals of *Canna indica* is due to anthocyanins. The following anthocyanins have been identified: 3-*O*-(6-*O*-acetyl-β-D-glucopyranoside)-5-*O*-β-D glucopyranoside; malvidin 3,5-*O*-β-D-diglucopyranoside; cyaniding-3-*O*-(6”-O-α rhamnopyranosyl-β-glucopyranoside; cyanidin-3-*O*-(6”-*O*-α-rhamnopyranosyl) βgalactopyranoside; cyanidin-3-*O*-β-glucopyranoside; cyanidin-*O*-β-galactopyranoside. Saponins, sterols, phenolic compounds, tannins and the water insoluble carotenoids have been extracted from the flowers. All these compounds will contribute to the chromatographic and spectroscopic characteristics of its extract.

5 APPARATUS

5.1 Analytical Balance, with resolution of 0.001 g

5.2 Spatula

5.3 Ultrasonic Water Bath (Sonicator)

5.4 Stoppered Conical Flask

5.5 Measuring Cylinder

5.6 Funnel

5.7 Qualitative Grade Filter Paper, Particle retention 11 µm.

6 REAGENTS

6.1 *Canna indica* Red Flowers, dried and powdered to serve as reference.

6.2 Methanol — Analytical grade.

7 PROCEDURE

7.1 Preparation of *Canna indica* Red Flower Dye Reference and Test Samples Dried and powdered red mature flowers from *Canna indica* plants should be used to prepare the reference sample. The reference stock solution of the natural dye is prepared by steeping 0.5 g of dry flower powder in 100 ml methanol in a stoppered conical flask. It is then agitated in a sonicator set at 45°C for about 20 min, after which the solution is filtered using a filter paper, and then transferred to another stoppered glass vessel for further analysis. If not used on the same day, the stock solution can be stored in a

refrigerator for about two weeks. Stock solution of the test sample is also prepared in the same way from dry, powdered plant material. If the test sample is a liquid concentrate or a spray dried extract, a shorter dissolution time and a smaller amount of sample (0.05 g) may be sufficient. If the test material is dyed textile, the method for the extraction of the natural dye from it is given in Annex E.

7.2 Analysis

The detection and identification of *Canna indica* red flower natural dye from prepared dye samples is carried out by using chromatographic techniques namely, TLC and HPLC as given in Annex B and C. These are also analyzed by UV-Visible spectrophotometry as given in
Annex D.

7.3 Qualification and Identification of *Canna indica* Red Flower Natural Dye Comparison between analyses of test sample and reference *Canna indica* red flower natural dye sample through **7.3** can identify whether the test sample is *Canna indica* red flower natural dye. The specific tests to be conducted are given in Table 1.

Table 1 Specific Tests Recommended for Identification of *Canna indica* Red Flower Natural Dye

(*Clause* 7.3)

Sl No	Test	Type of Test	Ref to Annex
(1)	(2)	(3)	(4)
i)	Chromatography tests:		
	a) Thin layer chromatography (TLC)	Mandatory test	B
	b) High performance liquid chromatography (HPLC)	Additional test (*See* Note)	C
ii)	Spectroscopy tests:		
	a) UV-visible spectroscopy	Confirmatory test	D
NOTE — HPLC may be performed wherever such instrumentation facilities are available			

8 CALIBRATION OF THE ANALYTICAL EQUIPMENT

The instruments/equipment being used (TLC, HPLC, UV-Visible spectrophotometer) should be calibrated using standard reference materials and procedures as recommended by the manufacturer to ensure their proper working before start of the analysis.

9 VALIDATION OF THE METHOD

The test methods employed should be validated using authentic reference dye sample for reliability and repeatability

10 REPORT

The report shall include the following information:

a) Reference to this standard,
b) Tests conducted and test conditions, and
c) Results on identification of the test sample

ANNEX A

(*Clause* 2)

LIST OF REFERRED INDIAN STANDARDS

IS NO	*TITLE*
IS 1070 : 2023	Reagent Grade Water Specification (*Fourth Revision*)

ANNEX B

(*Clauses* 1, 7.2 and Table 3)

IDENTIFICATION OF *CANNA INDICA* RED FLOWER NATURAL DYE BY THIN LAYER CHROMATOGRAPHY (TLC)

B-1 PRINCIPLE

TLC separates non-volatile constituent compounds in a mixture by virtue of differences in their affinity for the stationary solid phase which is spread as a thin layer on a sheet/plate and the selected mobile solvent phase which are then seen as different spots on the TLC plate. Positioning of a spot on the TLC plate denoted by its R_f value (distance travelled by the constituent / distance travelled by the solvent) depends on its chemical nature and is its characteristic property. Therefore, due to similarity in constituent chemical components, test and reference *Canna indica* red flower natural dye samples under similar TLC condition should result in spots of similar R_f values and color and thus a test sample can be identified as whether it is *Canna indica* red flower natural dye.

B-2 APPARATUS

B-2.1 Analytical thickness Silica gel G/ GF 254 coated TLC plates (may be cast in-house or procured from commercial suppliers).

B-2.2 Capillary for spotting TLC plates.

B-2.3 TLC development chamber (of a dimension suitable to accommodate TLC plate).

B-2.4 TLC spot visualization chamber containing crystals of iodine (of a dimension suitable to accommodate TLC plate).

B-2.5 Optionally, viewing chamber for TLC with 254 nm UV tubes which may also be fitted with daylight fluorescent tubes (CRI 95) and photographic camera for recording photographs.

B-3 REAGENTS

B-3.1 Stock Solutions, of test and reference *Canna indica* red flower natural dye prepared in **7.1**

B-3.2 Methanol
B-3.3 Ethyl Acetate

B-3.4 n-Hexane

NOTE — All reagents except item **B-3.1** should be of analytical reagent grade and all glassware should be cleaned and finally rinsed with reagent grade water and dried.

B-4 PROCEDURE

Both reference and the test sample extracts are spotted carefully, side-by-side, on the silica gel coated TLC plate. The spots are dried and the plate is placed in TLC development chamber, pre-equilibrated with the developing solvent consisting of 60 percent ethyl acetate and 40 percent n-hexane. After the solvent front has travelled to sufficient distance, the TLC plate is removed and the solvent is allowed to evaporate. Developed spots are visualized in daylight. The spots may optionally be visualized and photographed in the viewing chamber under daylight fluorescent tubes and if a silica gel GF254 plate is used, also under UV-254 nm light. The TLC plate is then placed in the iodine chamber and the spots are visualized in iodine. The constituents of the test sample present as spots on the TLC plate are compared with the reference dye sample. The color and R_f values of the spots in both the test and reference sample are noted.

B-5 OBSERVATION

Details of stock solution preparation and its dilution if any:

Type of TLC plate used: Silica gel G/ GF 254: in-house/ commercial Solvent system used: 60 percent ethyl acetate and 40 percent n-hexane

R_f of reference sample of *Canna indica* red flower dye extract	R_f and spot color of test sample extract	Comment
R_f 0.90, 0.80, 0.60, 0.40 and 0.20		

An indicative thin layer chromatogram with both the tracks being natural *Canna Indica* red flower dye extract is presented in Fig. 1. However, the position and number of spots may vary due to differences in sample preparation and chromatographic conditions.

B-6 CONCLUSION

Color of the spots and the R_f values of the reference and test samples are similar/different.

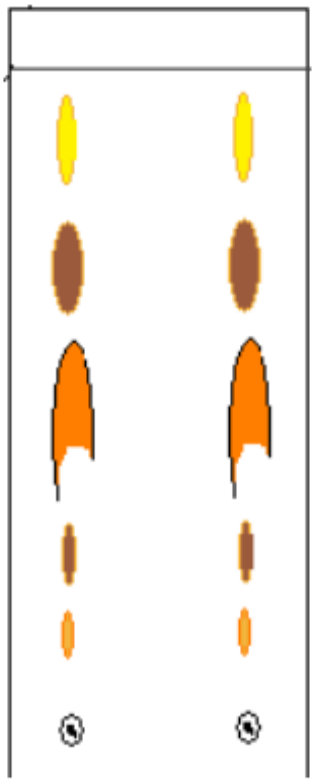

Fig. 1 Thin Layer Chromatogram of *Canna Indica* Red Flower Dye Extract

ANNEX C

(*Clauses* 1, 7.2 and Table 3)

IDENTIFICATION OF *CANNA INDICA* RED FLOWER DYE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

C-1 PRINCIPLE

HPLC is used to separate compounds in a mixture by virtue of differences in their affinity for the stationary phase which is packed in a column, and the mobile solvent phase that is pumped through it. The constituents, depending upon their chemical nature, are differently retarded during their passage through the column and are identified by comparing the time taken by a constituent compound in eluting from the column, as detected by a detector (retention time), with that of a standard reference compound. Selection of a detector is important as in the closed system, the eluting compounds cannot be seen by the eye and can only be detected by their characteristic properties. UV detector is a common detector employed as many organic compounds absorb in the UV region. Wavelength of the detector can be set to a value where absorption by the compounds of interest is high so as to achieve higher sensitivity. Various parameters of the HPLC assay are set in such a way that distinct peaks of the reference sample are observed in the chromatogram.

C-2 APPARATUS

C-2.1 HPLC System, with binary pump, UV/PDA detector and software.

C-2.2 C-18 Reverse Phase Column (RPC-C18) — 150 x 4.6 mm; 5 micron.

C-2.3 Guard Column, compatible with the analytical HPLC column.

C-2.4 Micro-Syringe

C-2.5 Membrane Filters (0.45 micron), for micro -syringe.

C-2.6 All Glass Filtration Assembly, for micro filtering solvents.

C- 2.7 Oil-Free Vacuum Pump

C-2.8 Membrane Filters (0.45 micron), for solvent filtration.

C-3 REAGENTS

C-3.1 Methanol

C-3.2 Deionized Water

C-3.3 Reference *Canna indica* red flower natural dye and test dye stock solutions prepared in **7.1**.

NOTE — All reagents except **C-3.3** should be of HPLC grade and need to be filtered through 0.45 micron membrane filter before use. All glassware used should be cleaned and finally rinsed with reagent grade water and dried.

C-4 PROCEDURE

C-4.1 Chromatographic conditions

a) Stationary phase : Column C18, 150 × 4.6 mm; 5 micron,
b) Eluent/ mobile phase: Methanol: Deionised water (80:20),
c) Flow rate: 1.0 ml/min,
d) Detection wavelength: 254 nm (band width 16 nm), and
e) Run time: 15 min

C-4.2 Method

C-4.2.1 HPLC system is started and the selected mobile phase is allowed to pass through the column till a stable baseline is obtained. Reference dye solution, after appropriate dilution, is then aspirated into the micro-syringe, a membrane pre-filter is fitted onto it and the solution is injected into the sample loop (10-20 micro liter capacity) in load position to fill it completely. The injection valve is then turned to inject position to introduce the sample onto the column. As the mobile phase is continuously passing through the column, the constituents of the injected dye extract get separated. These separated constituents exit the column and enter the UV/PDA Detector which detects them by recording the absorbance of the individual constituents as they pass through it. This absorbance is converted to a signal and peaks are seen on the signal intensity versus time plot which is continuously displayed on the monitor. When a UV-absorbing component passes through the detector a peak is seen on the plot. After completion of the run, a chromatogram along with a report of peak retention times, peak height and peak area for each peak is generated by the software. The test sample run is

also completed in the similar manner. Identity of the test sample is established by comparing the retention times of the peaks in reference and test chromatograms.
Peak height or its area provides additional quantitative information about the relative abundance of various constituents in test and reference samples.

C-4.2.2 An indicative chromatogram containing the peaks obtained after HPLC assay of the *Canna indica* red flower natural dye extract is shown in Fig. 2. Peak location/retention times and height may vary between the indicative chromatogram and the injected samples due to differences of the dye sample and the extract preparation conditions.

C-5 OBSERVATION

C-6 CONCLUSION

The retention time of the constituents of the reference and test sample are similar/different. The reference sample and test sample are therefore similar/different.

	In reference sample of *Canna indica* red flower natural dye extract	In test sample extract	Comment
Retention time of the peaks observed in HPLC chromatogram	Two main peaks at 2.2 and 2.6 min.		

