

Research Progress on Quality Control Methods of Donkey-hide Gelatin Preparations

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Abstract: Donkey-hide Gelatin is a solid gum made by decoction and concentration of the dried skin or fresh skin of an equine donkey, which was first published in the The Holy Husbandman's Classic on Roots and Herbs, and listed as the top product. It has the effect of nourishing blood and nourishing yin, moisturizing and stopping bleeding. Through literature search, it was found that Donkey-hide Gelatin mainly used thermogravimetric analysis, gel electrophoresis, infrared spectroscopy, X-ray fluorescence spectroscopy, circular dichromatography [9], liquid chromatography-mass spectrometry, DNA extraction and amplification in authenticity, chemical composition and content analysis. The foundation of the quality control method of Donkey-hide Gelatin preparation is relatively weak, therefore, by combing the quality control methods of Donkey-hide Gelatin preparation in the past ten years, the advantages and disadvantages of the quality control method are discussed and evaluated, which provides reference value for the establishment of a comprehensive multi-faceted quality control system for Donkey-hide Gelatin preparation.

Keywords: Donkey-hide Gelatin preparations; Quality control; Authenticity identification

1. Donkey-hide Gelatin, Donkey-hide Gelatin preparations, ingredient introduction

1.1 Donkey-hide Gelatin and Donkey-hide Gelatin preparations

Asini Corii Colla is a solid glue made by decocting and concentrating the dried skin or fresh skin of the equine donkey *Equus asinus L.*, which was first published in the The Holy Husbandman's Classic on Roots and Herbs, listed as the top grade. Together with ginseng and deer antler velvet, it is known as the "Three Treasures of Traditional Chinese Medicine". It has been used medicinally for more than 2,000 years. The 2020 edition of the Chinese People's Pharmacopoeia clarifies the sexual taste and return of Donkey-hide Gelatin: Sweet, Flat. Into the lungs, liver, kidney meridians. At the same time, the function and main treatment of Donkey-hide Gelatin are clarified: nourishing blood and yin, moisturizing, stopping bleeding, used for blood deficiency and chlorosis, dizziness, palpitations, muscle weakness, restlessness, weakness, dry lungs and cough, hemoptysis, vomiting blood and urine blood, blood leakage in the stool, pregnancy and fetal leakage. Due to inconvenient storage, inconvenient eating, poor taste and other reasons, Donkey-hide Gelatin preparations have been developed to overcome the inconvenience of traditional Donkey-hide Gelatin. Based on Donkey-hide Gelatin preparations, it solves the problems of the original Donkey-hide Gelatin taste and inconvenience of eating, and with the health care products using Donkey-hide Gelatin as raw materials, it is sought after by the public with its unique characteristics of nourishment and health. At present, the Donkey-hide Gelatin preparations included in the 2020 edition of the Chinese People's Pharmacopoeia include four preparations: Donkey-hide Gelatin three treasures ointment, Donkey-hide Gelatin blood tonic oral liquid, Donkey-hide Gelatin blood tonic ointment, and compound Donkey-hide Gelatin pulp.

1.2 Introduction to the ingredients of Donkey-hide Gelatin

Donkey-hide Gelatin is composed of donkey skin collagen and other substances hydrolysate, of which protein and peptide substances account for the largest proportion of about 63.1%~75.9%. Among them, the protein mainly includes donkey serum albumin, donkey collagen $\alpha 1(I)$ type and donkey collagen $\alpha 2(I)$ type 3 minutes, with the highest serum albumin content. Donkey-hide Gelatin contains at least 17 kinds of amino acids, the total content is between 56.73% ~ 82.03%, the main amino acids are glycine, proline, alanine, glutamic acid and arginine, followed by aspartic acid, lysine, histidine, etc. The results of X-ray fluorescence spectroscopy (XRF) showed that Donkey-hide Gelatin contained S, Cl, Ca, Fe, Na, Si, P, K, Mg, Zn, Al, Mn and other elements, of which S content was the highest, followed by Ca. Polysaccharides are important components in donkey skin, including dermatin sulfate (DS), a natural glycosaminoglycan. The substances with special fishy smell in Donkey-hide Gelatin were analyzed by gas chromatography, and 23 volatile substances including esters, ketones, halogenated hydrocarbons, heterocyclic compounds and other substances were analyzed.

