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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

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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).

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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.

Cut fibre snippets using a microtome device (6.3) in accordance with ISO 137:2015, 6.3. It is recommended that a representative sample have a total mass of approximatively 150 mg of fibre snippets. For fragmentation of fibres, it is possible to use any instrument to guarantee the homogeneous fragmentation of samples (scissors, microtome or mill).

7.4 Protein extraction

Add an extraction buffer [25 mmol/l tris, 2,4 mol/l thiourea (5.1), 5 M urea (5.2), 5 % dithiothreitol (DTT) pH 8,5 (5.3)] in a ratio of 9,5 ml for 150 mg of fibres.

Leave the buffer in contact with the fibres for two days at 50 °C under slow stirring.

Filter the solution on 5 µm pore size filters and centrifuge (6.5) it at 12 000 *g* for 20 min at room temperature.

Collect the supernatant is collected and keep it at 4 °C.

7.5 Trypsin digestion of the extracted proteins

Briefly sonicate the solution in order to dissolve the crystals of thiourea potentially present.

Afterwards, add 67 µl of the solution to an equal volume of 100 mmol/l NH_4HCO_3, and mix using a vortex mixer (6.6). Then, add 5 µl of a 200 mmol/l DTT solution (5.3) in 100 mmol/l NH_4HCO_3 (5.5), followed again by treatment with vortex.

Incubate the solution for 1 h at room temperature, then add 4 µl of iodoacetamide 1 mol/l (5.6) in 100 mmol/l NH_4HCO_3 (5.5), followed again by treatment with vortex.

Incubate again the solution for 1 h at room temperature, then eliminate the excess iodoacetamide by adding 20 µl of a 200 mmol/l DTT solution, followed again by treatment with vortex and incubation for 1 h at room temperature.

Add 818 µl of deionized water and 20 µl of trypsin solution (100 ng/µl in 1 % CH_3COOH, if needed), followed again by treatment with vortex.

Leave the solution at 37 °C for 4 h, then add 20 µl of hydrochloric acid 37 % (5.9) to stop the digestion and reduce the volume under nitrogen flux.

7.6 Tryptic peptides analysis by LC-ESI-MS

Dissolve the dried samples in 100 µl of eluent A [solution of water, 0,2 % acetonitrile (5.10) and 0,1 % formic acid (5.11)], treat them with vortex and transfer them to vials for the LC-ESI-MS analysis.

Some analytical parameters are shown in Annex A as an example.

7.7 Calculation and expression of results

For quantitative analyses, all markers are determined in mixed samples by Single Ion Recording (SIR) detection, specifically monitoring only the characteristic ions. The chromatographic areas associated with the markers are integrated in order to estimate the percentage of mixed samples.

The molecular mass, the retention time and the characteristic ions of each marker are reported in Annex B. Examples from an interlaboratory test carried out to validate the test method are given in Annex C.

The quantitative indices, given in percent, are calculated as follows:

$$w_{\text{yak}} = \frac{A_{\text{yak}}}{A_{\text{yak}} + A_{\text{sheep-goat}}} \times 100$$

$$w_{\text{wool1}} = \frac{A_{\text{sheep1}}}{A_{\text{sheep1}} + A_{\text{cashmere1}}} \times 100$$

$$w_{\text{wool2}} = \frac{A_{\text{sheep2}}}{A_{\text{sheep2}} + A_{\text{cashmere2}}} \times 100$$

$$w_{\text{wool}} = \frac{w_{\text{wool1}} + w_{\text{wool2}}}{2} \times \frac{100 - w_{\text{yak}}}{100}$$

$$w_{\text{cashmere}} = 100 - w_{\text{yak}} - w_{\text{wool}}$$

where

A_{yak}	is the specific marker of yak fibres;
A_{sheep1} and A_{sheep2}	are the specific markers of wool fibres;
$A_{\text{cashmere1}}$ and $A_{\text{cashmere2}}$	are the specific markers of cashmere fibres;
$A_{\text{sheep-goat}}$	is the specific marker of wool and cashmere fibres.

