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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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## ISO/DIS 20418-3:2019(E)

### 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)).

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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](http://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.

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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

### 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

**ISO/DIS 20418-3:2019(E)****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<sub>4</sub>HCO<sub>3</sub>) solution (25 mmol/l)**

— NH<sub>4</sub>HCO<sub>3</sub>, 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)**