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

Part 2: Peptide detection using MALDI-TOF MS

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

Partie 2: Détection des peptides par MALDI-TOF MS

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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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For an explanation of 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 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.

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

Animal hair fibres have been used for fabrics or furs. In general, fibres from different animals show distinct colours and morphologies, and in most cases can be distinguished to the species level under microscopic observation. In the textile industries, the identification and quantification of animal hair fibres are very important to guarantee the quality of textile products. Currently, the only practical way to identify animal hair fibres is the microscopic method. However, microscopic identification of animal species from hair fibres can be difficult in certain cases of highly processed fibres or ambiguous samples. Thus, microscopists in testing laboratories require vast experience and a high skill level.

In order to overcome the difficulties of the microscope method, some novel objective methods such as DNA method have been developed to identify animal hair fibres. This method is very sensitive and can be used for qualitative analysis. However, it has been reported that the quantitative analysis of some highly processed samples remains difficult with the DNA method.

It is well known that animal hairs are mostly composed of proteins, and that the amino acid sequences of these proteins are slightly different among different animal species. In the early 2000s, mass spectrometry (MS) was shown to be a very useful method for identifying protein structures. MS of the small peptides obtained by enzyme digestion of proteins can be used to clarify the differences in amino acid sequences among proteins. A particularly efficient qualitative and quantitative method was developed using matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) MS in 2014. 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 2:
Peptide detection using MALDI-TOF MS

1 Scope

This document specifies a qualitative and quantitative procedure to determine the composition of animal hair fibre blends by MALDI-TOF MS.

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

The method is based on a preliminary identification, by light microscopy, of all fibres in a blend on the basis of their morphology, according to ISO/TR 11827. In case 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. as described in 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 3696, *Water for analytical laboratory use — Specification and test methods*

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

EXAMPLE Some animal hair fibres come from camel, alpaca, and angora rabbit.

[SOURCE: ISO 20418-1:2018, 3.1, modified — examples of animal hair fibres have been added.]

3.2
protein
polymers of amino acids that play many critical roles in the body

3.3
peptide
small *proteins* (3.2) consisting of approximately less than 50 amino acids

3.4
buffer solution
solution used to keep pH at a desirable value of the reaction solution

3.5
marker
m/z of animal species specific monoisotopic peak used for identification and quantification

Note 1 to entry: See (Clause 4) for an explanation of *m/z*.

4 Abbreviated terms

SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis, a method used to separate proteins according to their molecular size
MALDI	matrix-assisted laser desorption/ionization, a soft ionization method for mass spectrometry
TOF MS	time of flight mass spectrometry, a type of mass spectrometry based on the time difference of ions to reach the target plate according to molecular mass
m/z	dimensionless quantity formed by dividing the ratio of the mass of an ion to the unified atomic mass unit, by its charge number (regardless of sign)

5 Principle

Proteins in animal hair fibres are extracted using SDS/dithiothreitol (DTT)/phosphate buffer. The extracted proteins are partially purified using SDS-PAGE. The proteins in the gel are enzymatically digested by trypsin. The ratios of animal species-specific peptides are analysed by MALDI-TOF MS. The percent composition of each animal hair fibre is calculated using the calibration curve.

6 Reagents

All reagents shall be of high enough quality suitable for biochemical analysis. Some of the media are available on the market.

6.1 Water, Grade 3 quality as specified in ISO 3696.

6.2 Sodium dihydrogen phosphate dihydrate, 99 % or higher in purity.

6.3 Disodium hydrogen phosphate, 99 % or higher in purity.

6.4 Sodium dihydrogen phosphate solution (0,2 mol/l).

— sodium dihydrogen phosphate dihydrate 31,2 g

Make up to 1 l by dissolving in water.

6.5 Disodium hydrogen phosphate solution (0,2 mol/l).

— disodium hydrogen phosphate 28,4 g

Make up to 1 l by dissolving in water.

6.6 Phosphate buffer (pH 7,8 and 0,2 mol/l).

— 0,2 mol/l sodium dihydrogen phosphate solution (6.4)

— 0,2 mol/l disodium hydrogen phosphate solution (6.5) 100 ml

Add 0,2 mol/l sodium dihydrogen phosphate solution (6.4) to 0,2 mol/l disodium hydrogen phosphate solution (6.5) to adjust the pH to 7,8.