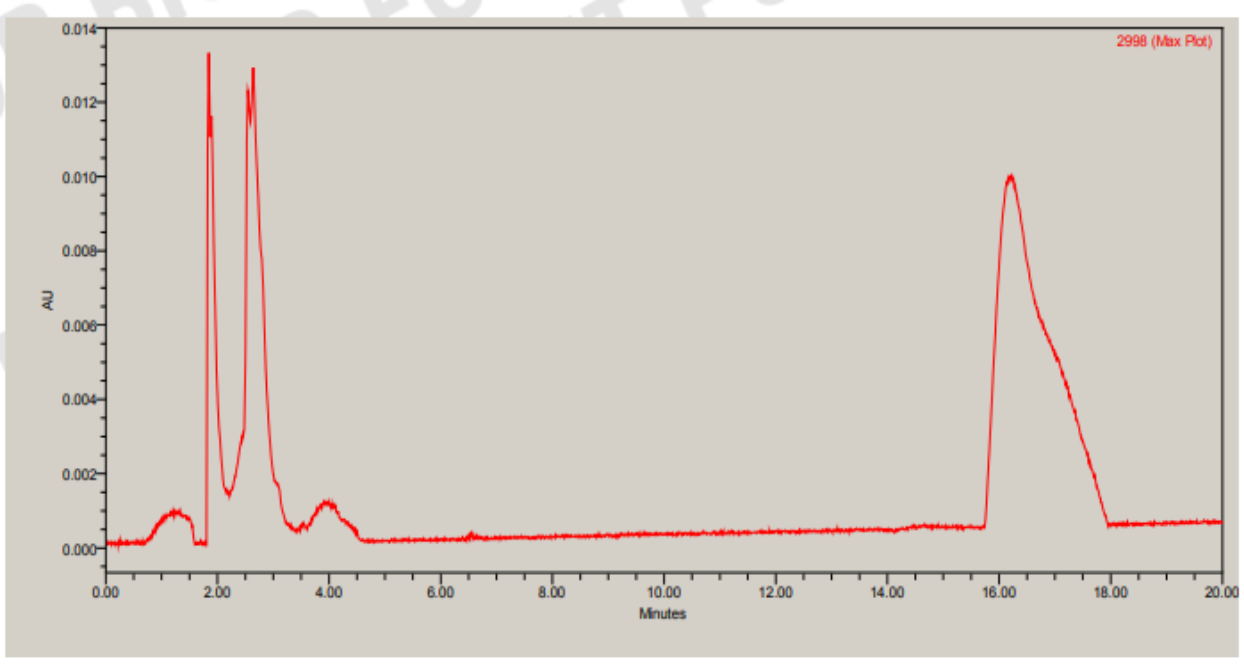

Fig. 2 High Performance Liquid Chromatogram of *Canna Indica* Red Flower Dye Extract

ANNEX D

(*Clauses* 1, 7.2 and Table 3)

IDENTIFICATION OF *CANNA INDICA* RED FLOWER DYE BY UV–VISIBLE SPECTROSCOPIC METHOD

D-1 PRINCIPLE

When sample molecules are exposed to light having an energy that matches a possible electronic transition within the molecule, some light energy is absorbed as the electron is promoted to a higher energy orbital. An optical spectrometer records the wavelengths at which absorption occurs, together with the quantum of absorption at each wavelength. The resulting spectrum is presented as a graph of absorbance (*A*) versus wavelength.

Many molecules absorb ultraviolet and/or visible light. Fine particles will scatter light and contribute to the absorbance. It is therefore essential to remove fine particles when measuring absorbance of dye solutions. Absorbance (*A*) is directly proportional to the path length, b, and the concentration, c, of the absorbing species. Beer's law states that:

$$A = \varepsilon bc$$

Where, ε is a constant of proportionality, called the absorptivity.

Different dye compounds within the natural dyestuff absorb radiations of different specific wavelengths. The spectral curve will thus be a summation of their individual absorbances. An absorption spectrum of the natural dyestuff will thus show a characteristic curve with peaks corresponding to structural groups present within the constituent molecules.

D-2 APPARATUS

D-2.1 Ultra Violet Visible Scanning Spectrophotometer

D-2.2 Quartz Sample Cuvettes — Matched pair of 1.00 cm light path;

D-3 REAGENTS

D-3.1 **Reference Canna indica Red Flower Natural Dye Extract**

D-3.2 Methanol

NOTE — All reagents should be of analytical reagent grade and all glassware should be cleaned and finally rinsed with reagent grade water and dried.

D- 4 PROCEDURE

D-4.1 The stock solution of the reference *Canna indica* red flower dye prepared in 7.1 may be used to record the spectrum in the UV-visible region (200 - 700 nm). If the absorbance in the UV region is too high, this solution may be suitably diluted for recording the spectrum in the UV region (200–400 nm). The test sample solution is also prepared in the same manner as the reference sample.

D-4.2 Spectra are recorded with pure methanol in reference holder of the spectrophotometer. Reference dye sample is first analyzed. Test sample is analyzed next and recorded spectra are compared for similarity of the spectral curve that is, peak positions and their intensity (absorbance value). The absorbance spectral curve of this dye extract has specific characteristics both in the UV and visible range and an indicative spectrum of the Canna indica red flower natural dye extract in UV-visible region is presented in Fig 3.

D-5 OBSERVATION

D-6 CONCLUSION

The nature of the spectral curve of the test sample in the UV and visible range is similar/different to the reference sample.

	In reference *Canna indica* red flower dye extract	In test sample extract	Comments (Describing difference/ similarity in spectral curve)
Peak position (nm) with absorbance	360 nm – 0.688A		
	463 nm – 0.456 A		

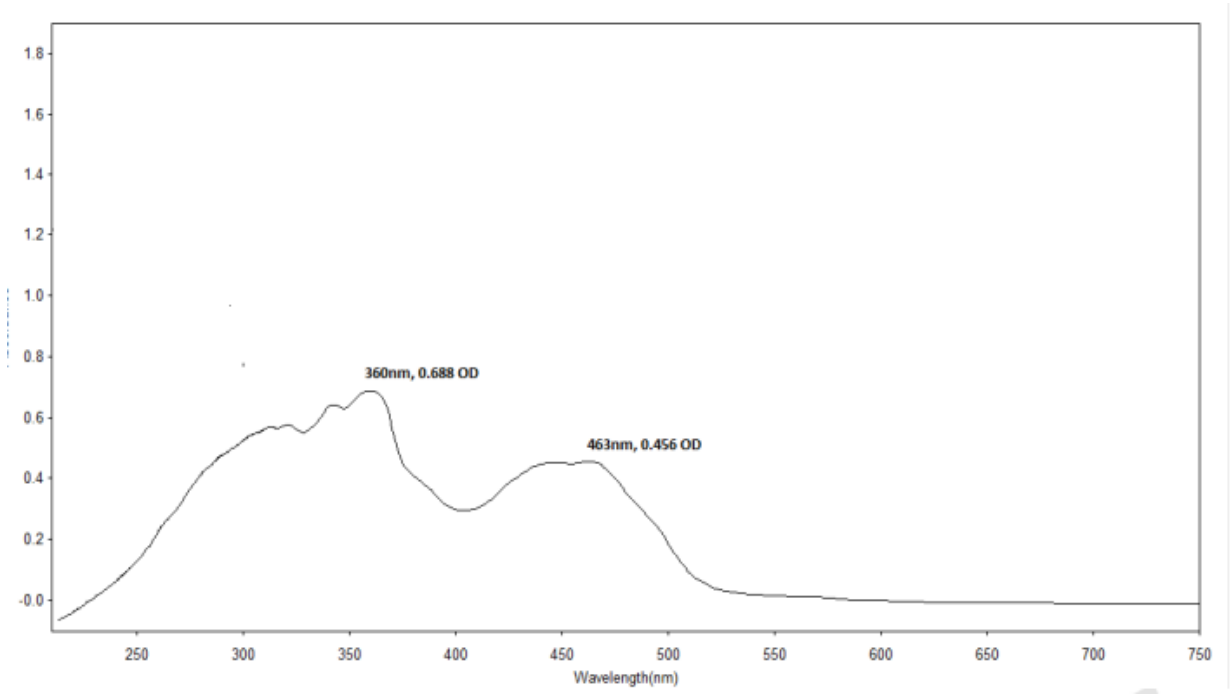

Fig. 3 UV-Visible Spectrum of *Canna Indica* Red Flower Dye Extract

ANNEX E

(*Clauses* 1 and 7.1)

EXTRACTION OF DYE FROM FABRIC/YARN DYED WITH *CANNA INDICA* RED FLOWER NATURAL DYE

E-1 PRINCIPLE

Extraction of dyed textile samples with a suitable solvent (methanol) is necessary to release the dyes from these samples.

E-2 APPARATUS

E-2.1 Soxhlet Extraction Assembly with Heating Bench

E-2.2 Scissors

E-2.3 Qualitative Filter Paper Sheet

E-2.4 Rotary Vacuum Evaporator with Evaporating Flask

E-2.5 Funnel

E-2.6 Conical Flasks

E-2.7 Measuring Cylinder

E-3 REAGENTS

E-3.1 Methanol (Analytical Reagent Grade)

E-4 PROCEDURE

About 50 g of dyed textile is cut into small pieces and packed in a filter paper. The packed filter paper column is put for soxhlet extraction with methanol as the solvent. The temperature is so adjusted to get about 8-10 cycles of the solvent per hour. Extraction is continued for 6-8 h, till most of the colour is discharged. The coloured solvent is filtered and is concentrated under vacuum in a rotary evaporator to about 20 ml. This concentrated extract can then be used for chromatographic and spectroscopic analysis as per the methods described in **7.3** after appropriate dilution, if required.

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft was finalized by Textile Specialty Chemicals and Dyestuffs Sectional Committee and had been approved by the Textile Division Council.

The revival of natural dyes has happened for two reasons:

a) Due to the harmful effect of synthetic dyes, safer natural dyes are being preferred globally, and
b) The ban on the use of azo dyes which release carcinogenic amines has also brought back natural dyes into the limelight. Natural dyes are being used extensively for the dyeing of cotton, linen, silk and wool substrates.

Impateins balsamina (Balsam, *Gulmehndi* in Hindi) is an annual plant extensively cultivated in gardens for its ornamental flowers which may be dark coloured in hues of red, purple and magenta or may be light coloured in tints of lavender and of pink to cream and white. The red flowered variety produces reddish brown dye which has been used for dyeing textiles and as a food dye as well.

In order to achieve optimum quality with minimum lot-to-lot shade variation, and little differences in colour when the same dyestuff is obtained from different manufacturers, it is essential to standardize this natural dyestuff. This will improve the cost benefit ratio and help the user adjust their dyeing or printing recipes appropriately.

It is for the first time that the standard for the natural dyestuff *Impateins balsamina* (red) flower dye has been written, for the benefit of buyers, sellers and users of this natural dyestuff. Standardization and identification methods will bring all the major parameters under scan and match. The main intention of this specification is to identify the natural dyestuff.

The composition of the Committee responsible for the formulation of this standard is given in Annex F.

In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 ‘Rules for rounding off numerical values (*revised*)’.

TEXTILE DYESTUFFS — NATURAL DYE FROM RED FLOWERS OF *IMPATEINS BALSAMINA* (BALSAM, GULMEHNDI) —IDENTIFICATION

1 SCOPE

This standard prescribes test methods and identification requirements of powder / aqueous extract of *Impateins balsamina* (Balsam, *Gulmehndi*) red flower dye, and of textiles colored with this dye. The detection and identification of the natural colourant from red flowers of *Impateins balsamina* is conducted using chromatographic techniques i.e., Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) as given in Annex B and Annex C and by UV-Visible spectroscopic technique as given in Annex D. The method for extraction of the dye from fabric dyed with *Impateins balsamina* red flower extracts is given in Annex E.

2 REFERENCE

The standards listed in Annex A 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 possibility of applying the most recent editions of the standards indicated in Annex A.

3 TERMS AND DEFINITIONS

For the purposes of this standard, the following terms and definitions apply.

3.1 *Impateins balsamina* Red Flower Dye — *Impateins balsamina* is the botanical name of the annual plant Balsam or *Gulmehndi* (Hindi) widely grown for its attractive ornamental flowers. It belongs to family Balsaminaceae. *Impateins balsamina* produces reddish brown dye from the red variety of its flowers, which has been used for dyeing textiles and as a food dye.

3.2 Natural Colourant — Colorants from natural origin, particularly materials obtained from plant parts, namely - stem, root and leaves, extracted without any chemical reaction primarily used in textiles for dyeing or printing.

4 PRINCIPLE

4.1 Natural dyestuffs are usually never a single compound; they are composed of several compounds many of which are structurally similar. Their individual character will determine how they bind to the substrate, and the ratios bound to the substrate may be different from their ratios in the extract. These individual components can be analyzed by spectroscopic and chromatographic techniques which can determine the authenticity of the natural colourant.

4.2 The colouration in red petals of *Impateins balsamina* is due to the anthocyanidin pelargonidin and its derivatives. The pelargonidin is highly conjugated with sugars (glucose, rhamnose) and acylated groups (coumaroyl, feruoyl). These anthocyanins and its esters are susceptible to hydrolysis, especially under alkaline conditions to yield the less soluble anthocyanidins. The petals also contain other coloured compounds such as the flavonol, kaempferol and its glycosides, and the naphtoquinones, lawsone and 2-methoxy-1,4-naphthoquinone. The sepals contain the conjugates of pelargonidin and kaempferol and also conjugates of the anthocyanidin, cyanidin and the flavonol, quercetin. An extract of red flowers of balsam will contain all these coloured compounds and also small amounts of various colourless phenolic compounds, tetralones and benzofurans which nevertheless will contribute to its chromatographic and spectroscopic characteristics.

