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**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB/T 42699.2-2023

**Textiles - Qualitative and quantitative proteomic analysis of
some animal hair fibres - Part 2: Peptide detection using
MALDI-TOF-MS**

纺织品 某些动物毛纤维蛋白质组定性和定量分析 第2部分：还原蛋白质多肽分析
基质辅助激光解吸电离飞行时间质谱（MALDI-TOF-MS）法

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1 Scope

GB/T 42699.2-2023 is Part 2 of the Chinese series on the proteomic analysis of animal hair fibres, and covers peptide detection by MALDI-TOF mass spectrometry. It exists because the price difference between cashmere, yak and fine wool is very large and microscopy cannot reliably separate them once the fibres are dehaired, descaled or blended - the morphology that distinguishes them has been processed away. The proteins have not: each species has keratin peptides with distinct masses, and the mass spectrum identifies and quantifies the species present. The standard sets the principle, the reagents, the instruments and apparatus, the test procedure, the precision and the interlaboratory tests, and the test report, with informative annexes giving example SDS-PAGE gel images, the species-specific mass peaks and the species-specific monoisotopic peaks. It took effect on 1 March 2024.

This document specifies a qualitative and quantitative method for determining the components of animal hair fibre mixtures in textiles using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). Non-animal hair fibre components can be tested according to GB/T 2910 (all parts), and the results can be combined to determine the overall fibre content. This method, following FZ/T 01057.3, uses an optical microscope to observe the morphology of mixed fibres for preliminary identification. This method is not applicable to situations where animal fibres of the same species coexist (e.g., a mixture of cashmere and mohair). Such samples can be analyzed and quantified using microscopic methods (e.g., the analytical methods described in GB/T 40905.1 and GB/T 40905.2).

2 Normative references

The provisions of the following documents constitute the essential clauses of this document through normative references in this text. Among them, for any dated reference, only the version corresponding to that date applies to this document; for any undated reference, the latest version (including all amendments) applies to this document.

GB/T 6682 Water for analytical laboratory use - Specification and test methods (GB/T 6682-2008, ISO 3696.1987, MOD)

3 Terms and definitions

The following terms and definitions apply to this document.

3.1 animal hair fibre Fibres with a multicellular structure and composed of keratin, which grow from animal hair follicles. EXAMPLE. cashmere, sheep wool, yak hair, etc.

3.2 protein An amino acid polymer that plays an important role in organisms.

3.3 peptide A small fragment protein composed of fewer than 50 amino acids through dehydration condensation (3.2).

3.4 buffer solution A solution that maintains the pH of the reaction solution within an appropriate range.

3.5 marker m/z of animal species-specific monoisotopic peaks used for qualitative and quantitative identification. NOTE. See Chapter 4 for an explanation of m/z .

4 Abbreviations

The following abbreviations apply to this document. SDS-PAGE. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis, which is a method for separating proteins based on their relative molecular mass. MALDI. Matrix-assisted laser desorption/ionization, which is a soft ionization method for mass spectrometry. TOFMS. Time-of-flight mass spectrometry, a mass spectrometry analysis method in which the flight times of ions in the

same channel vary due to different relative molecular masses. m/z . The mass-to-charge ratio of a charged particle, a dimensionless quantity.

5 Principle

Proteins in animal hair are extracted using sodium dodecyl sulfate (SDS)/ dithiothreitol (DTT)/phosphate buffer solution. The extracted proteins are purified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by trypsin digestion of the proteins in the gel. The proportions of species-specific proteins are analyzed using MALDI-TOF-MS. The content of each animal hair fibre in the mixture is calculated using a standard curve.

6 Reagents

Unless otherwise specified, only analytical grade reagents are used in the analysis. Some reagents are available commercially.

6.1 Water, Grade III water as specified in GB/T 6682.

6.2 Sodium dihydrogen phosphate, with a purity of $\geq 99\%$.

6.3 Disodium hydrogen phosphate, with a purity of $\geq 99\%$.

6.4 Sodium dihydrogen phosphate solution (

0.2 mol/L). -- Sodium dihydrogen phosphate 24 g Dissolve in water to a volume of 1 L.

6.5 Disodium hydrogen phosphate solution (

0.2 mol/L). -- Disodium hydrogen phosphate

28.4 g Dissolve in water to a volume of 1 L.

6.6 Phosphate buffer solution (pH=7.8,

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) and adjust the pH of the solution to 7.8.

6.7 Sodium dodecyl sulfonate (SDS), with a purity of $\geq 99.5\%$.

6.8 Dithiothreitol (DTT), with a purity of $\geq 97\%$.

6.9 SDS buffer solution. --

0.2 mol/L phosphate buffer solution (pH=7.8) (6.6) 50 mL

7.7 Pipette tip. The tip of the pipette is equipped with chromatography media for concentrating and purifying samples.

7.8 MALDI-TOF mass spectrometer. It is used for the detection of the relative molecular mass of peptides.

7.9 Centrifuge tube.

1.5 mL; it is capable of withstanding speeds of 6500 g and above. 7.10 96-well microplate. A plate with 96 wells made of inert material, suitable for micro-volume applications such as storage, and sample or reagent transfer.

7.11 pH meter. with an accuracy of 0.01.

7.12 Centrifuge. with a speed of 6500 g and above.

8.1 Sample preparation

8.1.1 Cut a representative and sufficient sample into small pieces with scissors and place (12.5±2.5) mg of the specimen into a

1.5 mL centrifuge tube (7.9) containing

0.25 mL of SDS buffer solution (6.9) and 6 grinding balls.

8.1.2 The fibre specimen is ground for 30 minutes in a ball mill (7.1) at a power of

8.2.1 Add

0.25 mL of the extraction solution (6.10) to a centrifuge tube (7.9) using a micropipette (7.6) and mix using a vortex mixer (7.3).

8.2.2 Place the centrifuge tube containing the specimen in a metal bath (7.2) at 95 °C for 15 min.

8.2.3 Centrifuge at 6500 g for 1 min at room temperature. Add 10 µL of DTT solution (6.12) and then react in a metal bath at 95 °C for 15 min.

8.2.4 At room temperature, add 50 µL of IAA solution (6.11) and react for 15 min to alkylate the protein and prevent the formation of disulfide bonds.

8.2.5 Add 20 µL of DTT solution (6.12) to terminate the reaction.

8.2.6 Centrifuge at 6500 g for 5 minutes at room temperature, and transfer the supernatant to another reaction tube to obtain the protein extract for later use.