SLOVENSKI STANDARD
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Tekstilije - Kvalitativna in kvantitativna proteomska analiza nekaterih živalskih vlaken - 3. del: Odkrivanje peptida z uporabo LC-MS brez zmanjšanja proteina (ISO/DIS 20418-3:2019)

Textiles - Qualitative and quantitative proteomic analysis of some animal hair fibers - Part 3: Peptide detection using LC-MS without protein reduction (ISO/DIS 20418-3:2019)

Textilien - Qualitative und quantitative Proteomanalyse einiger Tierhaarfasern - Teil 3: Peptiddetektion mit LC-ESI-MS ohne Proteinreduktion (ISO/DIS 20418-3:2019)

Textiles - Analyse protéomique qualitative et quantitative de certaines fibres animales - Partie 3: Détection des peptides par LC-MS sans réduction protéique (ISO/DIS 20418-3:2019)

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

Part 3:
Peptide detection using LC-MS without protein reduction

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

Partie 3: Détection peptidique par LC-MS sans réduction de protéines

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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).

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For an explanation on 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.

The committee responsible for this document is ISO/TC 38, *Textiles*.

A list of all parts in the ISO 20418- series can be found on the ISO website.

Introduction

Cashmere is a long slender fibre obtained from cashmere goat and is expensive because of the high quality and the rarity. Mislabelling or adulteration of cashmere products blended with other cheaper animal fibres such as sheep wool and yak has been repeatedly reported worldwide.

The current official methods to identify specific animal fibres are based on microscopic observation. In recent years, however, the microscopy-based identification is becoming more and more difficult as chemical or physical treatment in the manufacturing process, which often complicates microscopic identification of animal fibres, has become widely used.

Therefore, the development of novel identification methods has been desired. Though several methods such as near infrared spectroscopy and terahertz spectroscopy to distinguish the difference in fibre structures and the use of polymerase chain reaction to distinguish the difference in DNA sequences have been studied, each method has some difficulty in its practical application.

The animal fibres consist mainly of proteins called keratins and some associated proteins. Therefore, to analyse proteins contained in textiles is generally regarded as one of the most promising identification methods. The general method to analyse proteins includes the digestion of proteins by trypsin to convert to smaller molecules, i.e., peptides, followed by the detection of resulting peptides in mass spectrometers. Accordingly, identification method using either matrix-assisted laser desorption/ ionization time-of-flight mass spectrometer or liquid chromatography/electrospray ionization mass spectrometer (LC-MS) has been studied. The latter type of instrument is less expensive and more readily available in testing laboratories as a versatile analytical instrument. Moreover, LC-MS has high quantitative capability and is therefore preferable to calculate the blending ratio of animal fibres.

Keratins have many intermolecular and intramolecular disulfide bonds, which make the proteins hardly soluble. Therefore, keratins are generally extracted in the presence of reducing agents. This reducing step, however, needs much time and effort. In this part of the standard, an alternative method in which cysteine-free peptides are selected for identification markers, thereby eliminating the need for reducing step and enabling rapid preparation of LC-MS samples, is presented.

Both ISO 20418-1 and this document use LC-MS, but are different in the extracting method of peptides. In ISO 20418-1, proteins are first extracted from fibres by a thiourea/ urea/ dithiothreitol (DTT) solution, and then digested by trypsin to obtain peptides. In this document, peptides are directly extracted by trypsin digestion of mechanically powdered fibres. The method has been shown to be useful even for highly processed samples and is applicable to various types of animal hairs such as goat (cashmere or mohair), wool and yak.

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

Part 3:
Peptide detection using LC-MS without protein reduction

1 Scope

This document specifies a qualitative and quantitative procedure to determine the composition of animal hair fibre blends by LC-MS without protein reduction.

The composition of non-animal hair fibres can be measured by ISO 1833- series; then both results are combined to determine the whole composition of fibres.

The method is based on a preliminary identification of all fibres in the blend on the basis of their morphology, by light microscopy. In case of fibres of the same animal species are present (e.g. blends of cashmere and mohair), the method is not applicable and the quantitative analysis can be performed using microscopical analysis (e.g. ISO 17751- series).

