
**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

ISO 20418-3:2020

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ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Fax: +41 22 749 09 47
Email: copyright@iso.org
Website: www.iso.org

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Foreword

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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.