5 APPARATUS

5.1 Analytical Balance, with resolution of 0.001 g.

5.2 Spatula

5.3 Ultrasonic Water Bath (Sonicator)

5.4 Stoppered Conical Flask

5.5 Measuring Cylinder

5.6 Funnel

5.7 Qualitative Grade Filter Paper, Particle retention 11 µm.

6 REAGENTS

6.1 *Impateins balsamina* Red Flowers, dried and powdered to serve as reference

6.2 Methanol, analytical grade.

7 PROCEDURE

7.1 Preparation of *Impateins balsamina* Red Flower Dye Reference and Test Samples

Dried and powdered deep red mature flowers from *Impateins balsamina* (red variety) should be used to prepare the reference sample. The stock solution of the reference natural dye is prepared by steeping 0.5 g of dry flower powder in 100 ml methanol in a stoppered conical flask. It is then agitated in a sonicator set at 45°C for about 20 min, after which the solution is filtered using a filter paper, and then transferred to another stoppered glass vessel for further analysis. If not used on the same day, the stock solution can be stored in a refrigerator for about two weeks. Stock solution of the test sample is also prepared in the same way from dry, powdered plant material. If the test sample is a liquid

concentrate or a spray dried extract, a shorter dissolution time and a smaller amount of sample (0.05 g) may be sufficient. If the test material is dyed textile, the method for the extraction of the natural dye from it is given in Annex E

7.2 Analysis

The detection and identification of *Impateins balsamina* red flower natural dye from prepared dye samples is carried out by using chromatographic techniques viz., TLC and HPLC as given in Annex B and C. These are also analyzed by UV-Visible spectrophotometry as given in Annex D.

7.3 Qualification and Identification of *Impateins balsamina* Red Flower Natural Dye

Comparison between analyses of test sample and reference *Impateins balsamina* red flower natural dye sample through **7.3** can identify whether the test sample is *Impateins balsamina* red flower natural dye. The specific tests to be conducted are given in Table 1.

8 CALIBRATION OF THE ANALYTICAL EQUIPMENT

The instruments/equipment being used (TLC, HPLC, UV-Visible spectrophotometer) should be calibrated using standard reference materials and procedures as recommended by the manufacturer to ensure their proper working before start of the analysis.

9 VALIDATION OF THE METHOD

The test methods employed should be validated using authentic reference dye sample for reliability and repeatability

10 REPORT

The report shall include the following information:

a) Reference to this standard,
b) Tests conducted and test conditions, and
c) Results on identification of the test sample.

Table 1 Specific Tests Recommended for Identification of *Impateins balsamina*

Red Flower Natural Dye

(*Clause* 7.3)

Sl No.	Test	Type of Test	Ref to Annex
(1)	(2)	(3)	(4)
i)	Chromatography tests: a) Thin layer chromatography (TLC) b)High performance liquid chromatography (HPLC)	 Mandatory test Additional test (*See* Note)	 B C
ii)	Spectroscopy tests: a) UV-visible spectroscopy	Confirmatory test	D
NOTE — HPLC may be performed wherever such instrumentation facilities are available.			

ANNEX A

(*Clause* 2)

LIST OF REFFERED STANDARDS

IS No.	*Title*
1070 : 2023	Reagent grade water — Specification (third revision)

ANNEX B

(*Clauses* 1, 7.2 *and Table* 3)

IDENTIFICATION OF IMPATEINS BALSAMINA RED FLOWER NATURAL DYE BY THIN LAYER CHROMATOGRAPHY (TLC)

B-1 PRINCIPLE

TLC separates non-volatile constituent compounds in a mixture by virtue of differences in their affinity for the stationary solid phase which is spread as a thin layer on a sheet/plate and the selected mobile solvent phase which are then seen as different spots on the TLC plate. Positioning of a spot on the TLC plate denoted by its R_f value (distance travelled by the constituent / distance travelled by the solvent) depends on its chemical nature and is its characteristic property. Therefore, due to similarity in constituent chemical components, test and reference *Impateins balsamina* red flower natural dye samples under similar TLC condition should result in spots of similar R_f values and colour and thus a test sample can be identified as whether it is *Impateins balsamina* red flower natural dye.

B-2 APPARATUS

B-2.1 Analytical thickness Silica gel G/ GF 254 coated TLC plates (may be cast in-house or procured from commercial suppliers).

B-2.2 Capillary for spotting TLC plates.

B-2.3 TLC development chamber (of a dimension suitable to accommodate TLC plate).

B-2.4 TLC spot visualization chamber containing crystals of iodine (of a dimension suitable to accommodate TLC plate).

B-2.5 Optionally, a viewing chamber for TLC with 254 nm UV tubes which may also be fitted with daylight fluorescent tubes (CRI 95) and photographic camera for recording photographs.

B-3 REAGENTS

B-3.1 Stock Solutions, of test and reference *Impateins balsamina* red flower natural dye prepared in **7.1**.

B-3.2 Methanol

B-3.3 Ethyl Acetate

B-3.4 n-Hexane

All reagents except item **B-3.1** should be of analytical reagent grade and all glassware should be cleaned and finally rinsed with reagent grade water and dried.

B-4 PROCEDURE

Both reference and the test sample extracts are spotted carefully, side-by-side, on a silica gel coated TLC plate. The spots are dried and the plate is placed in TLC development chamber, pre-equilibrated with the developing solvent consisting of 60 percent Ethyl acetate and 40 percent n-Hexane (Solvent system-1). After the solvent front has travelled to sufficient distance, the TLC plate is removed from the chamber and the solvent is allowed to evaporate. Developed spots are visualized in daylight. The spots may optionally be visualized and photographed in the viewing chamber under daylight fluorescent tubes and if a silica gel GF254 plate is used, also under UV-254 nm light. The TLC plate is then placed in the iodine chamber and the spots are visualized in iodine. Another TLC separation can be performed in the solvent system consisting of 10 percent Methanol and 90 percent n-Hexane (Solvent system-2) where a greater number of spots are visualized. The constituents of the test sample present as spots on each TLC plate are compared with the reference dye sample and the color and R_f values of the spots in both the test and reference sample are noted.

B-5 OBSERVATION

Details of stock solution preparation and its dilution if any:

Type of TLC plate used: Silica gel G/ GF 254: in-house/commercial

Solvent system 1 used: 60 percent ethyl acetate and 40 percent n-hexane,

Solvent system 2 used: 10percent methanol and 90 percent n-hexane

	R_f of reference sample of *Impateins balsamina* red flower dye extract	R_f and spot color of test sample extract	Comment
Solvent system 1	R_f 0.50		
Solvent system 2	R_f 0.90, 0.60 and 0.20		

Two indicative thin layer chromatograms with both the tracks being *Impateins balsamina* red flower natural dye extract are presented in Fig. 1 and Fig. 2. However, the position and number of spots may vary due to differences in sample preparation and chromatographic conditions.

B-6 CONCLUSION

Colour of the spots and the R_f values of the reference and test samples are similar/different.

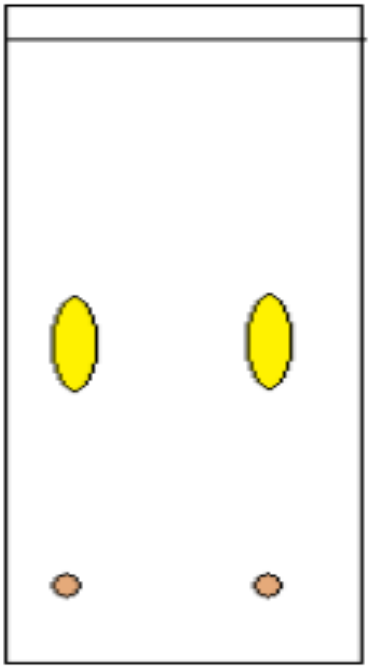

Fig. 1 Thin Layer Chromatogram of *Impateins Balsamina* Red Flower Natural Dye Extract (Solvent System 1)

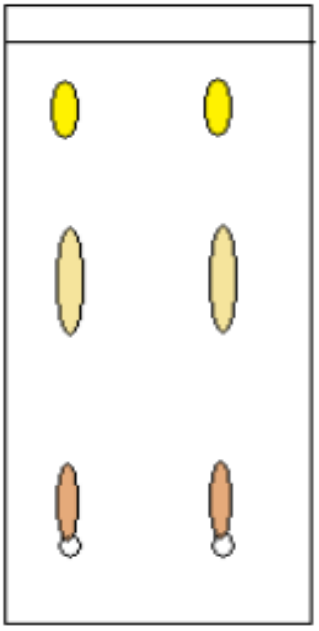

Fig. 2 Thin Layer Chromatogram of *Impateins Balsamina* Red Flower Natural Dye Extract (Solvent System 2

ANNEX C

(*Clauses* 1, 7.2 *and Table* 3)

IDENTIFICATION OF *IMPATEINS BALSAMINA* RED FLOWER DYE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

C-1 PRINCIPLE

HPLC is used to separate compounds in a mixture by virtue of differences in their affinity for the stationary phase which is packed in a column, and the mobile solvent phase that is pumped through it. The constituents, depending upon their chemical nature, are differently retarded during their passage through the column and are identified by comparing the time taken by a constituent compound in eluting from the column, as detected by a detector (retention time), with that of a standard reference compound. Selection of a detector is important as in the closed system, the eluting compounds cannot be seen by the eye and can only be detected by their characteristic properties. UV detector is a common detector employed as many organic compounds absorb in the UV region. Wavelength of the detector can be set to a value where absorption by the compounds of interest is high so as to achieve higher sensitivity. Various parameters of the HPLC assay are set in such a way that distinct peaks of the reference sample are observed in the chromatogram.

C-2 APPARATUS

C-2.1 HPLC System, with binary pump, UV/PDA detector and software.

C-2.2 C-18 Reverse Phase Column (RPC-C18) — 150 × 4.6 mm; 5 micron.

C-2.3 Guard Column, compatible with the analytical HPLC column.

C-2.4 Micro-Syringe

C-2.5 Membrane Filters (0.45 micron), for micro -syringe.

C-2.6 All Glass Filtration Assembly — for micro filtering solvents.

C- 2.7 Oil-Free Vacuum Pump

C-2.8 Membrane Filters (0.45 micron), for solvent filtration.

C-3 REAGENTS

C-3.1 Methanol

C-3.2 Deionized Water

C-3.3 Reference *Impateins balsamina* red flower natural dye and test dye stock solutions prepared in **7.1**.

NOTE — All reagents except **C-3.3** should be of HPLC grade and need to be filtered through 0.45 micron membrane filter before use. All glassware used should be cleaned and finally rinsed with reagent grade water and dried**.**

C-4 PROCEDURE

C-4.1 Chromatographic conditions

a)	Stationary phase:	Column C18, 150 × 4.6 mm; 5 micron,
b)	Eluent/ mobile phase:	Methanol: Deionised water (95:5),
c)	Flow rate:	1.0 ml/min,
d)	Detection Wavelength:	254 nm (band width 16 nm), and
e)	Run time:	15 min.

C-4.2 Method

C-4.2.1 HPLC system is started and the selected mobile phase is allowed to pass through the column till a stable baseline is obtained. Reference dye solution, after appropriate dilution, is then aspirated into the micro-syringe, a membrane pre-filter is fitted onto it and the solution is injected into the sample loop (10-20 micro litre capacity) in load position to fill it completely. The injection valve is then turned to inject position to introduce the sample onto the column. As the mobile phase is continuously passing through the column, the constituents of the injected dye extract get separated. These separated constituents exit the column and enter the UV/PDA Detector which detects them by recording the absorbance of the individual constituents as they pass through it. This absorbance is converted to a signal and peaks are seen on the signal intensity versus time plot which is continuously displayed on the monitor. When a UV-absorbing component passes through the detector a peak is seen on the plot. After completion of the run, a chromatogram along with a report of peak retention times, peak height and peak area for each peak is generated by the software. The test sample run is also completed in the similar manner. Identity of the test sample is established by comparing the retention times of the peaks in reference and test chromatograms. Peak height or its area provides additional quantitative information about the relative abundance of various constituents in test and reference samples.

C-4.2.2 An indicative chromatogram containing the peaks obtained after HPLC assay of the *Impateins balsamina* red flower natural dye extract is shown in Fig 3. Peak location/retention times and height may vary between the indicative chromatogram and the injected samples due to differences of the dye sample and the extract preparation conditions.

C-5 OBSERVATION

C-6 CONCLUSION

The retention time of the constituents of the reference and test sample are similar/different. The reference sample and test sample are therefore similar/different.

