



**International
Standard**

ISO 22195-7

**Textiles — Determination of index
ingredient from coloured textile —**

**Part 7:
Himalayan rhubarb**

*Textiles — Détermination d'indicateurs d'ingrédients de textiles
colorés —*

Partie 7: Rhubarbe de l'Himalaya

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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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

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

A list of all parts in the ISO 22195 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

There is no doubt that dyeing plays the most important role in expressing the colour of clothes. Until the invention of synthetic dyes capable of expressing diverse colours, materials obtained from nature to dye fabric have been used. Typically, colourants were obtained from plants or various materials were extracted from minerals or insects. When dyeing fabrics using materials derived from these natural substances, it becomes necessary to identify which substances the colourant was derived from. In other words, there has been a demand to confirm whether a fabric is dyed using a natural substance.

A test method is developed to identify which type of natural substances has been used.

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Textiles — Determination of index ingredient from coloured textile —

Part 7: Himalayan rhubarb

1 Scope

This document specifies a test method for the determination of the index ingredient of chemicals in coloured textile with aqueous extracts from the flowers of Himalayan rhubarb.

2 Normative references

The following document is referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696, *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 <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

3.1

Himalayan rhubarb

common name for a genus of flowering plant in the *Polygonaceae* family

Note 1 to entry: *Rheum australe* is synonymous to *Rheum emodi*. It is commonly known as Himalayan rhubarb, *Indian rhubarb* and *Red-veined pie plant*. The plant is usually found in the subalpine and alpine Himalayas at an altitude of 4 000 m. It gives deep golden tones varying from bright yellows to brown colour to the dyed textile. The plant's roots contain chemical compounds such as emodin, aloe-emodin, rhein and chrysophanol.

3.2

coloured

expressing of colours to textiles by dyeing or printing

3.3

natural colourant

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

4 Principle

Natural colourants usually contain several chemical constituents. Depending on the type of natural colourant, each contains a distinctive chemical. This characteristic chemical remains in the fabric dyed

with natural colourant. Therefore, analysis of natural coloured fabrics by chromatography can detect characteristic chemicals depending on the kind of natural colourant.

NOTE If the index component emodin is detected through this test method, it cannot be said that it is necessarily stained with Himalayan rhubarb alone. However, based on this principle, applying this test method to unknown coloured fabrics or textiles is useful to provide a minimum amount of information that can be used to confirm whether the fabric is coloured using Himalayan rhubarb.

5 Reagent

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

- 5.1 **Water**, glass double distilled water or grade 2 water in accordance with ISO 3696.
- 5.2 **Ethanol (CAS No. 64-17-5)**.
- 5.3 **Acetonitrile (CAS No. 75-05-8)**.
- 5.4 **Formic acid (CAS No. 64-18-6)**, volume fraction of 30 %.
- 5.5 **Emodin (CAS No. 518-82-1)**, reference standard with percentage purity indication, e.g. 90 % or more.

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 **Borosilicate glass container with stopper**, 50 ml.
- 6.4 **Membrane filter**, with 0,2 µm pore size.
- 6.5 **Liquid chromatograph (LC) with mass spectroscopy (MS)**.

7 Procedure

7.1 Standard preparation

Stock solution of emodin (5.5) is prepared in ethanol (5.2) containing 1 000 mg/l.

The diluted standards of emodin (5.5) at 1 mg/l, 10 mg/l, 20 mg/l, 50 mg/l and 100 mg/l are prepared for the calibration points.

The powdered Himalayan rhubarb colourant was dissolved in ethanol (5.2).

7.2 Preparation of test specimen

Cut the test specimen into pieces of approximately 5 mm × 5 mm and approximately 1 g. Weigh it to the nearest 0,01 g, and then place it into the glass container (6.3).

Pipette 10 ml of ethanol (5.2) each into the other glass container (6.3) and pour it into cut test specimen containing glass container and place it into an ultrasonic bath (6.2) 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 filter (6.4). The extracted solution may be diluted so that the peak of interest is detectable in the chromatogram. The dilution factor (f) should be noted.

7.3 Analysis

The detection and qualification of emodin is conducted using LC-MS (6.5) with ESI mass spectrometer. The recommended chromatographic conditions are given in Annex A.

7.4 Determination and calculation

7.4.1 Determination of emodin

Comparison between analyses of standard and test specimen through 7.3 may show the result of existence of emodin in test specimen.

Detection of emodin may vary due to conditions of coloured test specimen. In this case, the amount of test specimen and extraction solution can be modified, and concentration of extracted solution can be adopted. The modified test specimen preparation conditions should be described in test result.

7.4.2 Calibration curve

Calibration curves with standards of emodin at 1 mg/l, 10 mg/l, 20 mg/l, 50 mg/l and 100 mg/l are prepared with at least 5 calibration points. The area value of the peak is used for its quantification.

NOTE Concentration ranges for the calibration standards are subject to change depending on the need of each laboratory and equipment used.

For quantification, the calibration curve shall have a correlation coefficient greater than 0,995 (R^2 greater than 0,990).

7.4.3 Calculation of emodin

Calculate the concentration of each emodin in the test specimen, in $\mu\text{g/g}$ from Formula (1):

$$C_s = \frac{C_1}{W} \times f \times V \quad (1)$$

where

C_s is the concentration of each emodin in the test specimen, in $\mu\text{g/g}$;

C_1 is the concentration of each emodin in the test specimen solution, in $\mu\text{g/ml}$;

W is the mass of the test specimen, in g;

f is the dilution factor;

V is the final extraction volume, in ml.

8 Test report

The test report shall include the following information:

- a reference to this document, i.e. ISO 22195-7:2024;
- identification of the test specimen;
- detection result of emodin;

- d) conditions of chromatographic analysis;
- e) any deviation from the specified procedure in this document;
- f) date of the test.

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Annex A (informative)

Example of test result

A.1 Analysis of Himalayan rhubarb colourant

A.1.1 General

The powdered Himalayan rhubarb colourant was dissolved with ethanol tested according to 7.3. The result chromatogram is shown [Figure A.1](#).

A.1.2 Chromatographic conditions for the LC-MS

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

- Mobile Phase: (phase A) water (phase B) acetonitrile, both in 0,1 % formic acid
- Column: Phenomenex Omega 1,6 µm Pola C18 (150 mm × 2,1 mm)
- Column Oven: 35 °C
- Flow rate: 0,3 ml/min
- Injection: 3 µl
- Ionization: ESI-, ESI+
- Data acquisition: scan 200 m/z to 1 500 m/z
- Nebulizer gas flow: 1,5 l/min
- Interface temperature: 250 °C
- Interface voltage: 3 500 V