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

**Part 1:  
Madder**

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

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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 documents 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](http://www.iso.org/directives)).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see [www.iso.org/patents](http://www.iso.org/patents)).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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](http://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](http://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 have been used.

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# Textiles — Determination of index ingredient from coloured textiles —

## Part 1: Madder

### 1 Scope

This document specifies a test method which determines the index ingredient of chemicals in coloured fabric with madder.

### 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 (standards.iteh.ai)

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 <http://www.electropedia.org/>

#### 3.1 madder

common name for *Rubia* which is a genus of flowering plants in the *Rubiaceae* family

Note 1 to entry: *Rubia* is a genus of flowering plants in the *Rubiaceae* family. The genus and its best-known species are commonly known as madder, e.g. *Rubiatiectorum* (common madder), *Rubiaperegrina* (wild madder), and *Rubiaccordifolia* (Indian madder). The plant's roots contain an anthraquinone compound called alizarin that gives red colour to the dyed textile. *Rubiaccordifolia* contains more of anthraquinones munjistin and purpurin.

#### 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 alizarin is detected through this test method, it cannot be said that it is necessarily stained with madder 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 madder.

## 5 Reagent

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

- 5.1 **Water**, glass double distilled water or grade 2 water complying with ISO 3696.
- 5.2 **Methanol**.
- 5.3 **Acetonitrile**.
- 5.4 **Formic acid**, volume fraction of 30 %.
- 5.5 **Alizarin**, reference standard with percentage purity indication e.g. 95 % or more

## 6 Apparatus

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- 6.1 **Analytical balance**, resolution at 0,001 g.
- 6.2 **Ultrasonic water bath**, to be set up at  $(60 \pm 2) ^\circ\text{C}$ .  
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- 6.3 **Borosilicate glass container**, 50 ml.
- 6.4 **Rotary evaporator**.
- 6.5 **Membrane filter**, with 0,2  $\mu\text{m}$  pore size.
- 6.6 **Liquid chromatograph (LC) with mass spectroscopy (MS)**.

## 7 Procedure

### 7.1 Standard preparation

Stock solution of alizarin is prepared in methanol containing 1 000 mg/l.

### 7.2 Preparation of specimen

Cut the sample into pieces of approximately  $(5 \times 5)$  mm and approximately 2 g. Weigh it to the nearest 0,01 g, and then place it into the glass container (6.3).

Pipette 20 ml of water and methanol each into the other glass container and it poured to cut specimen containing glass container. Place the glass container containing the sample into an ultrasonic bath at  $(60 \pm 2) ^\circ\text{C}$  for  $(20 \pm 1)$  min. Afterwards, let the extract evaporate using by rotary evaporator (6.4) at  $(60 \pm 2) ^\circ\text{C}$  until all used water and methanol has evaporated.



Dilute the concentrated extract with water and methanol to a concentration of approximately 100 mg/l. Filter about 1 ml of the diluted solution into a HPLC vial using disposable syringe equipped with a membrane filter (6.5).

### 7.3 Analysis

The detection and qualification of alizarin is conducted using LC-MS (6.6) with ESI mass spectrometer.

The recommended chromatographic conditions are given in Annex A.

### 7.4 Determination of alizarin

Comparison between analyses of standard and sample through 7.3 may show the result of existence of alizarin in sample.

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

## 8 Test report

The test report shall include the following information:

- a) a reference to this document, i.e. ISO 22195-1:2020;
- b) identification of the sample;
- c) detection result of alizarin
- d) conditions of chromatographic analysis;
- e) any deviation from the specified procedure in this document;
- f) date of the test.