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 1833-1, *Textiles — Quantitative chemical analysis — Part 1: General principles of testing*

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 17751-1, *Textiles — Quantitative analysis of cashmere, wool, other specialty animal fibers and their blends — Part 1: Light microscopy method*

ISO 17751-2, *Textiles — Quantitative analysis of cashmere, wool, other specialty animal fibers and their blends — Part 2: Scanning electron microscopy method*

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:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at https://www.iso.org/obp

3.1
animal hair fibres
cashmere, sheep wool, yak, and some other animal hair fibres such as camel, alpaca, and angora rabbit

3.2
Bovidae
biological family of cloven-hoofed, ruminant mammals including cashmere goat, sheep and yak

3.3
Camelidae
biological family of even-toed ungulates mammals including camel and alpaca

3.4
proteins
polymers of amino acids that play many critical roles in the body

3.5
peptides
small proteins consisting of approximately less than 50 amino acids

3.6
marker peptides
specific peptides for the identification of animal hair fibres

3.7
LC-MS
high performance liquid chromatography–mass spectrometer equipped with an electrospray ionization ion source

3.8
mass chromatogram
chromatogram for a specific mass-to-charge ratio (m/z)

3.9
total ion chromatogram (TIC)
chromatogram with each data point created by summing up intensities of all mass spectral peaks belonging to the same scan

3.10
selected ion monitoring (SIM)
mass spectrometry scanning mode in which only a limited m/z range is transmitted/detected by the instrument

4 Symbols and abbreviated terms

A peak area

Br Bovidae rate

Cr Camelidae rate

ka correction factor for alpaca and camel

kc correction factor for cashmere

kr correction factor for angora rabbit

ky correction factor for yak

5 Principle

The mechanically powdered fibres are directly subjected to trypsin digestion without prior reduction. The analysis of the digested peptides is performed with LC-MS. The percent composition is calculated from the peak areas of the species-specific marker peptides.

6 Reagents

Following analytical grade reagents should be used.

6.1 Acetone, 99,5 % (GC)

6.2 Water, grade 3 quality specified in ISO 3696

6.3 Ammonium hydrogen carbonate (NH_4HCO_3) solution (25 mmol/l)

— NH_4HCO_3, 96,0 % (T) 197,5 mg

— Make up 100 ml by adding water (6.2)

6.4 Trypsin, sequencing-grade porcine trypsin modified by reductive methylation

6.5 Acetonitrile, 99,9 % (GC)

6.6 Formic acid, 98 % (T)

6.7 Trypsin solution

— Trypsin (6.4) 20 µg

— 0,1 % formic acid (6.6) 200 µl

7 Apparatus

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

7.1 Heating mantle, capable of operating at a temperature range of 50 °C to 150 °C

7.2 Mill, beads mill, cryogenic grinder or an equivalent, capable of crushing materials into an extremely fine powder

7.3 Membrane filter, for aqueous solutions, with a pore size of 0,45 µm

7.4 Heat block, capable of heating micro tubes at 37 °C

7.5 Tube mixer, capable of vortex micro tubes and LC vials for about 30 min

7.6 Centrifugal evaporator

7.7 LC-MS, capable of detecting *m/z* range from 200 to 1 500

7.8 LC vial, shall be manufactured from glass or polymethylpentane

7.9 LC column, octadecyl (C-18)-silica reversed phase column

7.10 Balance, with 1 mg readability or better

7.11 Recovery flask (eggplant flask or round bottom flask)

8 Test method

8.1 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 namely given in ISO 1833-1.

8.2 Preliminary identification

The preliminary qualitative analysis of the fibre composition is carried out on the basis of their morphology by light microscopy, according to ISO 17751- series.

8.3 Wash for degreasing

8.3.1 Reflux 1 g of the fibres in a recovery flask (7.11) on a heating mantle with 200 ml acetone for 30 min.

NOTE 1 Quantity of the fibres can be changed.

NOTE 2 This washing step may be omitted in the case of clean samples.

8.3.2 Take the degreased fibres out of a recovery flask and dry them in air.

NOTE Sample preparation method of ISO 20418-1 can be used alternatively.

8.4 Powderization of fibres

Crush about 1 g of the dried fibre sample using a mill (7.2) to a fine powder with an average length of 100 µm or less and mix thoroughly for securing representative sampling of the fibres.

8.5 Trypsin digestion

8.5.1 Weigh 10 mg of the crushed samples and place the test specimen into a micro tube. If more than 10 mg of the sample is used, increase the volumes of NH_4HCO_3 solution in 8.5.2 and Trypsin solution in 8.5.3 accordingly.

8.5.2 Add 300 µl NH_4HCO_3 solution (6.3) and vortex for 10 min to 30 min.

8.5.3 Add 10 µl Trypsin solution (6.7) to the sample and incubate at 37 °C for 20 h to 24 h.

8.5.4 Centrifuge the tryptic solution at 5 000 G for 3 min. Filter the supernatant through a membrane filter (7.3) to remove residual fibres.

NOTE Centrifugal filter, syringe filter or other means of filtration can be used.

8.5.5 Transfer the quantity of solution corresponding to 2 mg of fibre to a LC vial. Dry the sample in a centrifugal evaporator.

NOTE 1 When LC vial does not fit in a dryer, the solution can be dried in other types of container such as micro tubeand transfered sample to a LC vial after dissolution.

NOTE 2 Alternatively, freeze dryer or nitrogen flux can be used as the drying method, instead of centrifugal evaporator.

