## **EESTI STANDARD**

Textiles - Qualitative and quantitative proteomic analysis of some animal hair fibres - Part 3: Peptide detection using LC-MS without protein reduction (ISO 20418-3:2020) 



#### EESTI STANDARDI EESSÕNA

#### NATIONAL FOREWORD

See Eesti standard EVS-EN ISO 20418-3:2020 sisaldab Euroopa standardi EN ISO 20418-3:2020 ingliskeelset teksti.	This Estonian standard EVS-EN ISO 20418-3:2020 consists of the English text of the European standard EN ISO 20418-3:2020.				
Standard on jõustunud sellekohase teate avaldamisega EVS Teatajas.	This standard has been endorsed with a notification published in the official bulletin of the Estonian Centre for Standardisation.				
Euroopa standardimisorganisatsioonid on teinud Euroopa standardi rahvuslikele liikmetele kättesaadavaks 01.07.2020.	Date of Availability of the European standard is 01.07.2020.				
Standard on kättesaadav Eesti Standardikeskusest.	The standard is available from the Estonian Centre for Standardisation.				
agasisidet standardi sisu kohta on voimalik edasta	da, kasutades EVS-i veebilehel asuvat tagasiside vorm				

Tagasisidet standardi sisu kohta on võimalik edastada, kasutades EVS-i veebilehel asuvat tagasiside vormi või saates e-kirja meiliaadressile standardiosakond@evs.ee.

#### ICS 59.060.01

Standardite reprodutseerimise ja levitamise õigus kuulub Eesti Standardikeskusele

Andmete paljundamine, taastekitamine, kopeerimine, salvestamine elektroonsesse süsteemi või edastamine ükskõik millises vormis või millisel teel ilma Eesti Standardikeskuse kirjaliku loata on keelatud.

Kui Teil on küsimusi standardite autorikaitse kohta, võtke palun ühendust Eesti Standardikeskusega: Koduleht www.evs.ee; telefon 605 5050; e-post info@evs.ee

The right to reproduce and distribute standards belongs to the Estonian Centre for Standardisation

No part of this publication may be reproduced or utilized in any form or by any means, electronic or mechanical, including photocopying, without a written permission from the Estonian Centre for Standardisation.

If you have any questions about copyright, please contact Estonian Centre for Standardisation:

Homepage www.evs.ee; phone +372 605 5050; e-mail info@evs.ee

# **EUROPEAN STANDARD** NORME EUROPÉENNE **EUROPÄISCHE NORM**

# EN ISO 20418-3

July 2020

ICS 59.060.01

**English Version** 

## Textiles - Qualitative and quantitative proteomic analysis of some animal hair fibres - Part 3: Peptide detection using LC-MS without protein reduction (ISO 20418-3:2020)

Textiles - Analyse protéomique qualitative et quantitative de certaines fibres animales - Partie 3: Détection des peptides par LC-MS sans réduction protéique (ISO 20418-3:2020)

Textilien - Oualitative und quantitative Proteomanalyse einiger Tierhaarfasern - Teil 3: Peptiddetektion mit LC-MS ohne Proteinreduktion (ISO 20418-3:2020)

This European Standard was approved by CEN on 7 June 2020.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN-CENELEC Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.



EUROPEAN COMMITTEE FOR STANDARDIZATION COMITÉ EUROPÉEN DE NORMALISATION EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

#### **European foreword**

This document (EN ISO 20418-3:2020) has been prepared by Technical Committee ISO/TC 38 "Textiles" in collaboration with Technical Committee CEN/TC 248 "Textiles and textile products" the secretariat of which is held by BSI.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by January 2021, and conflicting national standards shall be withdrawn at the latest by January 2021.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

According to the CEN-CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

## **Endorsement notice**

The text of ISO 20418-3:2020 has been approved by CEN as EN ISO 20418-3:2020 without any modification.

## Contents

Page

Fore	word				iv
Intro	ductio	<b>1</b>			<b>v</b>
1	Scop	2			1
2	Normative references				1
3	Terms and definitions				
4	Symbols and abbreviated terms				2
5	Principle				2
6	Reagents				3
7	Apparatus				3
8	Test	Test method			
U	8.1	Sampli	ng		4
	8.2	Prelimi	inary identification		
	8.3	8.3 Wash for degreasing			
	8.4	8.4 Powderization of fibres			
	8.5	Trvpsir	n digestion		
	8.6	Marker	peptides		
	8.7	LC-MS	analysis		
	8.8	8.8 Evaluation of the validity of observed data			
	8.9 Calculation of correction factor				
		8.9.1	Correction factor		
		8.9.2	Correction factor for Cas1 against She1 and Y	/ak1 (kc)	
		8.9.3	Correction factor for Yak2 against She2. She3	and Cas2 (kv)	6
		8.9.4	Correction factor for alpaca and camel (ka)		6
		8.9.5	Correction factor for angora (kr)		6
	8.10 Calculation of blending ratio				6
	0.120	8.10.1	Calculation of blending ratio of cashmere, she	eep wool and vak	6
		8.10.2	Calculation by She1, Cas1, Yak1 and kc	cop moor and yan	7
		8.10.3	Calculation by She2, She3, Cas2, Yak2 and ky		7
		8.10.4	Calculation by She1, She2, She3, Cas2, Yak1		7
		8.10.5	Calculation of blending ratio of camel alpaca	and angora	8
9	Test	renort	careanation of pronancy rate of carrier, appear		9
Anne	<b>x A</b> (inf	formative	) Marker pentides of cashmere sheep wool	and vak fibres	10
Anno	$\mathbf{x} \mathbf{R}$ (inf	ormative	) Frample of I C-MS analysis conditions		13
A			) Analysis of some l almost and angene fibre		16
Anne		ormative	) Analysis of camer, arpaca and angola nore		10
Anne	ex D (inf	ormative	e) Example of marker peptide mass chroma	tograms	
Anne	<b>ex E</b> (inf	ormative	) Results of interlaboratory study		
Bibli	ograph	y			
				J.	

## 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 <a href="https://www.iso.org/directives">www.iso.org/directives</a>).

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 <a href="https://www.iso.org/patents">www.iso.org/patents</a>).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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 <a href="https://www.iso.org/iso/foreword.html">www.iso.org/iso/foreword.html</a>.

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 <u>www.iso.org/members.html</u>.

### 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<sup>[4]</sup>. 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 <u>https://www.iso.org/obp</u>
- IEC Electropedia: available at <u>http://www.electropedia.org/</u>

#### 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