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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 des peptides par LC-MS sans réduction protéique

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Foreword

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This document was prepared by Technical Committee ISO/TC 38, *Textiles*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 248, *Textiles and textile products*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

A list of all parts in the ISO 20418 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.

Introduction

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

Current official methods to identify specific animal fibres are based on microscopic observations. However, the microscopy-based identification is becoming increasingly difficult due to a wider use of chemical or physical treatments in the manufacturing process. Given these issues, several other methods have also been studied either to distinguish fibre structures by the use of near-infrared spectroscopy or terahertz spectroscopy, or to distinguish DNA sequences by the use of polymerase chain reaction. Nevertheless, each method has shown some complications when applied. Therefore, it is required to develop novel identification methods.

Animal fibres consist mainly of proteins called keratins and some associated proteins. Therefore, the most promising methods to identify fibres are based on the analysis of proteins contained in textiles. Commonly, proteins are analysed by being subjected to digestion by trypsin, resulting in smaller molecules, i.e. peptides, which will be later characterized through mass spectrometry. Accordingly, identification methods using either matrix-assisted laser desorption/ionization time-of-flight mass spectrometer or liquid chromatography/mass spectrometer (LC-MS) have been studied. When comparing these options, the latter type of instrument is less expensive and more readily available in testing laboratories as a versatile analytical instrument than the former. Moreover, LC-MS has a high quantitative capability, and is therefore preferable to calculate the blending ratio of animal fibres.

Keratins are highly insoluble due to the disulphide bonds they tend to form, both at an intramolecular as well as at an intermolecular level. Thus, keratins are generally extracted in the presence of reducing agents. However, this reducing step is considered as time-consuming and arduous. In this document, an alternative method in which cysteine-free peptides are selected for identification markers is used, thereby eliminating the need of the reducing step and enabling rapid preparation of LC-MS samples.

Both ISO 20418-1 and this document describe procedures using LC-MS, but they differ regarding the method utilized to extract the peptides. In ISO 20418-1, proteins are first extracted from fibres with a thiourea/urea/dithiothreitol (DTT) solution, and then digested by trypsin to obtain peptides. In the process described here, 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 (made of wool, cashmere, yak, alpaca, camel or angora) by LC-MS without protein reduction.

NOTE 1 The composition of non-animal hair fibres can be measured by ISO 1833 (all parts). Both results are combined to determine the total fibre composition.

The method is based on a preliminary identification, by light microscopy, of all fibres in the blend on the basis of their morphology, according to ISO/TR 11827[4]. It is not applicable if fibres of the same animal species (such as blends of cashmere and mohair) are present.

NOTE 2 In this case, the quantitative analysis is performed using microscopical analysis [for example, ISO 17751 (all parts)].

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 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 (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, such as wool, cashmere, yak, alpaca, camel or angora

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

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

3.4
protein
polymer of amino acids that play many critical roles in the body

3.5
peptide
small *protein* (3.4) consisting of approximately less than 50 amino acids

3.6
marker peptide
portion of a *protein* (3.4) used for its identification, recovery and purification

3.7
mass chromatogram
chromatogram for a specific mass-to-charge ratio

3.8
total ion chromatogram
TIC
chromatogram with each data point created by summing up intensities of all mass spectral peaks belonging to the same scan

3.9
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
W	blending ratio
Br	Bovidae rate
Cr	Camelidae rate
ka	correction factor for alpaca and camel
kc	correction factor for cashmere
kr	correction factor for angora
ky	correction factor for yak
m/z	mass to charge ratio, where *m* is the mass, expressed in atomic mass unit, and *z* is the charge number of ions

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

The following analytical grade reagents shall be used.

6.1 Acetone, with purity greater than or equal to 99,5 %.

6.2 Water, grade 3 quality specified in ISO 3696.

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

— 197,5 mg of NH_4HCO_3, with purity greater than or equal to 96,0 %.

— Make up 100 ml by adding water (6.2).

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

6.5 Acetonitrile, with purity greater than or equal to 99,8 %.

6.6 Formic acid, with purity greater than or equal to 98 %.

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 microtubes at 37 °C.

7.5 Tube mixer, capable of vortex microtubes and LC vials for about 30 min.

7.6 Centrifugal evaporator, capable to deliver 5 000 *g*.

7.7 LC-MS, liquid chromatography–mass spectrometer, capable of detecting *m/z* (u) range from 200 *m/z* (u) to 1 500 *m/z* (u).