6.7 Sodium dodecyl sulfate (SDS), 99,5 % or higher in purity.

6.8 Dithiothreitol (DTT), 97 % or higher in purity.

6.9 SDS-buffer solution.

— 0,2 mol/l phosphate buffer (pH 7,8) (6.6) 50 ml

— SDS 4,0 g

Make up to 100 ml by adding water.

6.10 Extraction buffer.

— SDS-buffer solution (6.9) 0,25 ml

— DTT 1,9 mg

Dissolve the DTT in SDS-buffer solution just prior to use.

6.11 Iodoacetamide (IAA) solution.

— IAA, 98 % or higher in purity 4,7 mg

Dissolve in 50 µl water just prior to use.

6.12 DTT solution.

— DTT 1,9 mg

Dissolve in 10 µl water just prior to use.

6.13 Polyacrylamide gels of mini size.

6.14 Tris(hydroxymethyl)amino methane (Tris), 99,9 % or higher in purity.

6.15 MOPS (3-Morpholinopropanesulfonic acid), 99,5 % or higher in purity.

6.16 Tris-MOPS buffer.

—	Tris	6,06 g
—	MOPS	10,46 g
—	SDS	1,0 g
—	EDTA	0,3 g
—	Water	900 ml

Make up to 1 l by adding water.

6.17 Tris buffer (0,5 mol/l).

—	Tris	6,05 g
—	Water	80 ml

Adjust the pH of the solution to 6,8 by adding 1 mol/l hydrochloric acid.

Make up to 100 ml by adding water.

6.18 Sample buffer.

—	0,5 mol/l Tris buffer (pH 6,8) (6.17)	4 ml
—	10 % SDS	1 ml
—	Glycerol, 99 % or higher in purity	4 ml

Make up to 10 ml by adding water.

6.19 Coomassie brilliant blue (CBB) solution.[1)]

6.20 Ammonium bicarbonate (NH_4HCO_3), 96 % or higher in purity.

6.21 Ammonium bicarbonate (100 mmol/l).

—	Ammonium bicarbonate (6.20)	7,91 g
—	Pure water	900 ml

Make up to 1 l by adding pure water.

6.22 Acetonitrile, 99,8 % or higher in purity.

6.23 Washing buffer.

—	100 mmol/l ammonium bicarbonate (6.21)	50 ml
—	Acetonitrile	50 ml

1) AE-1340 EzStain Aqua is an example of a suitable product available commercially. This information is given for the convenience of users and does not constitute an endorsement by ISO of this product.

6.24 5-Cyclohexyl-1-Pentyl-β-D-Maltoside (CYMAL-5), a detergent that stabilizes trypsin and decreases the adsorption of tryptic peptides on the surface of the microplate well.

6.25 Sequencing-grade modified trypsin.[2)]

6.26 Digestion buffer.

—	100 mmol/l ammonium bicarbonate (6.21)	50 ml
—	CYMAL-5	10 mg

Make up to 100 ml by adding water.

6.27 Trypsin solution.

—	Sequencing-grade modified trypsin (6.25)	20 µg
—	0,01 mol/l hydrochloric acid	300 µl
—	Digestion buffer (6.26)	4 ml

Cool the digestion buffer on ice and add 100 µl of trypsin dissolved in 0,01 mol/l hydrochloric acid prior to use.

6.28 Trifluoroacetic acid (TFA), 99,8 % or higher in purity.

6.29 α-cyano-4-hyroxycinnamic acid (CHCA), 99,5 % or higher in purity, used as a matrix for peptides in MALDI MS analyses.

6.30 Matrix solution.

—	CHCA	25 mg
—	Acetonitrile/0,1 % TFA (7.3)	5 ml

7 Apparatus

7.1 Ball mill, to grind materials into an extremely fine powder.

7.2 Aluminium block bath, to keep the reaction temperature constant, +30 °C to +100 °C.

7.3 Vortex mixer, to mix small vials of liquid.

7.4 Mini-slab size electrophoresis system.

7.5 Gel cutter[3)] or **razor blade**, for cutting out the part of the gels containing the target proteins.

A 200 µl pipette with pipette tip, and whose tip is cut about 2 cm may be used as gel spot cutter.

2) Sequencing grade modified trypsin is an example of a suitable product available commercially. This information is given for the convenience of users and does not constitute an endorsement by ISO of this product.

