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

**Part 2:
Peptide detection using MALDI-TOF MS**

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*Textiles — Analyse protéomique qualitative et quantitative de
certaines fibres animales —
Partie 2: Détection des peptides par MALDI-TOF MS*

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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 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.

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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).

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2 Normative references (standards.iteh.ai)

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
<i>m/z</i>	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.

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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 (NH₄HCO₃), 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 μ g
- 0,01 mol/l hydrochloric acid 300 μ l
- Digestion buffer (6.26) 4 ml

Cool the digestion buffer on ice and add 100 μ l 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-hydroxycinnamic acid (CHCA), 99,5 % or higher in purity, used as a matrix for peptides in MALDI MS analyses.

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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 cutter³⁾ or **razor blade**, for cutting out the part of the gels containing the target proteins.

A 200 μ l 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 ($\pm 0,20 \mu\text{l}$), (20 to 200) μl ($\pm 1,60 \mu\text{l}$), (200 to 1 000) μl ($\pm 8 \mu\text{l}$).

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 $(12,5 \pm 2,5)$ 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).