	In reference sample of *Impateins balsamina* red flower natural dye extract	In test sample extract	Comment
Retention time of the peaks observed in HPLC chromatogram	2 min 2.5 min 3.6 min		Characteristic 3 peaks in the ratio of 1:1.5:2

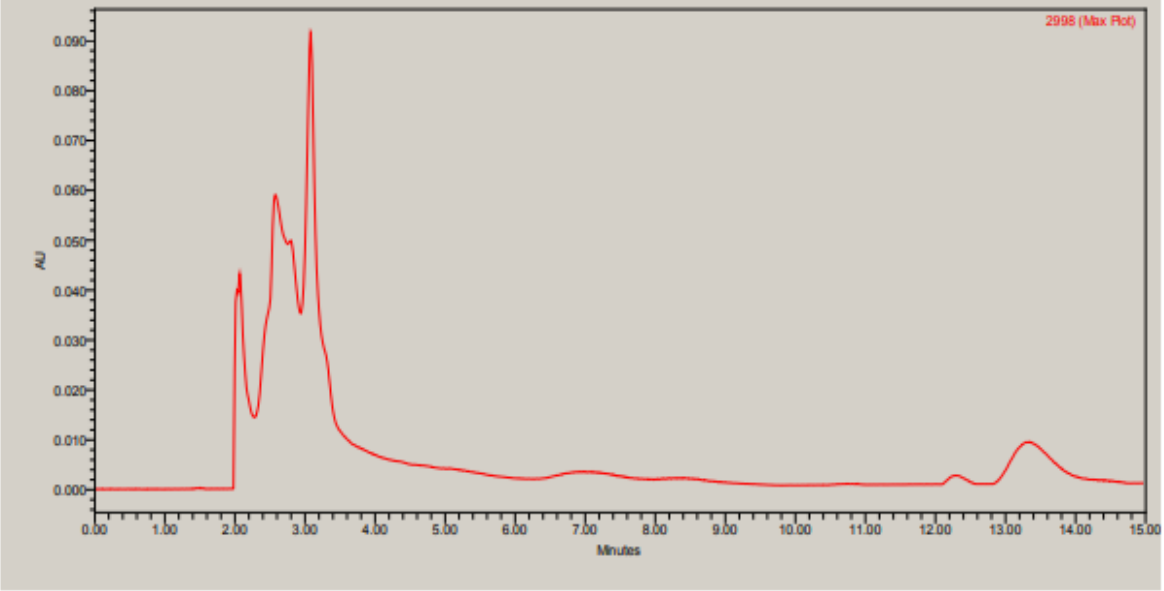

Fig. 3 High Performance Liquid Chromatogram of *Impateins Balsamina* Red Flower Natural Dye Extract

ANNEX D

(*Clauses* 1, 7.2 *and Table* 3)

IDENTIFICATION OF IMPATEINS BALSAMINA RED FLOWER DYE BY

UV–VISIBLE SPECTROSCOPIC METHOD

D-1 PRINCIPLE

D-1.1 When sample molecules are exposed to light having an energy that matches a possible electronic transition within the molecule, some light energy is absorbed as the electron is promoted to a higher energy orbital. An optical spectrometer records the wavelengths at which absorption occurs, together with the quantum of absorption at each wavelength. The resulting spectrum is presented as a graph of absorbance (*A*) versus wavelength.

D-1.2 Many molecules absorb ultraviolet and/or visible light. Fine particles will scatter light and contribute to the absorbance. It is therefore essential to remove fine particles when measuring absorbance of dye solutions. Absorbance (*A*) is directly proportional to the path length, b, and the concentration, c, of the absorbing species. Beer's law states that

$$A = \mathcal{E}bc$$

Where, Ɛ is a constant of proportionality, called the absorptivity.

D-1.3 Different dye compounds within the natural dyestuff absorb radiations of different specific wavelengths. The spectral curve will thus be a summation of their individual absorbances. An absorption spectrum of the natural dyestuff will thus show a characteristic curve with peaks corresponding to structural groups present within the constituent molecules.

D-2 APPARATUS

D-2.1 Ultra Violet Visible Scanning Spectrophotometer

D-2.2 Quartz Sample Cuvettes — Matched pair of 1.00 cm light path.

D-3 REAGENTS

D-3.1 Reference *Impateins balsamina* Red Flower Natural Dye Extract

D-3.2 Methanol

NOTE — All reagents should be of analytical reagent grade and all glassware should be cleaned and finally rinsed with reagent grade water and dried.

D- 4 PROCEDURE

D-4.1 The stock solution of the reference *Impateins balsamina* red flower dye prepared in 7.1 may be used to record the spectrum in the visible region (400 – 700 nm). This solution after diluting five-

fold may be suitable for recording the spectrum in the UV region (200 – 400 nm). The test sample solution is also prepared in the same manner as the reference sample.

D-4.2 Both UV and visible spectra are recorded with pure methanol in reference holder of the spectrophotometer. Reference dye sample is first analysed. Test sample is analysed next and recorded spectra are compared for similarity of the spectral curve that is, peak positions and their intensity (absorbance value). Indicative UV and visible spectra of the *Impateins balsamina* red flower natural dye extract is presented in Figs. 4 and 5, respectively. The absorbance spectral curve of this dye extract has specific characteristics both in the UV and visible range as shown in these Figures.

D-5 OBSERVATION

D-6 CONCLUSION

The nature of the spectral curve of the test sample in the UV and visible range is similar/different to the reference sample.

	In reference *Impateins balsamina* red flower dye extract	In test sample extract	Comments (Describing difference/ similarity in spectral curve)
Peak position (nm) with absorbance	210 nm — 2.40 A 275 nm — 1.60 A 360 nm — 1.16 A		
	510 nm — 1.40A		

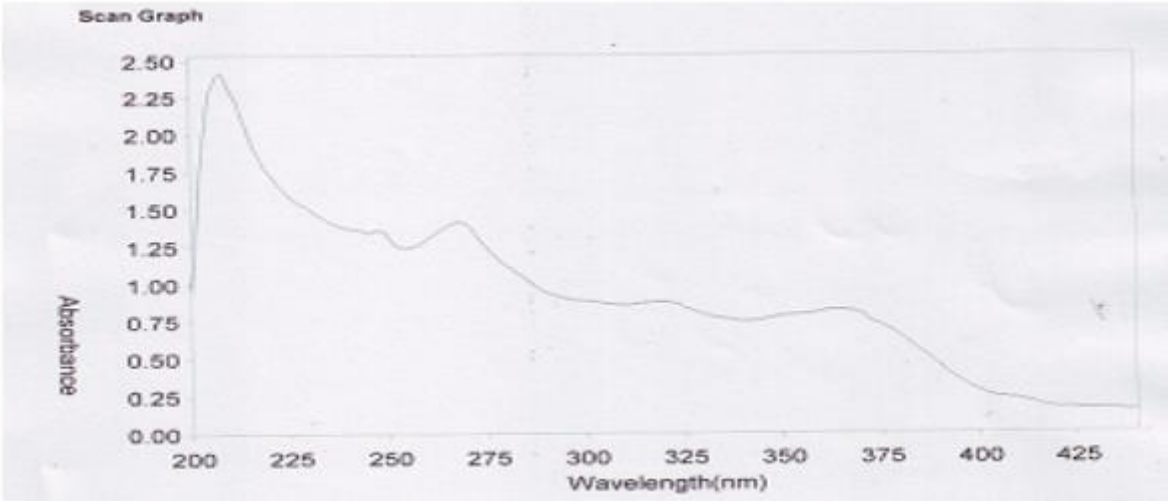

Fig. 4 UV Spectrum of *Impateins Balsamina* Red Flower Natural Dye Extract

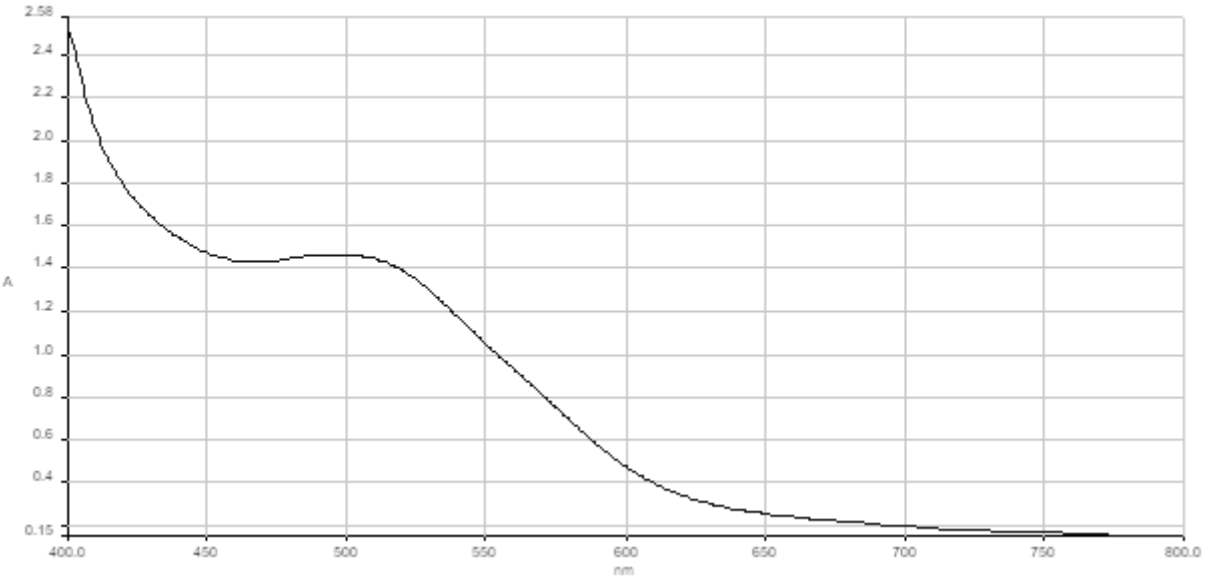

Fig. 5 Visible Spectrum of *Impateins Balsamina* Red Flower Natural Dye Extract

ANNEX E

(*Clauses* 1 *and* 7.1)

EXTRACTION OF DYE FROM FABRIC/YARN DYED WITH IMPATEINS BALSAMINA RED FLOWER NATURAL DYE

E-1 PRINCIPLE

Extraction of dyed textile samples with a suitable solvent (methanol) is necessary to release the dyes from these samples.

E-2 APPARATUS

E-2.1 Soxhlet Extraction Assembly with Heating Bench

E-2.2 Scissors

E-2.3 Qualitative Filter Paper Sheet

E-2.4 Rotary Vacuum Evaporator with Evaporating Flask

E-2.5 Funnel

E-2.6 Conical Flasks

E-2.7 Measuring Cylinder

E-3 REAGENTS

E-3.1 Methanol (Analytical Reagent Grade)

E-4 PROCEDURE

About 50 g of dyed textile is cut into small pieces and packed in a filter paper. The packed filter paper column is put for soxhlet extraction with methanol as the solvent. The temperature is so adjusted to get about 8-10 cycles of the solvent per hour. Extraction is continued for 6-8 h, till most of the colour is discharged. The coloured solvent is filtered and is concentrated under vacuum in a rotary evaporator to about 20 ml. This concentrated extract can then be used for chromatographic and spectroscopic analysis as per the methods described in **7.3** after appropriate dilution, if required.

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft was finalized by Textile Specialty Chemicals and Dyestuffs Sectional Committee and had been approved by the Textile Division Council.

The revival of natural dyes has happened for two reasons:

a) Due to the harmful effect of synthetic dyes, safer natural dyes are being preferred globally, and
b) The ban on the use of azo dyes which release carcinogenic amines has also brought back natural dyes into the limelight. Natural dyes are being used extensively for the dyeing of cotton, linen, silk and wool substrates.

Tectona grandis (Teak, *Sagaun* in Hindi) leaves have been used to dye yarn and fabric in a reddish brown shade by many dyers in India. The tender leaves are reddish, and turn green as they grow. Mature leaves of only some plants yield the reddish dye and these are valuable as a dye source.

In order to achieve optimum quality with minimum lot-to-lot shade variation, and little differences in colour when the same dyestuff is obtained from different manufacturers, it is essential to standardize this natural dyestuff. This will improve the cost benefit ratio and help the user adjust their dyeing or printing recipes appropriately.

It is for the first time that the standard for natural dyestuff *Tectona grandis* leaves has been written, for the benefit of buyers, sellers and users of this natural dyestuff. Standardization and identification methods will bring all the major parameters under scan and match. The main intention of this specification is to identify the natural dyestuff.

The composition of the Committee responsible for the formulation of this standard is given in Annex F.

In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 ‘Rules for rounding off numerical values (*revised*)’.

Indian Standard

TEXTILE DYESTUFFS — NATURAL DYE FROM TECTONA GRANDIS (SAGAUN) LEAVES — IDENTIFICATION

1 SCOPE

This standard prescribes test methods and identification requirements of powder / aqueous extract of *Tectona grandis* (*Sagaun*) leaf dye, and of textiles coloured with this dye. The detection and identification of the natural colourant from *Tectona grandis* leaves is conducted using chromatographic techniques, that is, Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) as given in Annex B and C and by UV-Visible spectroscopic technique as given in Annex D. The method for extraction of the dye from textile dyed with *Tectona grandis* leaf extracts is given in Annex E.

2 REFERENCE

The standards listed in Annex A 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 possibility of applying the most recent editions of the standards indicated in Annex A.