3) Gel spot cutter, 1,8 mm in diameter, is an example of a suitable product available commercially. This information is given for the convenience of users and does not constitute an endorsement by ISO of this product.

7.6 Pipette, capable of measuring and taking (0 to 20) µl (±0,20 µl), (20 to 200) µl (±1,60 µl), (200 to 1 000) µl (±8 µl).

7.7 Pipette tip with a bed of chromatography medium fixed at its end, for concentrating and purifying samples.

7.8 MALDI-TOF mass spectrometer, for measuring molecular weight of peptides.

8 Test methods

8.1 Sample preparation

8.1.1 Cut a sufficient amount of the sample to typify the contents of the sample into small pieces with scissors. Put (12,5 ± 2,5) mg of the cut sample into a reaction tube with 0,25 ml SDS buffer solution (6.9) and six small zirconia balls.

8.1.2 Grind the hair fibres with a ball mill (7.1) at a rate of 25 Hz for 30 min.

8.2 Protein extraction

8.2.1 Add 0,25 ml extraction buffer (6.10) with pipette (7.6) to the reaction tube and mix well with a vortex mixer (7.3).

8.2.2 Keep the sample tube in the aluminium block bath (7.2) at 95 °C for 15 min.

8.2.3 Centrifuge the solution at 6 500 g for 1 min at room temperature. Add 10 µl DTT solution (6.12) and keep the reaction tube at 95 °C for another 15 min.

8.2.4 Alkylate the reaction mixture with 50 µl IAA solution (6.11) for 15 min at room temperature to block disulfide bond formation.

8.2.5 Stop the reaction by adding 20 µl DTT solution (6.12).

8.2.6 Centrifuge the solution at 6 500 g for 5 min at room temperature. Transfer the supernatant to another reaction tube. Keep the protein extract for further analysis.

8.3 Partial purification of extracted proteins using SDS-PAGE

8.3.1 Partially purify the protein extract (8.2.6) with SDS-PAGE using mini-slab size electrophoresis system (7.4) and polyacrylamide gels of mini size (6.13) in order to remove the lower and higher molecular-weight components.

8.3.2 Dilute the extract (8.2.6) three times with sample buffer (6.18) and load 5 µl to 10 µl diluted sample to the well of the polyacrylamide gels of mini size (6.13).

8.3.3 After 15 min of electrophoresis, take out the polyacrylamide gel. Dye the proteins on the gel with CBB (6.19).

8.3.4 The protein bands are observed at the top, middle, and bottom part of the gel as shown in Figure A.1. Take out circular gel more than 1 mm in diameter at the middle part by cutting with gel cutter or razor blade (7.5) (see the example in Annex A).

8.3.5 Put the cut gel into 96 well microplate and wash with 100 µl washing buffer (6.23) once for 10 min and twice for one min to remove the CBB. Add 100 µl acetonitrile on the gel and remove it after one min. Dry the gel for (10 ± 5) min.

8.4 Trypsin digestion of the extracted proteins

8.4.1 After drying the gel, add 35 µl of trypsin solution (6.27) on the gel piece and maintain at 50 °C for 1 h.

8.4.2 Purify the tryptic digests with pipette tip with a bed of chromatography medium fixed at its end (7.7) and elute it with 1,5 µl matrix solution (6.30) on the target plate for MALDI-TOF MS.

8.5 MALDI-TOF MS analysis of the tryptic peptides

8.5.1 Analyse the tryptic peptides using the MALDI-TOF mass spectrometer (7.8) in reflection mode. Set the laser power to the threshold level required to generate a signal. The accelerating voltage is set to 20 kV.

8.5.2 Obtain the average of at least 50 scans for each spectrum, and conduct each experiment three separate times at different locations on the sample spot to average the results and ensure reproducibility.

8.5.3 The animal species-specific markers are observed in the range of 2 450 m/z to 2 750 m/z. Each species-specific marker is shown as an example in Annexes B and C. Before quantitative analysis of animal hair fibres, determine which type of animal hair fibres are present in the sample by the markers.