NOTE (u) is for unified atomic mass unit, SI unit.

7.8 LC vial, shall be glass or polymethylpentane.

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

7.10 Balance, with a resolution of at least 0,001 g.

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 to obtain a fibre test specimen differs depending on the sample form. The terms relating to sampling for the various types of samples shall be in accordance with ISO 1833-1.

8.2 Preliminary identification

The preliminary qualitative analysis of the animal hair fibre shall be carried out based on their morphology, which is determined using light microscopy, according to ISO 17751 (all parts), after removal of non-animal fibre.

8.3 Wash for degreasing

8.3.1 Reflux 1 g of the fibres in a recovery flask (7.11) on a heating mantle (7.1) with 200 ml of acetone (6.1) for 30 min. This washing step may be omitted in the case of clean samples. Quantity of the fibres can be changed.

8.3.2 Take the degreased fibres out of the recovery flask and dry them in the air. Alternatively, the sample preparation method of ISO 20418-1[5] can be used.

8.4 Powderization of fibres

Crush the dried fibre sample (8.3.2) using a mill (7.2) to get a fine powder with an average length of 100 µm or less by checking under the microscope and mix thoroughly for securing representative sampling of the fibres.

8.5 Trypsin digestion

8.5.1 Weigh about 10 mg of the crushed sample and place it into a microtube. If more than 10 mg of the sample is used, increase the volumes of the NH_4HCO_3 solution in 8.5.2 and Trypsin solution in 8.5.3 proportionally.

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

8.5.3 Add 10 µl of the 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 for 3 min using the centrifugal evaporator (7.6). 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 solution to an LC vial, then dry it using the centrifugal evaporator (7.6). If the LC vial does not fit in the dryer, the solution can be dried in other types of container such as a microtube. The sample is transferred to an LC vial after dissolution. Alternatively, a freeze dryer or nitrogen flux can be used as the drying method, instead of the centrifugal evaporator.

8.5.6 Add 40 µl of water containing 0,1 % formic acid and 5 % acetonitrile and vortex for 30 min, for subsequent LC-MS measurements. Sonication shall not be a substitute for vortex when LC-MS sample is dissolved.

8.6 Marker peptides

8.6.1 Select the peptides which are used as markers for differential identification of fibres, as specified in Annex A and Annex C. The result of the preliminary identification by microscopy (8.2) and Table 1 can be used as references for this selection.

Table 1 — Correlation of marker peptides and identifiable animal taxa

Fibre	Species		Family		Class	
	Name	Marker[a]	Name	Marker	Name	Marker
Sheep wool	*Ovis aries*	She1, (She2, She3)	Bovidae	Fbv1	Mammalia	Cmm1
Cashmere/ mohair	*Capra hircus*	Cas1, (Cas2)				
Yak	*Bos grunniens*	Yak1, (Yak2)				
Alpaca	*Vicugna pacos*	Alp1, (Alp2)	Camelidae	Fcm1		
Camel	*Camelus ferus*	Cam1, (Cam2)				
Angora rabbit	*Oryctolgus cuniculus*	Ang1, (Ang2)	Leporidae	—		

[a] Marker peptides with suffix 1 are preferable when conducting quantitative analysis.

8.6.2 Optimize LC-MS parameters and confirm retention times of target peaks by using either synthesized peptides (with amino acid sequences shown in Annex A and Annex C) or peptides extracted from pure animal hair fibre samples.

8.7 LC-MS analysis

8.7.1 Inject 5 µl of the sample onto an LC column (7.9). Use water containing 0,1 % formic acid and acetonitrile containing 0,1 % formic acid to form a gradient with increasing concentration of acetonitrile for chromatography. The initial concentration of acetonitrile is 5 %. An example of LC parameters is indicated in Annex B.

8.7.2 Operate mass spectrometer in SIM mode. The selected markers (preferably those with suffix 1), which are described in Annex A and Annex C, shall be monitored. An example of MS parameters is indicated in Annex B.

8.7.3 Integrate the peak area of each marker peptide. The peaks of additional marker peptides (those with suffix 2 and 3), which are also described in Annex A and Annex C, can be used when it is difficult to use the target peak.

8.8 Evaluation of the validity of observed data

See Annex C.

8.9 Calculation of correction factor

8.9.1 Correction factor

Peak areas of marker peptides are not expected to be proportional to the amounts of corresponding animal fibres in the following combinations of marker peptides, for reasons such as the difference in