3 TERMS AND DEFINITIONS

For the purpose of this standard, the following terms and definitions apply:

3.1 *Tectona grandis* Leaves Dye — *Tectona grandis* is the botanical name of the Teak or *Sagaun* (Hindi) tree, well known for its good quality timber. It belongs to family Lamiaceae. The leaves of this plant are used variously as a food additive, source of tannin, and dye. Leaves of *Tectona grandis* produce a reddish brown dye which has been used for dyeing textiles since ancient times.

3.2 Natural Colourant — Colourants from natural origin, particularly materials obtained from plant parts, namely - stem, root and leaves, extracted without any chemical reaction primarily used in textiles for dyeing or printing.

4 PRINCIPLE

4.1 Natural dyestuffs are usually never a single compound; they are composed of several compounds many of which are structurally similar. Their individual character will determine how they bind to the substrate, and the ratios bound to the substrate may be different from their ratios in the extract. These individual components can be analyzed by spectroscopic and chromatographic techniques which can determine the authenticity of the natural colourant.

4.2 Anthraquinones are reported to be responsible for reddish brown dye. In particular, Tectoleafquinone (1,4,5,8-tetrahydroxy-2-iso-pentadienylanthraquinone) is considered to be the main reddish colourant. Other anthraquinones whose occurrence is reported in leaves of *Tectona grandis* are Anthratectone, Naphthotectone, Grandiquinone A, Quinizarine, Tectograndone, Tectone, 5,8-dihydroxy-2-methylanthraquinone and 3-hydroxy-2-methylanthraquinone. Naphtaquinones and flavonoids are the other class of coloured compounds present in leaves of *Tectona grandis*. Tannic acid and gallic acid have also been detected. All these compounds will contribute to the chromatographic and spectroscopic characteristics of its extract.

5 APPARATUS

5.1 Analytical Balance, with resolution of 0.001 g.

5.2 Spatula

5.3 Ultrasonic Water Bath (Sonicator)

5.4 Stoppered Conical Flask

5.5 Measuring Cylinder

5.6 Funnel

5.7 Qualitative Grade Filter Paper — Particle retention 11 µm.

6 REAGENTS

6.1 *Tectona grandis* Leaves, dried and powdered to serve as reference.

6.2 Methanol, analytical grade

7 PROCEDURE

7.1 Preparation of *Tectona grandis* Leaf Dye Reference and Test Samples

Authentic leaf powder made by pulverizing only those *Tectona grandis* leaves that dye textile substrates in reddish brown colour should be used to prepare the reference sample. The stock solution of the reference natural dye is prepared by steeping 0.5 g of dry leaf powder in 100 ml methanol in a stoppered conical flask. It is then agitated in a sonicator set at 45°C for about 20 min after which the solution is filtered using a filter paper, and then transferred to another stoppered glass vessel for further analysis. If not used on the same day, the stock solution can be stored in a refrigerator for about two weeks. Stock solution of the test sample is also prepared in the same way from dry, powdered plant material. If the test sample is a liquid concentrate or a spray dried extract, a shorter

dissolution time and a smaller amount of sample (0.05 g) may be sufficient. If the test material is dyed textile, the method for the extraction of the natural dye from it is given in Annex E

7.2 Analysis

The detection and identification of *Tectona grandis* leaf natural dye from prepared dye samples is carried out by using chromatographic techniques namely, TLC and HPLC as given in Annex B and C. These are also analyzed by UV-Visible spectrophotometry as given in Annex D.

7.3 Qualification and Identification of *Tectona grandis* Leaf Natural Dye

Comparison between analyses of test sample and reference *Tectona grandis* leaf dye sample through **7.3** can identify whether the test sample is natural *Tectona grandis* leaf dye. The specific tests to be conducted are given in Table 1.

Table 1 Specific Tests Recommended for Identification of *Tectona grandis* leaf dye
(*Clause* 7.3)

Sl No.	Test	Type of Test	Ref to Annex
(1)	(2)	(3)	(4)
i)	Chromatography tests: a) Thin layer chromatography (TLC) b) High performance liquid chromatography (HPLC)	 Mandatory test Additional test (*See* Note)	 B C
ii)	Spectroscopy tests: a) UV-Visible spectroscopy	Confirmatory test	D
NOTE — HPLC may be performed wherever such instrumentation facilities are available.			

8 CALIBRATION OF THE ANALYTICAL EQUIPMENT

The instruments/equipment being used (TLC, HPLC, UV-Visible spectrophotometer) should be calibrated using standard reference materials and procedures as recommended by the manufacturer to ensure their proper working before start of the analysis.

9 VALIDATION OF THE METHOD

The test methods employed should be validated using authentic reference dye sample for reliability and repeatability

10 REPORT

The report shall include the following information:

a) Reference to this standard,
b) Tests conducted and test conditions, and

c) Results on identification of the test sample.

ANNEX A

(*Clause* 2)

LIST OF REFFERED STANDARDS

IS No.	*Title*
1070 : 2023	Reagent grade water — Specification (third revision)

ANNEX B

(*Clauses* 1, 7.2 *and Table* 3)

IDENTIFICATION OF *TECTONA GRANDIS* LEAF NATURAL DYE BY THIN LAYER CHROMATOGRAPHY (TLC)

B-1 PRINCIPLE

TLC separates non-volatile constituent compounds in a mixture by virtue of differences in their affinity for the stationary solid phase which is spread as a thin layer on a sheet/plate and the selected mobile solvent phase which are then seen as different spots on the TLC plate. Positioning of a spot on the TLC plate denoted by its R_f value (distance travelled by the constituent / distance travelled by the solvent) depends on its chemical nature and is its characteristic property. Therefore, due to similarity in constituent chemical components, test and reference e *Tectona grandis l*eaf natural dye samples under similar TLC condition should result in spots of similar R_f values and colour and thus a test sample can be identified as whether it is *Tectona grandis* leaf natural dye.

B-2 APPARATUS

B-2.1 Analytical thickness Silica gel G/ GF 254 coated TLC plates (may be cast in-house or procured from commercial suppliers).

B-2.2 Capillary for spotting TLC plates.

B-2.3 TLC development chamber (of a dimension suitable to accommodate TLC plate).

B-2.4 TLC spot visualization chamber containing crystals of iodine (of a dimension suitable to accommodate TLC plate).

B-2.5 Optionally, a viewing chamber for TLC with 254 nm UV tubes which may also be fitted with daylight fluorescent tubes (CRI 95) and photographic camera for recording photographs.

B-3 REAGENTS

B-3.1 Stock Solutions, of test and reference *Tectona grandis* leaf natural dye prepared in **7.1**.

B-3.2 Methanol

B-3.3 Ethyl Acetate

B-3.4 n-Hexane

All reagents except item **B-3.1** should be of analytical reagent grade and all glassware should be cleaned and finally rinsed with reagent grade water and dried.

B-4 PROCEDURE

Both reference and the test sample extracts are spotted carefully, side-by-side, on the silica gel coated TLC plate. The spots are dried and the plate is placed in TLC development chamber, pre-equilibrated with the developing solvent consisting of 80 percent ethyl acetate and 20 percent n-hexane. After the solvent front has travelled to sufficient distance, the TLC plate is removed and the solvent is allowed to evaporate. Developed spots are visualized in daylight. The spots may optionally be visualized and photographed in the viewing chamber under daylight fluorescent tubes and if a silica gel GF254 plate is used, also under UV-254 nm light. The TLC plate is then placed in the iodine chamber and the spots are visualized in iodine. The constituents of the test sample present as spots on the TLC plate are compared with the reference dye sample. The colour and R_f values of the spots in both the test and reference sample are noted.

B-5 OBSERVATION

Details of stock solution preparation and its dilution if any:

Type of TLC plate used: Silica gel G/ GF 254: in-house/commercial

Solvent system used: 80 percent ethyl acetate and 20 percent n-hexane,

Of reference sample of

R_f and spot colour in *Tectona grandis* leaf natural dye	R_f and spot colour of test sample	Comment
R_f 0.60 major and 0.80 minor		

An indicative thin layer chromatogram with both the tracks being natural *Tectona grandis* leaf natural dye extract is presented in Fig. 1. However, the position and number of spots may vary due to differences in sample preparation and chromatographic conditions.

B-6 CONCLUSION

Colour of the spots and the R_f values of the reference and test samples are similar/different.

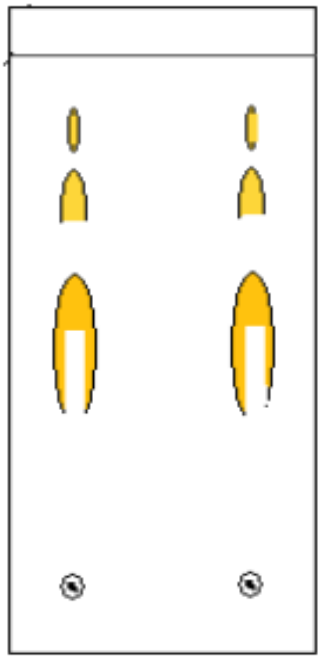

Fig. 1 Thin Layer Chromatogram of *Tectona Grandis* Red Leaf Natural Dye Extract

ANNEX C

(*Clauses* 1, 7.2 *and Table* 3)

IDENTIFICATION OF *TECTONA GRANDIS* LEAF DYE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

C-1 PRINCIPLE

HPLC is used to separate compounds in a mixture by virtue of differences in their affinity for the stationary phase which is packed in a column, and the mobile solvent phase that is pumped through it. The constituents, depending upon their chemical nature, are differently retarded during their passage through the column and are identified by comparing the time taken by a constituent compound in eluting from the column, as detected by a detector (retention time), with that of a standard reference compound. Selection of a detector is important as in the closed system, the eluting compounds cannot be seen by the eye and can only be detected by their characteristic properties. UV detector is a common detector employed as many organic compounds absorb in the UV region. Wavelength of the detector can be set to a value where absorption by the compounds of interest is high so as to achieve higher sensitivity. Various parameters of the HPLC assay are set in such a way that distinct peaks of the reference sample are observed in the chromatogram.

C-2 APPARATUS

C-2.1 HPLC System, with binary pump, UV/PDA detector and software.

C-2.2 C-18 Reverse Phase Column (RPC-C18) — 150 × 4.6 mm; 5 micron.

C-2.3 Guard Column, compatible with the analytical HPLC column.

C-2.4 Micro-Syringe

C-2.5 Membrane Filters (0.45 micron), for micro -syringe.

C-2.6 All Glass Filtration Assembly — for micro filtering solvents.

C- 2.7 Oil-Free Vacuum Pump

C-2.8 Membrane Filters (0.45 micron), for solvent filtration.

C-3 REAGENTS

C-3.1 Methanol

C-3.2 Deionized Water

C-3.3 Reference *Tectona grandis* leaf natural dye and test dye stock solutions prepared in **7.1**.

NOTE — All reagents except **C-3.3** should be of HPLC grade and need to be filtered through 0.45 micron membrane filter before use. All glassware used should be cleaned and finally rinsed with reagent grade water and dried**.**

C-4 PROCEDURE

C-4.1 Chromatographic conditions

a)	Stationary phase:	Column C18, 150 × 4.6 mm; 5 micron,
b)	Eluent/ mobile phase:	Methanol: Deionised water (80:20),
c)	Flow rate:	1.0 ml/min,
d)	Detection Wavelength:	254 nm (band width 16 nm), and
e)	Run time:	10 min.

C-4.2 Method

C-4.2.1 HPLC system is started and the selected mobile phase is allowed to pass through the column till a stable baseline is obtained. Reference dye solution, after appropriate dilution, is then aspirated into the micro-syringe, a membrane pre-filter is fitted onto it and the solution is injected into the sample loop (10-20 micro litre capacity) in load position to fill it completely. The injection valve is then turned to inject position to introduce the sample onto the column. As the mobile phase is continuously passing through the column, the constituents of the injected dye extract get separated. These separated constituents exit the column and enter the UV/PDA Detector which detects them by recording the absorbance of the individual constituents as they pass through it. This absorbance is converted to a signal and peaks are seen on the signal intensity versus time plot which is continuously displayed on the monitor. When a UV-absorbing component passes through the detector a peak is seen on the plot. After completion of the run, a chromatogram along with a report of peak retention times, peak height and peak area for each peak is generated by the software. The test sample run is also completed in the similar manner. Identity of the test sample is established by comparing the retention times of the peaks in reference and test chromatograms. Peak height or its area provides additional quantitative information about the relative abundance of various constituents in test and reference samples.

C-4.2.2 An indicative chromatogram containing the peaks obtained after HPLC assay of the *Tectona grandis* leaf natural dye extract is shown in Fig 2. Peak location/retention times and height may vary between the indicative chromatogram and the injected samples due to differences of the dye sample and the extract preparation conditions.

C-5 OBSERVATION

C-6 CONCLUSION

The retention time of the constituents of the reference and test sample are similar/different. The reference sample and test sample are therefore similar/different.