8.6 Calculation of the weight percentages

8.6.1 Using the peak height of goat (h_g), wool (h_w), and yak (h_y), the peak ratios of goat, wool, and yak are calculated using Formulae (1), (2) and (3), respectively:

$$G_p = \frac{h_g \times 100}{h_g + h_w + h_y} \quad (1)$$

$$W_p = \frac{h_w \times 100}{h_g + h_w + h_y} \quad (2)$$

$$Y_p = \frac{h_y \times 100}{h_g + h_w + h_y} \quad (3)$$

where

G_p is the goat peak (%);

W_p is the wool peak (%);

Y_p is the yak peak (%).

The content (%) of goat, wool, and yak is calculated using the calibration curves shown in 8.7.

8.6.2 Similar methods can be applied to samples containing other animal hairs such as angora rabbit and alpaca. Generally, using the peak height of fibre 1 (h_1) and fibre 2 (h_2), the peak ratio of fibre 1 and fibre 2 is calculated using Formulae (4) and (5):

$$F_{1p} = \frac{h_1 \times 100}{h_1 + h_2} \tag{4}$$

$$F_{2p} = \frac{h_2 \times 100}{h_1 + h_2} \tag{5}$$

where

F_{1p} is the fibre 1 peak (%);

F_{2p} is the fibre 2 peak (%).

8.7 Calibration curves

8.7.1 Prepare standard samples by mixing goat (cashmere or mohair) and wool or goat (cashmere or mohair) and yak fibres at the ratios of 1:9, 3:7, 5:5, 7:3 and 9:1. Grind and mix the standard samples completely using a ball mill (7.1).

8.7.2 Solubilize and analyse each standard sample at least twice using the method described in 8.2 to 8.6.

8.7.3 Plot the cashmere peak (%) against content (%) of cashmere in the blends (see the examples in Annex D and Annex E). The calibration curves for wool-yak and other fibres can be prepared using the same method.

8.8 Calculation of the content (%) of each animal hair in binary mixtures

Using the peak (%) (8.6.1 to 8.6.2) and calibration curves (8.7.3), calculate the content (%) of goat, wool, and yak.

8.9 Calculation of the content (%) of each animal hair in ternary mixtures

In case of samples containing three types of fibres such as goat, wool and yak, calculate the content (%) of goat, wool and yak by using the calibration curves for both cashmere-wool and cashmere-yak. In general, calculate R_{12} and R_{13} using each content ratio of fibre 1 and 2, and fibre 1 and 3 obtained by 8.8 by using Formulae (6) and (7):

$$R_{12} = \frac{C_{12}F_2}{C_{12}F_1} \tag{6}$$

$$R_{13} = \frac{C_{13}F_3}{C_{13}F_1} \tag{7}$$

where

$C_{12}F_2$ is the content $(\%)_{12}$ of fibre 2 in binary mixture of fibre 1 and fibre 2;

$C_{12}F_1$ is the content $(\%)_{12}$ of fibre 1 in binary mixture of fibre 1 and fibre 2;

$C_{13}F_3$ is the content $(\%)_{13}$ of fibre 3 in binary mixture of fibre 1 and fibre 3;

$C_{13}F_1$ is the content $(\%)_{13}$ of fibre 1 in binary mixture of fibre 1 and fibre 3.

The content (%) of three components can be calculated using Formulae (8), (9) and (10):

$$CF_1 = \frac{100}{1 + R_{12} + R_{13}} \tag{8}$$

$$CF_2 = \frac{R_{12} \times 100}{1 + R_{12} + R_{13}} \tag{9}$$

$$CF_3 = \frac{R_{13} \times 100}{1 + R_{12} + R_{13}} \tag{10}$$

where

CF_1 is the content (%) of fibre 1;

CF_2 is the content (%) of fibre 2;

CF_3 is the content (%) of fibre 3.

9 Precision

This test method was applied to actual samples. The repeatability and reproducibility were obtained as shown in Annex F. Tests for the samples whose compositions are known were performed between 2013 and 2015. The results are shown in Annex G.

10 Interlaboratory trial

Two laboratories in Japan, one laboratory in Korea and one laboratory in China joined the trial from December 2016 to February 2017. Ten standard samples were used for preparing calibration curves of cashmere-wool and cashmere-yak. Six unknown samples were tested. The results are shown in Annex H.

11 Test report

The test report shall include the following information:

a) a reference to this document, i.e. ISO 20418-2:2018;

b) details of the sample fibres to be tested;

c) details of the testing results, including mass spectra;

d) details of any deviation from the specified procedure.