1.3 Current status of quality control of Donkey-hide Gelatin preparations

With the development of the times, people's pursuit of material things is not limited to simple clothing, food, housing and transportation, but also pays more attention to health care and prolonging life. Traditional Chinese medicine with health preservation effects has begun to attract attention. Donkey-hide Gelatin, has always been considered to be the best product to replenish blood and qi, in recent years, highly sought after, resulting in the shortage of donkey skin resources can not meet the demand, and the use of other animal leather boiled into rubber is difficult to distinguish from Donkey-hide Gelatin in appearance and shape, so the market counterfeit and shoddy Donkey-hide Gelatin sales incidents are endless, by the public's attention.

Compared with the existing literature, the identification methods of Donkey-hide Gelatin include: thermogravimetric analysis, gel electrophoresis, infrared spectroscopy, X-ray fluorescence spectroscopy, circular dichromatography, liquid chromatography-mass spectrometry, DNA extraction and amplification, etc. It is mainly analyzed by proteins, peptides, amino acids, trace elements and other components. However, there are relatively few methods for quality control of Donkey-hide Gelatin preparations, and most of the preparations included in the 2020 edition of the Chinese Pharmacopoeia do not involve the quality control standards of Donkey-hide Gelatin, and most of the existing standards only detect a single ingredient. This paper discusses the above issues to provide a horizontal direction for improving the quality control of Donkey-hide Gelatin preparations.

2. Quality control method of Donkey-hide Gelatin preparation

2.1 High performance liquid chromatography

Wang Mingzhi^[1] et al. used RP-HPLC to determine the contents of L-hydroxyproline, glycine, alanine and L-proline in Donkey-hide Gelatin Blood Supplementation Oral Liquid, and the average contents of alanine, glycine, L-hydroxyproline and L-proline in Donkey-hide Gelatin Blood Supplement Oral Liquid were 3.75, 7.83, 3.97 and 4.74mg·mL⁻¹, respectively. RP-HPLC method verifies that the precision, repeatability, stability and recovery rate are good, and can be used as a method for determining the content of glycine, alanine, L-hydroxyproline and L-proline in Donkey-hide Gelatin blood supplement oral liquid, which can better reflect the quality of the preparation than the nitrogen determination method in pharmacopoeia. Yan Dan^[2] et al. use HPLC-ELSD to determine the composition and content analysis of 17 amino acids in Donkey-hide Gelatin, the sample can be directly injected and analyzed without derivatization, eliminating cumbersome pretreatment, avoiding the screening of derivatization conditions when derivatization determination of amino acids, reducing the measurement error, easy to operate, reliable results, and can be used for quality control inspection. Yin Ningning^[3] et al. used HPLC to determine the four amino acids L-hydroxyproline, glycine, alanine and L-proline in compound Donkey-hide Gelatin blood supplementation granules, and established a content determination method with strong exclusivity, which has good column durability and wide range of application, which provides a reliable method for the quality control of compound Donkey-hide Gelatin preparations.

2.2 High performance liquid chromatography-mass spectrometry tandem method

Zhang Yuting et al. took Donkey-hide Gelatin reference substances to check the Donkey-hide Gelatin components of the samples of five manufacturers in turn, and used ultra-high performance liquid chromatography-mass spectrometry to detect the characteristic ions of Donkey-hide Gelatin in Jiao, which was simple to operate and highly exclusive, and was used for the qualitative identification of Donkey-hide Gelatin components^[4]. Li Guanghua et al. identified Donkey-hide Gelatin in Nuangong Pregnancy Pill, and established high performance liquid chromatography-triple quadrupole rod mass spectrometry (UPLC-QQQ MS), using mass spectrometry detector, electrospray positive ion mode (ESI+), multi-reaction monitoring (MRM), and using m/z539.8 (double charge) → 612.4 and 539.8 (double charge) → 923.8 as the detection ion pair, the sample is detected after trypsin enzymatic hydrolysis, the method is highly specific and reproducible, can be used for the identification of Donkey-hide Gelatin in warm uterine pregnancy pills^[5], can effectively control the quality of Donkey-hide Gelatin preparations. Hang Baojian et al. used ultra-high performance liquid chromatography-mass spectrometry to detect the characteristic peptide ion pairs of several heterodermal rubber samples, and determined the source components of horse, deer, cattle, sheep and camel skins, with simple sample preparation, good sensitivity, specificity and precision, which can meet the detection requirements of heterodermal source components in Donkey-hide Gelatin, and provide support for the quality monitoring of Donkey-hide Gelatin preparations^[6]. The Donkey-hide Gelatin in Donkey-hide Gelatin blood supplement granules was enzymatically hydrolyzed by trypsin, and the Donkey-hide Gelatin-specific characteristic peptides were detected by UPLC-QQQ MS, and the characteristic peptides of Donkey-hide Gelatin were detected in three batches of commercially available samples. This method is methodologically verified and has strong specificity, which can be used for the detection of Donkey-hide Gelatin components in Donkey-hide Gelatin blood supplementation granules^[7]. Yang Jiangang et al. used UPLC-QQQ MS to detect the characteristic peptides of Donkey-hide Gelatin and doped pig skin source components in Zhikang granules, which could not only accurately identify the Donkey-hide Gelatin components in Zhikang granules, but also detect the adulteration of pig skin source components,