	In reference sample of *Tectona grandis* leaf natural dye extract	In test sample extract	Comment
Retention time of the peaks observed in HPLC chromatogram	1.5 min 2.8 min 3.2 min		

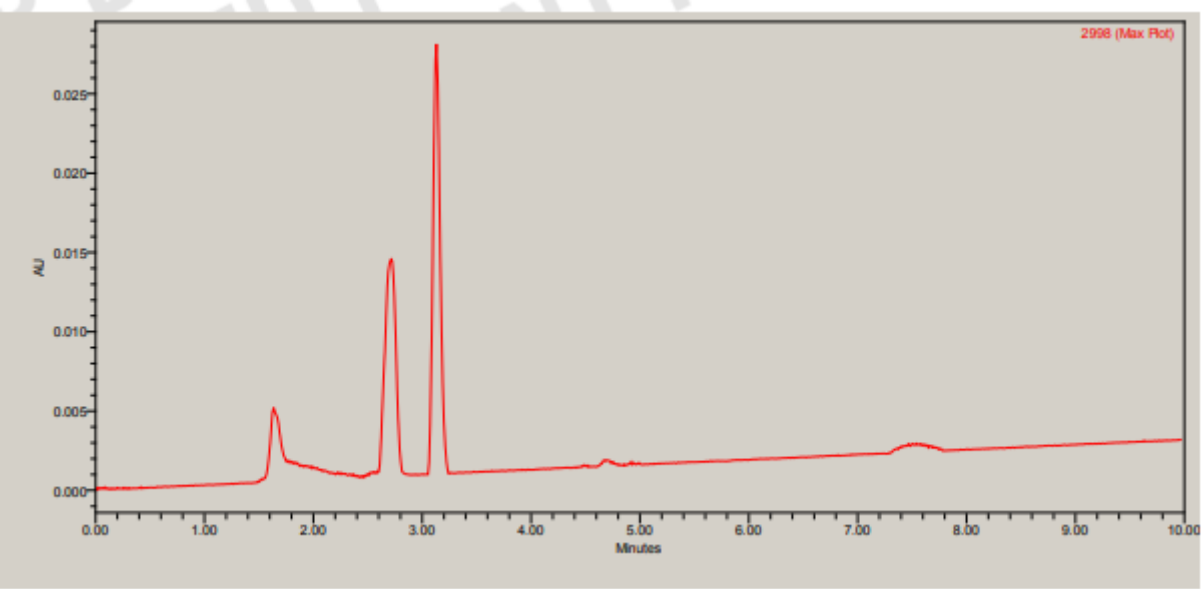

Fig. 2 High Performance Liquid Chromatogram of *Tectona Grandis* Leaf Natural Dye Extract

ANNEX D

(*Clauses* 1, 7.2 *and Table* 3)

IDENTIFICATION OF *TECTONA GRANDIS* LEAF DYE BY UV–VISIBLE SPECTROSCOPIC METHOD

D-1 PRINCIPLE

When sample molecules are exposed to light having an energy that matches a possible electronic transition within the molecule, some light energy is absorbed as the electron is promoted to a higher energy orbital. An optical spectrometer records the wavelengths at which absorption occurs, together with the quantum of absorption at each wavelength. The resulting spectrum is presented as a graph of absorbance (*A*) versus wavelength.

Many molecules absorb ultraviolet and/or visible light. Fine particles will scatter light and contribute to the absorbance. It is therefore essential to remove fine particles when measuring absorbance of dye solutions. Absorbance (*A*) is directly proportional to the path length, b, and the concentration, c, of the absorbing species. Beer's law states that

$$A = \mathcal{E}bc$$

Where, Ɛ is a constant of proportionality, called the absorptivity.

Different dye compounds within the natural dyestuff absorb radiations of different specific wavelengths. The spectral curve will thus be a summation of their individual absorbances. An absorption spectrum of the natural dyestuff will thus show a characteristic curve with peaks corresponding to structural groups present within the constituent molecules.

D-2 APPARATUS

D-2.1 Ultra Violet Visible Scanning Spectrophotometer

D-2.2 Quartz Sample Cuvettes — Matched pair of 1.00 cm light path.

D-3 REAGENTS

D-3.1 Reference *TECTONA GRANDIS* LEAF Natural Dye Extract

D-3.2 Methanol

NOTE — All reagents should be of analytical reagent grade and all glassware should be cleaned and finally rinsed with reagent grade water and dried.

D- 4 PROCEDURE

D-4.1 The stock solution of the reference *Tectona grandis* leaf dye prepared in **7.1** may be used to record the spectrum in the visible region (400 – 700 nm). This solution after diluting five-fold may be suitable for recording the spectrum in the UV region (200 – 400 nm). The test sample solution is also prepared in the same manner as the reference sample.

D-4.2 Both visible and UV spectra are recorded with pure methanol in reference holder of the spectrophotometer. Reference dye sample is first analysed. Test sample is analysed next and recorded spectra are compared for similarity of the spectral curve that is, peak positions and their intensity (absorbance value). Indicative UV and visible spectra of the *Tectona grandis* leaf natural dye extract is presented in Figs. 3 and 4, respectively. The absorbance spectral curve of this dye extract has specific characteristics both in the UV and visible range as shown in these figures.

D-5 OBSERVATION

D-6 CONCLUSION

The nature of the spectral curve of the test sample in the UV and visible range is similar/different to the reference sample.

	In reference *Tectona grandis* leaf natural dye extract	In test sample extract	Comments (Describing difference/ similarity in spectral curve)
Peak position (nm) with absorbance	209 nm — 2.90 A 286 nm — 1.06 A 328 nm — 1.12 A 608 nm—0.12 A 664 nm—0.36 A		

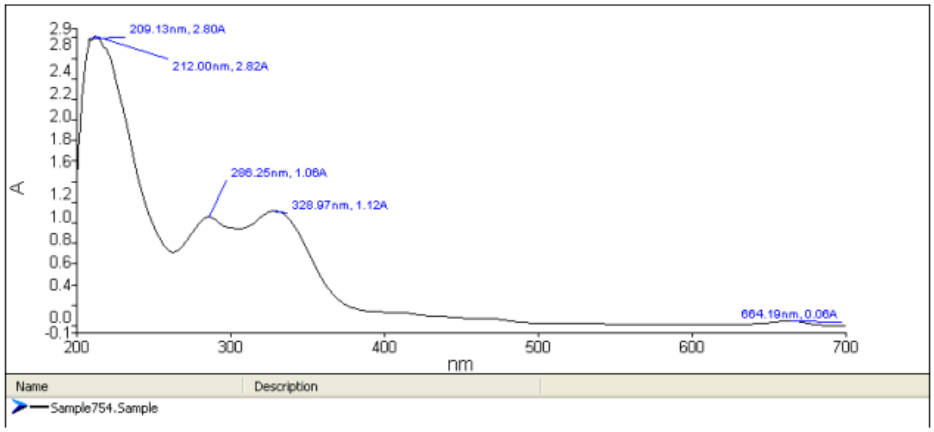

Fig. 3 UV Spectrum of *Tectona Grandis* Leaf Natural Dye Extract

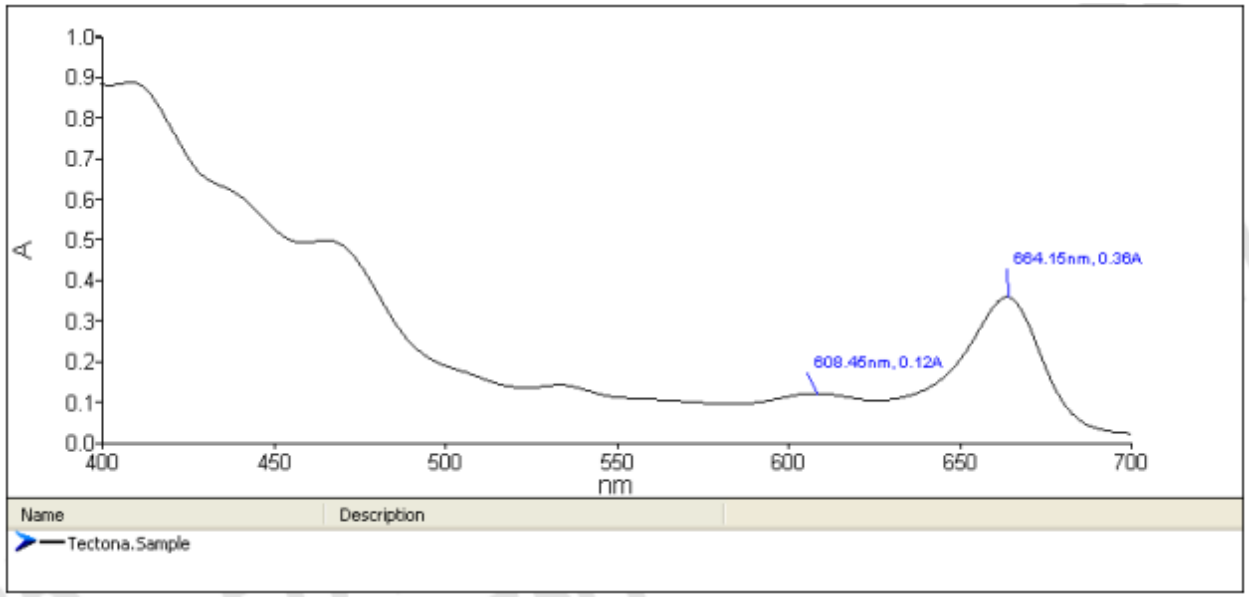

Fig. 4 Visible Spectrum of *Tectona Grandis* Leaf Natural Dye Extract

ANNEX E

(*Clauses* 1 *and* 7.1)

EXTRACTION OF DYE FROM FABRIC/YARN DYED WITH *TECTONA GRANDIS* LEAF DYE

E-1 PRINCIPLE

Extraction of dyed textile samples with a suitable solvent (methanol) is necessary to release the dyes from these samples.

E-2 APPARATUS

E-2.1 Soxhlet Extraction Assembly with Heating Bench

E-2.2 Scissors

E-2.3 Qualitative Filter Paper Sheet

E-2.4 Rotary Vacuum Evaporator with Evaporating Flask

E-2.5 Funnel

E-2.6 Conical Flasks

E-2.7 Measuring Cylinder

E-3 REAGENTS

E-3.1 Methanol (Analytical Reagent Grade)

E-4 PROCEDURE

About 50 g of dyed textile is cut into small pieces and packed in a filter paper. The packed filter paper column is put for soxhlet extraction with methanol as the solvent. The temperature is so adjusted to get about 8-10 cycles of the solvent per hour. Extraction is continued for 6-8 h, till most of the colour is discharged. The coloured solvent is filtered and is concentrated under vacuum in a rotary evaporator to about 20 ml. This concentrated extract can then be used for chromatographic and spectroscopic analysis as per the methods described in **7.3** after appropriate dilution, if required.

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft finalized by Textile Speciality Chemicals and Dyestuffs Sectional Committee had been approved by the Textile Division Council.

The revival of natural dyes has been for two reasons:

a) Due to the harmful effect of synthetic dyes, safer natural dyes are being preferred globally, and
b) Ban on the use of azo dyes which release carcinogenic amines has also brought back natural dyes in limelight. Natural dyes are being used extensively for dyeing of cotton, linen, silk and wool substrates.

Terminalia arjuna (Arjuna, *Arjun* in Hindi) bark has been variously used for medicinal purposes in improving cardio vascular health, and for tanning and dyeing purposes. It has been used for dyeing textiles in shades of brown since ancient times.

In order to achieve optimum quality with minimum lot-to-lot shade variation, and little differences in colour when the same dyestuff is obtained from different manufacturers, it is essential to standardize this natural dyestuff. This will improve the cost benefit ratio and help the user adjust their dyeing or printing recipes appropriately.

It is for the first time that the standard for natural dyestuff *Terminalia arjuna* (*Arjun*) bark dye has been written, it is mainly for the ease of buyers, seller and users of this natural dyestuff. Standardization and identification methods will bring all the major parameters under scan and match. The main intention of this specification is to identify the natural dyestuff

The composition of the Committee responsible for the formulation of this standard is given in Annex G.

In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 ‘Rules for rounding off numerical values (*revised*)’.

Indian Standard

TEXTILE DYESTUFFS — NATURAL DYE FROM *TERMINALIA ARJUNA* (*ARJUN*) BARK — IDENTIFICATION

1 SCOPE

This standard prescribes test method and identification requirement of powder/ aqueous extract of *Terminalia arjuna* (*Arjun*) bark dye, and of textiles colored with this dye. The detection and identification of the natural colorant from *Terminalia arjuna* (*Arjun*) bark is conducted using Kit method given in Annex B, chromatographic techniques, that is Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) as given in Annex C and D and by UV-Visible spectroscopic technique as given in Annex E. The method for extraction of the dye from textile dyed with *Terminalia arjuna* (*Arjun*) bark extract is given in Annex F

2 REFERENCE

The standards listed in Annex A 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 possibility of applying the most recent editions of the standards indicated in Annex A.