Annex A
(informative)

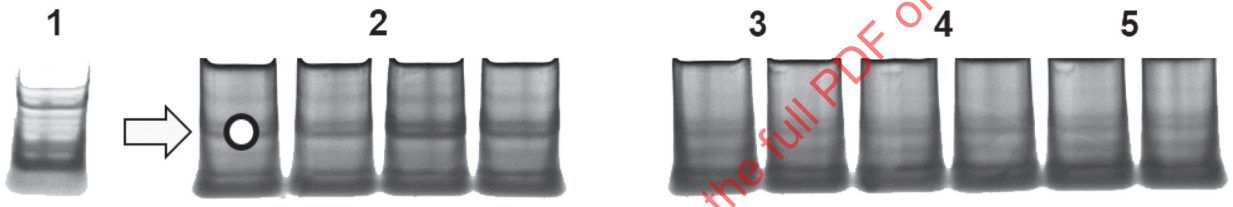
Example of SDS-PAGE image of extracted proteins

A.1 General

Pure animal hair fibres were dissolved in extraction buffer and the extracted proteins were partially purified with SDS-PAGE.

A.2 Cut of the SDS-PAGE gel

The protein band containing keratin type I and type II shown by the arrow in Figure A.1 is cut with gel cutter or razor blade (7.5) and subjected to trypsin treatment.



Key

1 marker
2 samples
3 cashmere
4 wool
5 yak

Figure A.1 — Example of SDS-PAGE image of extracted proteins and the position where the gel is cut

Annex B
(informative)

Animal species-specific peaks in MALDI-TOF MS analysis

B.1 General

Pure animal hair fibres were dissolved in extraction buffer and the extracted proteins were treated with trypsin after partial purification with SDS-PAGE. The peptides in tryptic digests were analysed with MALDI-TOF MS.

B.2 Animal species-specific marker

Around the area between 2 450 *m/z* to 2 750 *m/z*, animal species-specific marker were observed in MALDI-TOF MS analysis. The *m/z* of monoisotopic peak for each animal hair fibre is listed in Table B.1.

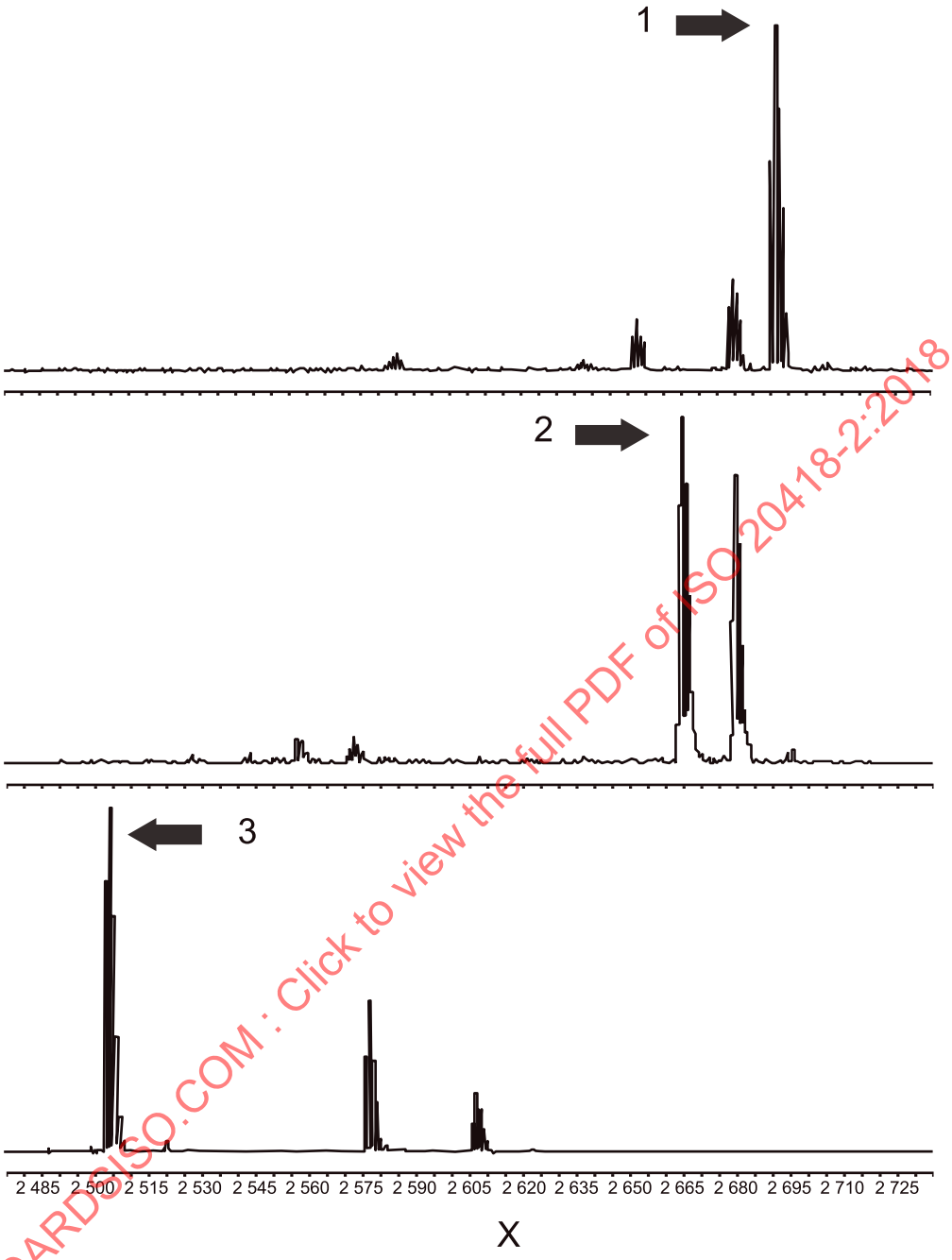
Table B.1 — Animal species-specific marker in MALDI-TOF MS analysis

