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Textiles — Qualitative and quantitative proteomic analysis of some animal hair fibres —

Part 1: Peptide detection using LC-ESI-MS with protein reduction

Textiles — Analyse protéomique qualitative et quantitative de certaines fibres animales —

Partie 1: Détection des peptides par LC-ESI-MS avec réduction protéique

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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).

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).

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

For an explanation on 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 the following URL: 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 20418 series can be found on the ISO website.

Introduction

International producers of textiles in cashmere and other speciality fibres have to be aware of and be able to guarantee the fibre content in order to protect consumers and defend themselves from common frauds. Therefore, it is important to have a harmonized method of analysis at an international level to avoid different interpretations of the results and related conflicts between stakeholders.

The innovations in the method described in this document are

— objective qualitative determination of the presence of fibres derived from animal species and

— quantitative assessment of the relative percentages present in blends.

Textiles — Qualitative and quantitative proteomic analysis of some animal hair fibres —

Part 1:
Peptide detection using LC-ESI-MS with protein reduction

1 Scope

This document specifies a qualitative and quantitative testing method to determine the content of wool, cashmere, yak fibres and their blends in textiles by microscope preliminary screening, protein extraction, enzymatic digestion and specific peptides detection using a liquid chromatography-mass spectrometer equipped with electrospray ionization source (LCI-ESI-MC).

This method can be applied to relevant textile products at each process stage (i.e. from raw material to garment) with a homogeneous distribution of the components. It can be applied to different types of textile materials (e.g. staples, tops, yarns and fabrics) that contain wool, cashmere or yak fibres and their blends. The method is based on a preliminary identification of all fibres in the blend on the basis of their morphology, by light microscopy. The proteins are then extracted by a thiourea/urea/dithiothreitol (DTT) solution. An enzymatic digestion by trypsin of the protein extracted from the fibres is carried out. Analysis of the specific markers is performed by LC-MS and the percent composition is calculated.

This method is applicable to samples containing other kinds of fibres than wool, cashmere and yak, by combining its results with the results obtained using the ISO 1833 series and/or the ISO 17751 series.

This document does not apply if fibres of the same animal species are present (e.g. blends of cashmere and mohair); in this case, the quantitative analysis can be performed using microscopic analysis (e.g. ISO 17751 series).

2 Normative references

The following documents are 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 137:2015, *Wool — Determination of fibre diameter — Projection microscope method*

ISO 1833 (all parts), *Textiles — Quantitative chemical analysis*

ISO 17751 (all parts), *Textiles — Quantitative analysis of cashmere, wool, other specialty animal fibers and their blends*

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 http://www.electropedia.org/

3.1
animal hair fibre
type of keratin fibre for textile use: wool, cashmere and yak

3.2
liquid chromatography – mass spectrometry
LC-ESI-MS
analytical chemistry technique that combines the physical separation capabilities of liquid chromatography with the mass analysis capabilities of mass spectrometry

3.3
fibre test specimen
portion taken from fibre snippets randomly cut from a laboratory sample for measurement purposes

3.4
trypsin digestion
part of the sample preparation for the mass spectrometric identification which consists in enzymatically cutting protein by trypsin into a limited number of shorter fragments

3.5
peptide
short fragment derived from a protein after enzymatic digestion

3.6
marker peptide
portion of a protein used for its identification, recovery and purification

3.7
proteomic analysis
systematic identification and quantification of the complete complement of proteins of a biological system using different techniques of separation and analysis such as mass spectrometry

4 Abbreviated terms

WS cashmere

WO wool

WY yak

5 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and distilled or demineralized water or water of equivalent purity.

5.1 Thiourea, 99 %.

5.2 Urea, 99,5 % (reagent plus).

5.3 DL,-dithiothreitol (DTT) >98 % (TLC), >99 % (titration).

5.4 Tris, (hydroxymethyl) aminomethane (Tris), >99,9 %.

5.5 Ammonium bicarbonate, (NH_4HCO_3) eluent additive for LC-MS.

5.6 Iodoacetamide, (IAM), > 99 % (NMR).

5.7 Trypsin Type II, from porcine pancreas (1 000 to 2 000) units/mg dry solid or with an equivalent level.

5.8 Water, LC-MS for LC-MS.

5.9 Hydrochloric acid, 37 % (HCl) >97 %.

5.10 Acetonitrile, LC-MS for LC-MS.

5.11 Formic acid, (HCOOH) reagent grade >95 %.

6 Apparatus

The usual laboratory apparatus and, in particular, the following.

6.1 Light microscope (LM) in accordance with ISO 17751-1.

6.2 Soxhlet apparatus consisting of a condenser, an extractor, a round boiling flask and a thimble (usually made of thick filter paper) which retains the sample.

6.3 Microtome in accordance with ISO 137.

6.4 Thermostatic bath with controllable heating and a shaking speed range of 20 r/min to 200 r/min.

6.5 Centrifuge with a speed range of 200 r/min to 20 000 r/min.

6.6 Vortex mixer, with speed settings ranging from 100 r/min to 3 200 r/min.

6.7 Sample concentrator under nitrogen flow, with maximum gas pressure of 2 psi, equipped with steel needles.

6.8 Liquid chromatography – mass spectrometer, equipped with electrospray ionization source (LC-ESI-MS).

7 Test method

7.1 Sampling

The general requirement is that the test specimen shall be representative for the lot of material from which it is taken. The method of obtaining a fibre test specimen differs depending upon the sample form. The terms relating to sampling for the various types of samples are given in ISO 1833-1.

7.2 Preliminary identification

The preliminary qualitative analysis of the fibre composition shall be carried out on the basis of the morphology of hair fibres by light microscopy, in accordance with ISO 17751-1.

7.3 Sample preparation

Scour and dehair the samples. Clean them either with petroleum-ether for 2 h in a Soxhlet extractor, or by immersion. Rinse them for 1 h in water at room temperature. Then rinse them for 1 h in water at 50 °C. Dry the fibres in an oven at 50 °C. Condition them for 24 h at a standard atmosphere of 20 °C and 65 % RH.