3 TERMS AND DEFINITIONS

For the purposes of this standard, the following terms and definitions apply.

3.1 *Terminalia arjuna* (*Arjun*) Dye — The botanical name of Arjun tree is *Terminalia arjuna*, it belongs to family Combretaceae. *Terminalia arjuna* bark is used variously as medicine for heart problems and high blood pressure, astringent, tannin source, and dye. It has been used for dyeing textiles in shades of brown since ancient times.

3.2 Natural Colourant — Colourants from natural origin, particularly materials obtained from plant parts, namely - stem, root and leaves, extracted without any chemical reaction primarily used in textiles for dyeing or printing.

4 PRINCIPLE

Natural colorants are usually never a single compound, they are composed of several compounds many of which are structurally similar and therefore visual response to one or more common laboratory chemicals may give a preliminary indication about the presence of those specific type of compounds found in a particular natural dye. The individual character of different compounds present in a natural colorant will determine how they bind to the textile substrate, and the ratios bound to the

substrate may be different from their ratios in the extract. These individual components can be analyzed out by spectroscopic and chromatographic techniques which can establish the authenticity of the natural colourant. *Terminalia arjuna* bark is rich in tannins and flavonoids which contribute to the chromatographic and spectroscopic characteristics of its extract.

4.1 Flavonoids are reported in Arjuna bark powder. These include quercetin, kaempferol, luteolin, and pellargonidin. Some Triterpenes such as arjunic acid, arjunolic acid, arjungenin, and arjunetin**.** Tannins also constitute a major part

5 APPARATUS

5.1 Analytical Balance, with resolution of 0.001 g.

5.2 Spatula

5.3 Ultrasonic Water Bath (Sonicator)

5.4 Stoppered Conical Flask

5.5 Measuring Cylinder

5.6 Funnel

5.7 Qualitative Grade Filter Paper, Particle retention 11 µm.

6 REAGENTS

6.1 *Terminalia arjuna* Bark, dried and powdered to serve as reference

6.2 Methanol, analytical grade.

7 PROCEDURE

7.1 Preparation of *Terminalia arjuna* Bark Dye Reference and Test Samples

Powder made by pulverizing authentic *Terminalia arjuna* bark material is used to prepare the reference sample. The stock solution of the reference natural dye is prepared by steeping 0.5 g of dry bark powder in 100 ml methanol in a stoppered conical flask. It is then agitated in a sonicator set at 45°C for about 20 min after which the solution is filtered using a filter paper, and then transferred to another stoppered glass vessel for further analysis. If not used on the same day, the stock solution can be stored in a refrigerator for about two weeks. Stock solution of the test sample is also prepared in the same way from dry, powdered plant material. If the test sample is a liquid concentrate or a spray dried extract, a shorter dissolution time and a smaller amount of sample (0.05 g) may be sufficient. If

the test material is dyed textile, the method for the extraction of the natural dye from it is given in Annex F.

7.2 Analysis

The detection and identification of natural *Terminalia arjuna* (*Arjun*) dye from prepared dye samples is conducted using Kit method given in Annex B, by using chromatographic techniques viz., TLC and HPLC as given in Annex C and D as well as by UV-Visible spectroscopy as given in Annex E.

7.3 Qualification and Identification of *Terminalia arjuna* (*Arjun*) Natural Dye

Comparison between analyses of test sample and reference *Terminalia arjuna* dye sample through **7.3** can identify whether the test sample is *Terminalia arjuna* dye. The specific tests to be conducted are given in Table 1.

8 CALIBRATION OF THE ANALYTICAL EQUIPMENT

The instruments/equipment being used (TLC, HPLC, UV-Visible spectrophotometer) should be calibrated using standard reference materials and procedures as recommended by the manufacturer to ensure their proper working before start of the analysis.

9 VALIDATION OF THE METHOD

The test methods employed should be validated using authentic reference dye sample for reliability and repeatability

10 REPORT

The report shall include the following information:

d) Reference to this standard,
e) Tests conducted and test conditions, and
f) Results on identification of the test sample.

Table 1 Specific Tests Recommended for Identification of *Terminalia arjuna* Bark Dye
(*Clause* 7.3)

Sl No.	Test	Type of Test	Ref to Annex
(1)	(2)	(3)	(4)
i)	Kit test	Preliminary test	B
i)	Chromatography tests: a) Thin layer chromatography (TLC) b) High performance liquid chromatography (HPLC)	 Mandatory test Additional (*See* Note)	 C D
ii)	Spectroscopy tests:	Confirmatory test	E

	a) UV-visible spectroscopy		
NOTE — HPLC may be performed wherever such instrumentation facilities are available.			

ANNEX A

(*Clause* 2)

LIST OF REFFERED STANDARDS

IS No.	*Title*
1070 : 2023	Reagent grade water — Specification (third revision)

ANNEX B

(*Clauses* 1, 7.2 *and Table* 3)

KIT TEST FOR IDENTIFICATION OF *TERMINALIA ARJUNA* (*ARJUN*) BARK DYE

B-1 PRINCIPLE

Each natural dye has its characteristic molecular composition which is the distinctive feature of that dyestuff. The *Terminalia arjuna* (*Arjun*) dye gives a characteristic reaction with sodium hydroxide solution which is used for its identification.

B-2 APPARATUS

B-2.1 Test Tubes

B-2.2 Test Tube Holder

B-2.3 pH Meter

B-2.4 Pipette

B-2.5 Measuring Cylinder

B-3 REAGENTS

B-3.1 Distilled Water

B-3.2 Sodium Hydroxide, Analytical Grade

B-3.3 Stock Solutions of Test and Reference *Terminalia arjuna* (*Arjun*) Dye

B-4 PROCEDURE

The stock solution of both the reference and test *Terminalia arjuna* (*Arjun*) dye is diluted 10 times with water and 10 ml of the diluted solution is taken in a test tube. This solution is then made alkaline (pH 10.6) by using 10 percent solution of sodium hydroxide (NaOH) and observed for any colour change.

B-5 OBSERVATION

A dark maroon colour develops in the solution of reference *Terminalia arjuna* dye. If a similar colour change is observed in test sample also, it is likely to be *Terminalia arjuna* dye.

B-5 CONCLUSION

The development of dark maroon colour in test sample gives an indication that it is *Terminalia arjuna* dye.

ANNEX C

(*Clauses* 1, 7.2 *and Table* 3)

IDENTIFICATION OF TERMINALIA ARJUNA BARK NATURAL DYE BY THIN LAYER CHROMATOGRAPHY (TLC)

C-1 PRINCIPLE

TLC separates non-volatile constituent compounds in a mixture by virtue of differences in their affinity for the stationary solid phase which is spread as a thin layer on a sheet/plate and the selected mobile solvent phase which are then seen as different spots on the TLC plate. Positioning of a spot on the TLC plate denoted by its R_f value (distance travelled by the constituent / distance travelled by the solvent) depends on its chemical nature and is its characteristic property. Therefore, due to similarity in constituent chemical components, test and reference *Terminalia arjuna* dye samples under similar TLC condition should result in spots of similar R_f values and colour and thus a test sample can be identified as whether it is *Terminalia arjuna* bark natural dye.

C-2 APPARATUS

C-2.1 Analytical thickness silica gel G/ GF 254 coated TLC plates (may be cast in-house or procured from commercial suppliers)

C-2.2 Capillary for spotting TLC plates

C-2.3 TLC development chamber (of a dimension suitable to accommodate TLC plate)

C-2.4 TLC spot visualization chamber containing crystals of iodine (of a dimension suitable to accommodate TLC plate)

C-2.5 Optionally, a viewing chamber for TLC with 254 nm UV tubes which may also be fitted with daylight fluorescent tubes (CRI 95) and photographic camera for recording photographs

C-3 REAGENTS

C-3.1 Stock Solutions, of test and reference Terminalia arjuna bark natural dye prepared in **7.1**.

C-3.2 Methanol

C-3.3 Ethyl Acetate

C-3.4 n-Hexane

NOTE — All reagents except **C-3.1** should be of analytical reagent grade and all glassware should be cleaned and finally rinsed with reagent grade water and dried.

C-4 PROCEDURE

Both reference and the test sample extracts are spotted carefully, side-by-side, on the silica gel coated TLC plate. The spots are dried and the plate is placed in TLC development chamber, pre-equilibrated with the developing solvent consisting of 60 percent ethyl acetate and 40 percent n-hexane. After the solvent front has travelled to sufficient distance, the TLC plate is removed and the solvent is allowed to evaporate. Developed spots are visualized in daylight. The spots may optionally be visualized and photographed in the viewing chamber under daylight fluorescent tubes and if a silica gel GF254 plate is used, also under UV-254 nm light. The TLC plate is then placed in the iodine chamber and the spots are visualized in iodine. The constituents of the test sample present as spots on the TLC plate are compared with the reference dye sample. The colour and R_f values of the spots in both the test and reference sample are noted.

C-5 OBSERVATION

Details of stock solution preparation and its dilution if any,

Type of TLC plate used: Silica gel G/ GF 254; in-house/commercial

Solvent system used: 80 percent ethyl acetate and 20 percent n-hexane

R_f and spot colour in reference sample of *Terminalia arjuna* bark dye	R_f and spot colour of test sample	Comment
R_f 0.60 major and 0.20 minor		

C-6 CONCLUSION

Colour of the spots and the *Rf* values of the reference and test samples are similar/different.

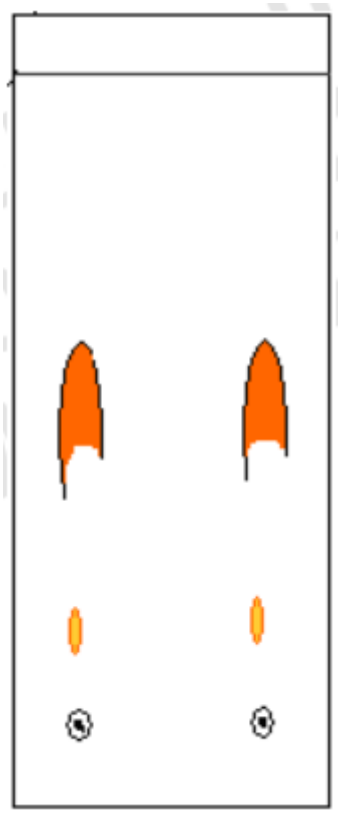

Fig. 1 Thin Layer Chromatogram of *Terminalia Arjuna* Bark Dye

ANNEX D

(*Clauses* 1, 7.2 *and Table* 3)

IDENTIFICATION OF *TERMINALIA ARJUNA* BARK DYE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

D-1 PRINCIPLE

HPLC is used to separate compounds in a mixture by virtue of differences in their affinity for the stationary phase which is packed in a column, and the mobile solvent phase that is pumped through it. The constituents, depending upon their chemical nature, are differently retarded during their passage through the column and are identified by comparing the time taken by a constituent compound in eluting from the column, as detected by a detector (retention time), with that of a standard reference compound. Selection of a detector is important as in the closed system, the eluting compounds cannot be seen by the eye and can only be detected by their characteristic properties. UV detector is a common detector employed as many organic compounds absorb in the UV region. Wavelength of the detector can be set to a value where absorption by the compounds of interest is high so as to achieve higher sensitivity. Various parameters of the HPLC assay are set in such a way that distinct peaks of the reference sample are observed in the chromatogram.

D-2 APPARATUS

D-2.1 HPLC System, with binary pump, UV/PDA detector and software.

D-2.2 C-18 Reverse Phase Column (RPC-C18) — 150 × 4.6 mm; 5 micron.

D-2.3 Guard Column, compatible with the analytical HPLC column.

D-2.4 Micro-Syringe

D-2.5 Membrane Filters (0.45 micron), for micro -syringe.

D-2.6 All Glass Filtration Assembly — for micro filtering solvents.

D- 2.7 Oil-Free Vacuum Pump

D-2.8 Membrane Filters (0.45 micron), for solvent filtration.

D-3 REAGENTS

D-3.1 Methanol

D-3.2 Deionized Water

D-3.3 Reference *Terminalia Arjuna* bark natural dye and test dye stock solutions prepared in **7.1**.

NOTE — All reagents except **D-3.3** should be of HPLC grade and need to be filtered through 0.45 micron membrane filter before use. All glassware used should be cleaned and finally rinsed with reagent grade water and dried**.**

D-4 PROCEDURE

D-4.1 Chromatographic conditions

a)	Stationary phase:	Column C18, 150 × 4.6 mm; 5 micron,
b)	Eluent/ mobile phase:	Methanol: Deionised water (95:5),
c)	Flow rate:	1.0 ml/min,
d)	Detection Wavelength:	254 nm (band width 16 nm), and
e)	Run time:	15 min.