Marker (*m/z*)	Genus or species	Textile fibre
2 503	*Bos grunniens*	yak
2 556	*Chinchilla*	long-tailed chinchilla
2 563	*Equus caballus*	horse
2 583	*Camellus bactianus* or *Lama pacos*	camel or alpaca
2 638	*Vulpes*	fox
2 656	*Oryctolagus cuniculus*	rabbit
2 664	*Ovis aries*	wool
2 684	*Procyon lotor*	common raccoon
2 691	*Capra hircus*	goat (e.g. cashmere, mohair)
2 698	*Martes*	marten or sable
2 711	*Nyctereutes procyonoides*	raccoon dog

Annex C
(informative)

Animal species-specific peaks in MALDI-TOF MS analysis

Animal species-specific peaks of cashmere, wool, and yak are shown in Figure C.1. No overlaps of the peak positions were observed among cashmere, wool and yak.



Key

X m/z

1 cashmere, monoisotopic peak: m/z 2 691,4; amino acid sequence: YSCQLNQVQSLIVNVESQLAEIR

2 wool, monoisotopic peak: m/z 2 664,4; amino acid sequence: YSCQLNQVQSLIVSVESQLAEIR

3 yak, monoisotopic peak: m/z 2 503,3; amino acid sequence: YSSQLAQVQGLIGNVESQLAEIR

Figure C.1 — Peak positions and amino acid sequences of animal species-specific peptides of cashmere, wool and yak

Annex D
(informative)