Depending on the equipment used by the laboratory, the yak fibre quantification can be overestimated. If this is the case, a corrective factor shall be determined.

NOTE The corrective factor can be determined based on internal reference materials used as quality control.

If other fibres than wool, cashmere and yak are present, the entire sample fabric composition shall be calculated by combining the results of ISO 20418-1 with the results obtained using the ISO 1833 series and/or the ISO 17751 series.

7.8 Test report

The test report shall include at least the following information:

a) reference to this document, i.e. ISO 20418-1;

b) the nature of the sample (e.g. fibre, yarn, swatch of fabric, ready-made product);

c) the identification of the sample (e.g. lot number, article number);

d) the equipment used;

e) results reported as percentage of wool, cashmere and yak, with the confidence limit in case of blend.

Annex A
(informative)

Parameters

A.1 Chromatographic parameters (Acquity UPLC® separation system, Waters®)

— Column: ACQUITY UPLC®BEH300 C18 1,7µm, (2,1 × 150 mm) (Waters®[1])

— Column temperature: 35 °C

— Eluent A: water+ 0,2 % acetonitrile + 0,1 % formic acid

— Eluent B: acetonitrile + 0,1 % formic acid

— Gradient: from 0 to 7 min, isocratic 100 % A

  — from 7 min to 50 min from 100 % to 50 % A

  — from 50 min to 52,6 min isocratic 50 % A

  — from 52,6 min to 53 min from 50 % A to 0 % A

  — from 53 min to 58,2 min isocratic 0 % A

  — from 58,2 min to 59 min from 0 % A to 100 % A

  — from 59 min to 67 min isocratic 100 % A

— Flux: 0,2 ml/min

— Injection volume: 5 µl

A.2 Acquisition parameters (SQD2, Waters®)

— Detection by ESI-MS positive ion mode

— Capillary voltage: 3,20 kV

— Cone voltage: 31 V

— Extractor voltage: 3 V

— Source temperature: 150 °C

— Desolvation temperature: 500 °C

— Cone gas flow (N_2): 50 l/h

— Desolvation gas flow (N_2): 1 000 l/h

Full scan

Full scan mode acquisition: from 100 *m/z* to 2 000 *m/z*

1) Acquity ULPC separation system, Waters® and SQD2, Waters ® are examples of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

Scan time: 1 s

Interscan delay: 0,1 s

Solvent delay: 7 min

SIR

Ions monitored: see Annex B

Span: 1

Solvent delay: 7 min

It is also possible to quantify by MRM analysis (A.3).

A.3 Acquisition parameters (6495 Triple Quad, Agilent Technologies®[2])

— Detection by AJS-ESI-MS positive ion mode

— Capillary voltage: 3,50 kV

— Nozzle voltage: 1 000 V

— Source temperature: 180 °C

— Gas flow (N_2): 960 l/min, nebulizer 276 kPa

— Sheath gas temperature: 250 °C

— Sheath gas flow (N_2): 720 l/h

— iFunnel parameter: high pressure RF 130 V, low pressure RF 80 V

MRM

Ions monitored: see Annex B.

2) 6495 Triple Quad, Agilent Technologies® is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

Annex B
(informative)

Markers

The peptides were identified by common methods of proteomics discovery. After a liquid chromatography separation and identification with high resolution mass spectrometry, the collected data were identified using a library database and the possible pool of markers were compared with another collected data set. See Table B.1.

Table B.1 — Markers

Marker	Molecular mass[8] MW	Ion Q1 *m/z*	Ion Q3 *m/z*
Yak	2 504	835,5	1 499,7 – 1 215,7
Sheep - Goat	2 234	745,7	534,3
Cashmere 1	2 634	879,0	1 371,4
Sheep 1	2 607	870,1	1 880,5
Cashmere 2	2 691	898,1	1 527,6 – 816,3
Sheep 2	2 664	889,0	1 294,6 – 946,2