D-4.2 Method

D-4.2.1 HPLC system is started and the selected mobile phase is allowed to pass through the column till a stable baseline is obtained. Reference dye solution, after appropriate dilution, is then aspirated into the micro-syringe, a membrane pre-filter is fitted onto it and the solution is injected into the sample loop (10-20 micro litre capacity) in load position to fill it completely. The injection valve is then turned to inject position to introduce the sample onto the column. As the mobile phase is continuously passing through the column, the constituents of the injected dye extract get separated. These separated constituents exit the column and enter the UV/PDA Detector which detects them by recording the absorbance of the individual constituents as they pass through it. This absorbance is converted to a signal and peaks are seen on the signal intensity versus time plot which is continuously displayed on the monitor. When a UV-absorbing component passes through the detector a peak is seen on the plot. After completion of the run, a chromatogram along with a report of peak retention times, peak height and peak area for each peak is generated by the software. The test sample run is also completed in the similar manner. Identity of the test sample is established by comparing the retention times of the peaks in reference and test chromatograms. Peak height or its area provides additional quantitative information about the relative abundance of various constituents in test and reference samples.

D-4.2.2 An indicative chromatogram containing the peaks obtained after HPLC assay of the *Terminalia arjuna* bark natural dye extract is shown in Fig 2. Peak location/retention times and height may vary between the indicative chromatogram and the injected samples due to differences of the dye sample and the extract preparation conditions.

D-5 OBSERVATION

D-6 CONCLUSION

The retention time of the constituents of the reference and test sample are similar/different. The reference sample and test sample are therefore similar/different.

	In reference sample of n *Terminalia arjuna* bark natural dye	In test sample	Comment
Retention time of the peaks observed in HPLC chromatogram	1.8 min 3.0 min 3.3 min		

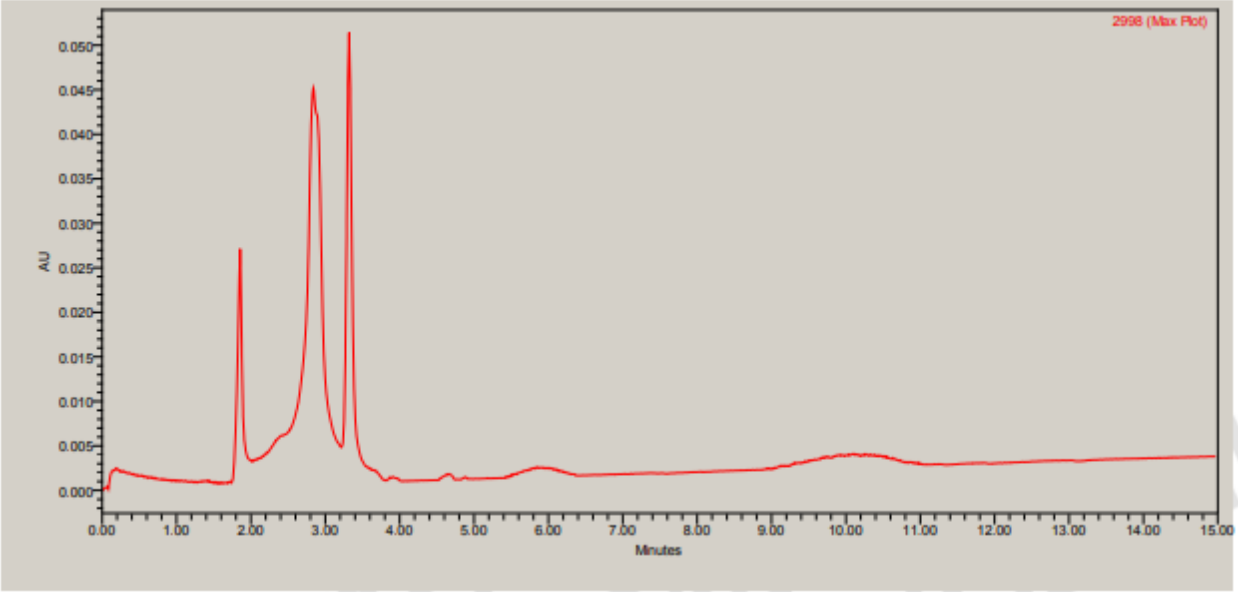

Fig. 2 Chromatogram of The *Terminalia Arjuna* Bark Dye

ANNEX E

(*Clauses* 1, 7.2 *and Table* 3)

IDENTIFICATION OF *TERMINALIA ARJUNA* BARK DYE BY UV–VISIBLE SPECTROSCOPIC METHOD

E-1 PRINCIPLE

When sample molecules are exposed to light having an energy that matches a possible electronic transition within the molecule, some light energy is absorbed as the electron is promoted to a higher energy orbital. An optical spectrometer records the wavelengths at which absorption occurs, together with the quantum of absorption at each wavelength. The resulting spectrum is presented as a graph of absorbance (*A*) versus wavelength.

Many molecules absorb ultraviolet and/or visible light. Fine particles will scatter light and contribute to the absorbance. It is therefore essential to remove fine particles when measuring absorbance of dye solutions. Absorbance (*A*) is directly proportional to the path length, b, and the concentration, c, of the absorbing species. Beer's law states that

$$A = \mathcal{E}bc$$

Where, Ɛ is a constant of proportionality, called the absorptivity.

Different dye compounds within the natural dyestuff absorb radiations of different specific wavelengths. The spectral curve will thus be a summation of their individual absorbances. An absorption spectrum of the natural dyestuff will thus show a characteristic curve with peaks corresponding to structural groups present within the constituent molecules.

E-2 APPARATUS

E-2.1 Ultra Violet Visible Scanning Spectrophotometer

E-2.2 Quartz Sample Cuvettes — Matched pair of 1.00 cm light path.

E-3 REAGENTS

E-3.1 Reference *Terminalia arjuna* Bark Dye Extract

E-3.2 Methanol

NOTE — All reagents should be of analytical reagent grade and all glassware should be cleaned and finally rinsed with reagent grade water and dried.

E- 4 PROCEDURE

E-4.1 The stock solution of the reference *Terminalia arjuna* Bark dye prepared in **7.1** may be used to record the spectrum in the visible region (200 – 700 nm). This solution after diluting five-fold may be suitable for recording the spectrum in the UV region (200 – 400 nm). The test sample solution is also prepared in the same manner as the reference sample.

E-4.2 Both visible and UV spectra are recorded with pure methanol in reference holder of the spectrophotometer. Reference dye sample is first analysed. Test sample is analysed next and recorded spectra are compared for similarity of the spectral curve that is, peak positions and their intensity (absorbance value). Indicative UV and visible spectra of the *Terminalia arjuna* leaf dye extract is presented in Figs. 3 and 4, respectively. The absorbance spectral curve of this dye extract has specific characteristics both in the UV and visible range as shown in these Fig. 3.

E-5 OBSERVATION

E-6 CONCLUSION

The nature of the spectral curve of the test sample in the UV and visible range is similar/different to the reference sample.

	In reference *Terminalia arjuna* Bark extract	**In test sample extract**	**Comments (Describing difference/ similarity in spectral curve)**
Peak position (nm) with absorbance	232 nm — 1.56 A 278 nm — 1.70 A 364 nm — 0.16 A 410 nm — 0.31 A 575 nm— 1.68 A		

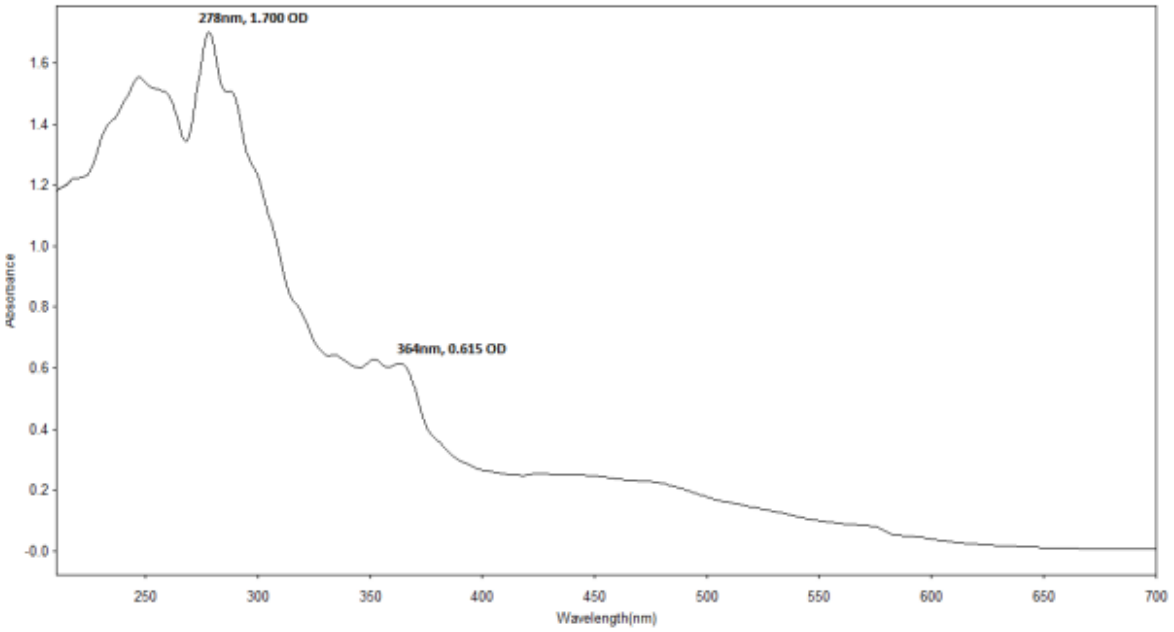

Fig. 3 UV - Visible Spectrum of the *Terminalia Arjuna* Bark Dye

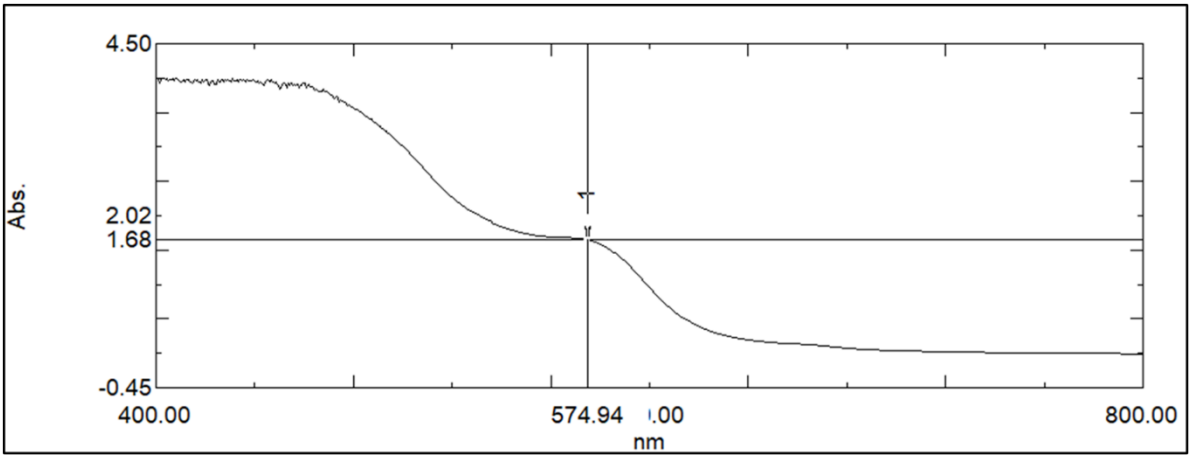

Fig. 4. Visible Spectrum of the Terminalia Arjuna Bark Dye

ANNEX F

(*Clauses* 1 *and* 7.1)

EXTRACTION OF DYE FROM FABRIC/YARN DYED WITH *TERMINALIA ARJUNA* BARK DYE

F-1 PRINCIPLE

Extraction of dyed textile samples with a suitable solvent (methanol) is necessary to release the dyes from these samples.

F-2 APPARATUS

F-2.1 Soxhlet Extraction Assembly with Heating Bench

F-2.2 Scissors

F-2.3 Qualitative Filter Paper Sheet

F-2.4 Rotary Vacuum Evaporator with Evaporating Flask

F-2.5 Funnel

F-2.6 Conical Flasks

F-2.7 Measuring Cylinder

F-3 REAGENTS

F-3.1 Methanol (Analytical Reagent Grade)

F-4 PROCEDURE

About 50 g of dyed textile is cut into small pieces and packed in a filter paper. The packed filter paper column is put for soxhlet extraction with methanol as the solvent. The temperature is so adjusted to get about 8-10 cycles of the solvent per hour. Extraction is continued for 6-8 h, till most of the colour is discharged. The coloured solvent is filtered and is concentrated under vacuum in a rotary evaporator to about 20 ml. This concentrated extract can then be used for chromatographic and spectroscopic analysis as per the methods described in **7.3** after appropriate dilution, if required

Commentator: Shri Gaurav Gupta, Office of Textile Commissioner, Mumbai
Comment:

In IS 4360 : 2020 Method for determination of strength of fast bases :- In Para 7.1 Procedure Notes 1 line 2

For :- After cooling the solution to ***200C***
To be :-After cooling the solution to ***20 ^{o}C***

Please check.
Regards
(Gaurav Gupta)