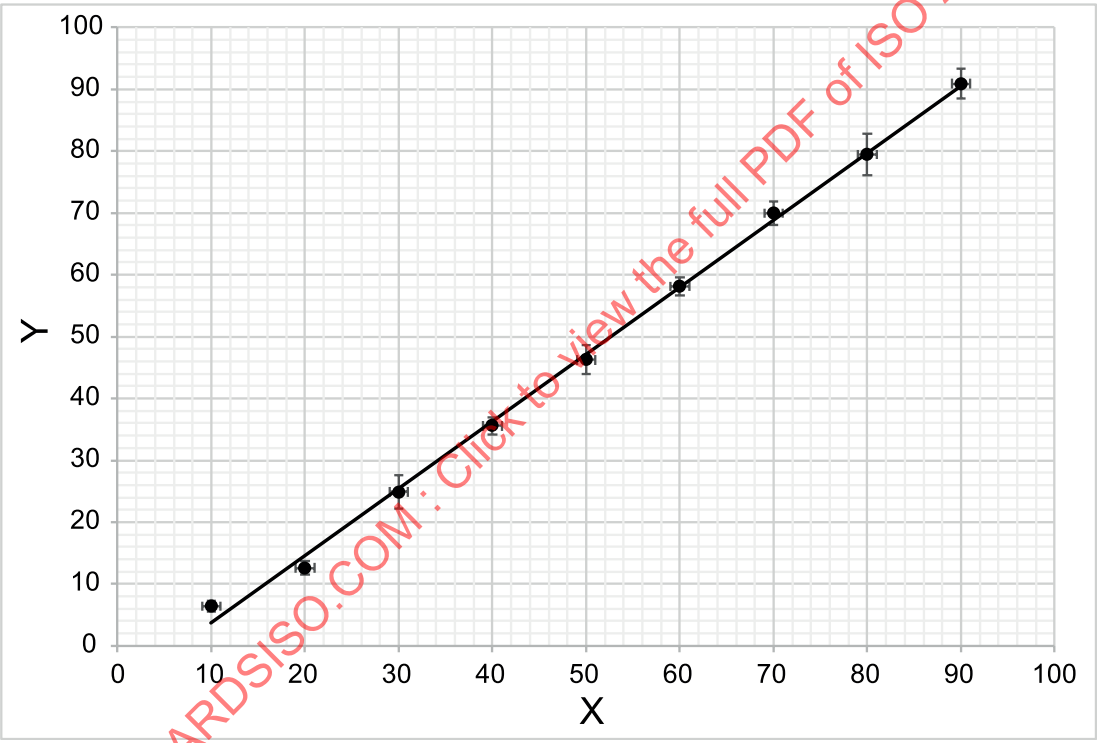
Calibration curve for the cashmere-wool blend

D.1 Samples

The mixtures of cashmere and wool with the mix ratio from 10 % to 90 % were used.

D.2 Preparation of calibration curves

An example of the calibration curve for the mixtures of cashmere and wool is shown in Figure D.1.



Key

X	content (%) of cashmere
Y	cashmere peak (%)
Y = 1,0854X-7,131	approximate curve
R^2 = 0,998	coefficient of determination

Figure D.1 — Calibration curve for the cashmere-wool blend

Annex E
(informative)

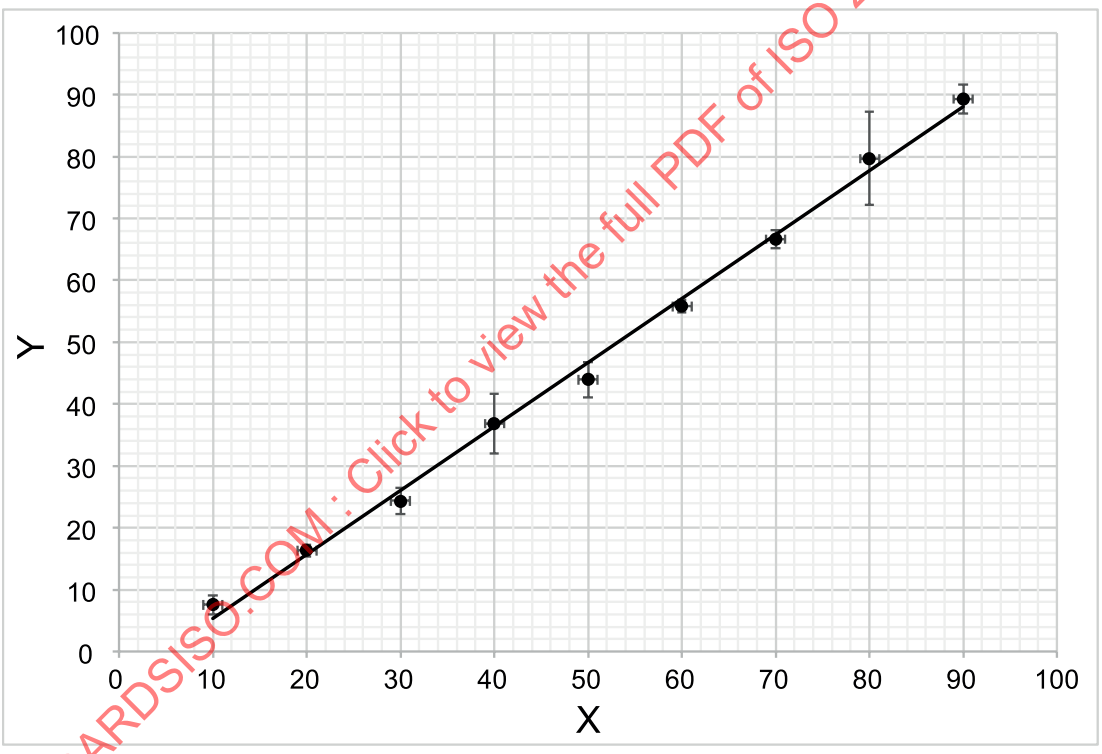
Calibration curve for the cashmere-yak blend