which provided a quality detection method for Donkey-hide Gelatin preparations. Zhang Guifeng^[8] et al. used trypsin to enzymatically cut Donkey-hide Gelatin, bovine skin gum and pig skin gum, and compared the peptide components of the three collase-producing species by high performance liquid chromatography-mass spectrometry, and found that there were characteristic polypeptides with different amino acid sequences in the enzymatic hydrolysate of Donkey-hide Gelatin, bovine skin gum and pig skin gum, which could identify the sample glue species by detecting the characteristic polypeptide sequences in the sample gum, and laid a technical foundation for the examination of Donkey-hide Gelatin preparations.

2.3 Thin layer chromatography with DNA extraction

Zhang Quanfang^[9] et al. selected four different Donkey-hide Gelatin products, including common Donkey-hide Gelatin oral liquid, compound Donkey-hide Gelatin pulp, Donkey-hide Gelatin paste and ready-to-eat Donkey-hide Gelatin block, extracted DNA by SDS-protease K combined with Chelex-100 method, and purified DNA by glass milk. This method can extract trace DNA from different Donkey-hide Gelatin products, obtain fragments by PCR amplification, and use agarose gel electrophoresis and DNA sequencing to verify that the target fragments are consistent, the method is simple, economical, efficient, and less pollution, and the DNA purity is high, suitable for DNA extraction of various Donkey-hide Gelatin products with low nucleic acid content and high degradation due to deep processing, which provides the basis for the identification of Donkey-hide Gelatin. Zhang Jie^[10] used thin layer chromatography to identify glycine, alanine, valine and leucine in Donkey-hide Gelatin, and showed the same purple-red spots under chromatography, the results of this method were clear, negative and no interference, and could sensitively and easily identify Donkey-hide Gelatin medicinal materials, which provided a method for the qualitative amino acid content of Donkey-hide Gelatin products.

3. Conclusion and outlook

At present, the identification methods of Donkey-hide Gelatin that have been documented include: thermogravimetric analysis, gel electrophoresis, infrared spectroscopy, X-ray fluorescence spectroscopy, circular dichromatography, liquid chromatography-mass spectrometry, DNA extraction and amplification, etc. It is mainly analyzed by proteins and peptides, amino acids, trace elements and other components to achieve the purpose of quantitative and qualitative purposes. There are relatively few quality control methods for Donkey-hide Gelatin preparations, mainly including high performance liquid chromatography, ultra-performance liquid chromatography-mass spectrometry, DNA extraction and thin layer chromatography. High performance liquid chromatography mainly reacts to the quality of Donkey-hide Gelatin components by determining the amino acid content. The former thin layer chromatography and DNA extraction method use the method of color development of four amino acids under chromatography for qualitative analysis, and the latter extracts Donkey-hide Gelatin trace DNA by PCR technology for amplification, and the DNA fragment is compared with the target fragment, which has the characteristics of less pollution and can be used for the identification of Donkey-hide Gelatin. Ultra-performance liquid chromatography-mass spectrometry mainly detects the characteristic peptide ions or characteristic peptides of Donkey-hide Gelatin in Donkey-hide Gelatin preparations, which has the characteristics of high specificity, high sensitivity and high precision, and can be used for content determination and authenticity identification. Therefore, the next stage should conduct a comprehensive and thorough study on the control methods of Donkey-hide Gelatin preparations, and strive to use multi-directional technical means to establish a method of controlling the components of Donkey-hide Gelatin preparations with good exclusivity, high accuracy and strong universality in different types of compound preparations, so as to better control and improve the quality of Donkey-hide Gelatin preparations, ensure clinical efficacy and protect the rights and interests of consumers.

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