E.1 Samples

The mixtures of cashmere and yak with the mix ratio from 10 % to 90 % were used.

E.2 Preparation of calibration curves

An example of the calibration curve for the mixtures of cashmere and yak is shown in Figure E.1



Key

X	content (%) of cashmere
Y	cashmere peak (%)
Y = 1,0341X-4,9921	approximate curve
R^2 = 0,996 4	coefficient of determination

Figure E.1 — Calibration curve for the cashmere-yak blend

Annex F
(informative)

Repeatability and reproducibility

F.1 Samples

The mixtures of cashmere (Ca) and wool (W) with the ratio of 30 %, 50 % and 70 % were used.

F.2 Repeatability

Measurements for three samples with the different mixtures of cashmere and wool were done 10 times each by one operator on the same day. The results are shown in Table F.1.

F.3 Reproducibility

Measurement of the mixtures of cashmere and wool were repeated in our laboratory from March 2013 to August 2015. The results are shown in Table F.2.

Table F.1 — Repeatability of MALDI-TOF MS method

Actual content	Measured content of goat %											
	1	2	3	4	5	6	7	8	9	10	Average	Standard deviation
Ca 30 % W 70 %	26,8	25,9	26,3	26,2	26,6	27,1	25,4	26,5	25,7	26,3	26,3	0,51
Ca 50 % W 50 %	47,0	46,8	46,6	46,8	47,0	46,5	46,7	45,9	46,9	46,7	46,7	0,33
Ca 70 % W 30 %	69,5	69,4	69,6	70,7	69,9	70,1	70,1	70,7	70,7	70,7	70,1	0,53

Table F.2 — Reproducibility of MALDI-TOF MS method

	Measured content of goat %								
Year	**2013**								
Month	**Mar**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
Ca 30 %, W 70 %	28,4	26,9	28,8	27,1	28,6	30,5	28,1	29,1	29,4
Ca 50 %, W 50 %	47,3	48,8	47,9	48,0	47,3	48,8	45,5	48,6	48,5
Ca 70 %, W 30 %	68,4	72,8	70,8	68,7	69,8	69,1	68,0	65,6	69,3

	Measured content of goat %								
Year	**2014**					**2015**		**Average**	**Standard deviation**
Month	**Jan**	**Mar**	**Apr**	**Sep**	**Nov**	**May**	**Aug**		
Ca 30 %, W 70 %	27,6	29,0	30,6	28,0	28,0	27,2	27,1	28,4	1,13
Ca 50 %, W 50 %	46,8	48,3	48,8	51,8	50,6	45,8	46,8	48,1	1,59
Ca 70 %, W 30 %	70,9	72,2	67,3	71,7	75,8	68,7	69,8	69,9	2,43

Annex G
(informative)

Analysis of blind test samples using MALDI-TOF MS method

The unknown samples for the testing operator were tested by MALDI-TOF MS Method. The results were shown in Table G.1 with the declared composition by the sample supplier.

Table G.1 — Analysis of blind test samples

Sample	Colour	Content of goat %		Content of wool %		Content of yak %	
		Observed	Declared	Observed	Declared	Observed	Declared
A	Gray	9,4[a]	10	90,6	90	—	—
B	Purple	39,6	40	60,4	60	—	—
C	Black	29,0	30	43,5	40	27,5	30
D	Brown	73,8	75	—	—	26,2	25
E	Black	67,3	70	—	—	32,7	30
F	Brown	75,8	80	—	—	24,2	20
G	Dark Blue	46,8	50	28,2	25	25,0	25
H	Orange	100,0	100	—	—	—	—
I	Brown	60,0	60	20,1	20	19,9	20

[a] Corrected using calibration curve.