Advances in Quality Control Methods for Colla Corii Asini Preparations

Ruoyu Wang
Huzhou College, Huzhou 313000, Zhejiang, China

Abstract: The main raw material of Colla Corii Asini is donkey skin, which has the effects of tonifying blood and nourishing yin, moistening dryness and stopping bleeding, and is widely used. Literature search revealed that the quality control methods of Colla Corii Asini and its preparations in authentication, chemical composition analysis and content determination include high performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), nuclear magnetic resonance (NMR), and NIR spectroscopy analysis. This paper summarizes the quality control methods for Colla Corii Asini and its preparations in recent years, with a view to providing reference for the further development of quality control of Colla Corii Asini and its preparations.

***Keywords*:** colla corii asini, preparation, quality control methods

1. Source, composition and efficacy of Colla Corii Asini

Colla Corii Asini (Asini Corii Colla, ACC) is a solid gum made from the dried or fresh skin of Equus asinus L., a donkey of the family Equidae, by decocting and concentrating. Colla Corii Colla is mostly composed of collagen, which is hydrolyzed to obtain a variety of amino acids, such as lysine, arginine, histidine, cystine, tryptophan, hydroxyproline, aspartic acid, threonine, serine, glutamic acid, proline, glycine, and alanine. It is flat in nature, sweet in taste, belonging to lung, liver and kidney meridians, tonifying blood and nourishing yin, moistening dryness and stopping bleeding. It is used for blood deficiency and atrophy, dizziness and palpitation, muscle impotence, insomnia, deficiency wind, lung dryness and cough, labor cough and hemoptysis, vomiting and urinating blood, blood in stool and leakage, and fetal leakage in pregnancy[1]. Colla Corii Asini has a history of more than 2,000 years in China, and it has been verified that the earliest records of "Colla Corii Asini" in ancient books appeared in the Warring States period, and the term "Colla Corii Asini" was first used in the Qin and Han Dynasties[2]. It has been used in a variety of prescriptions. For example, the 2020 edition of the Pharmacopoeia of the People's Republic of China contains the following prescriptions: Nourishing Heart and Palpitations Cream, Colla Corii Asini Sambucus Cream, Donkey Colla Corii Asini Blood Replenishing Pellet, Angelica Sinensis Blood Replenishing Pill, Jiawei Biochemistry Pellet, and Gynecological Stopping Tape Capsule. Colla Corii Asini has a wide range of efficacy and is well loved by people, so the demand for Colla Corii Asini is increasing, how to maximize the efficacy of Colla Corii Asini in the preparation needs to be further explored, the following are introduced to several Colla Corii Asini and its preparations of quality control methods, in order to provide a reference for the Colla Corii Asini preparations of quality control methods for further research.

2. Colla Corii Asini quality control methods

2.1 High performance liquid chromatography

High performance liquid chromatography (HPLC) is a more commonly used method in the quality control of Colla Corii Asini, mainly used to detect the content and type of amino acids in Colla Corii Asini. Li Wan Si et al used hydrochloric acid hydrolysis, pre-column derivatization and high performance liquid chromatography (HPLC) to determine the content of 17 amino acids in Colla Corii Asini, the experimental results showed that the amino acid derivatization solution in Colla Corii Asini remained stable for 36 h, the correlation coefficient of the 17 amino acids within the linear range of r>0.995, and the recoveries of the spiked samples ranged from 85.8% to 120.2%; the amino acid fingerprints of the 10 batches of Colla Corii Asini had a proprietary amino acid profile and the amino acid fingerprints of the 10 batches of Colla Corii Asini had a proprietary amino acid profile. The amino acid fingerprints of 10 batches of Colla Corii Asini were exclusive, and the similarity was >0.95, and the results of cluster analysis were similar to the similarity results. Fu Yingjie et al[3] took Colla Corii Asini as the basis, used different proteases to enzymatically digest different batches of Colla Corii Asini from different manufacturers, took the enzyme digest as the samples, established the HPLC fingerprints, and carried out similarity, clustering and principal component analysis, and the experiments showed that the method was stable and reliable, and could be used for the quality control of Colla Corii Asini. For the separation and determination of amino acids by high performance liquid chromatography, the composition and ratio of the mobile phase have a greater impact on the separation of amino acids,

and the optimized submicellar liquid chromatography can achieve rapid and accurate separation of mixtures, which is of good value for the analysis of the complex composition of the products such as Colla Corii Asini, but the analysis time is relatively long[4].

2.2 Gas chromatography-mass spectrometry technology

Gas chromatography-mass spectrometry technology is the use of gas chromatography excellent separability and mass spectrometry identification of high selectivity, to achieve the qualitative and quantitative determination of organic substances in complex artifacts system. In Colla Corii Asini it is based on the principle that the amino acid sequences of proteins from different species are different. Pengyun Zhang et al[5] used head space-solid phase micro-extraction (HS-SPME) to extract the volatile components of Colla Corii Asini, and utilized one-way and orthogonal tests to study the effects of sample volume, extraction temperature, extraction time, equilibrium time, and desorption time on the extraction effect. And to determine the more optimal conditions for HS-SPME-GC-MS to analyze the volatile substances of Colla Corii Asini. Sui Liqiang et al. used GC-MS to identify and compare the chemical compositions of each sample before and after preparation, and applied the peak area normalization method to calculate the relative content of each component, and identified 39 compounds from the volatile components of Colla Corii Asini.

2.3 Nuclear magnetic resonance technique

Nuclear magnetic resonance (NMR) is a technique that can directly study the structure of proteins, nucleic acids and other molecules with small relative molecular mass in solution and living cells without damaging the cells. It is widely used in gum identification. Cui Li et al applied low-field nuclear magnetic resonance (LF-NMR) combined with principal component analysis (PCA) and stepwise discriminant analysis (Step-LDA) to the determination of gum doped with yellow gelatin, new gum, and poor-quality gum, and found that the application of low-field nuclear magnetic resonance combined with stoichiometry can realize the distinction between gum doped with yellow gelatin, new gum, and poor-quality gum, and the accuracy of the identification of gum doped with yellow gelatin was better by combining LF-NMR and Step-LDA, and the accuracy of identification was better by combining LF-NMR and Step-LDA. LDA identification accuracy is better. Deng Shuhong et al[6] used nuclear magnetic resonance hydrogen spectroscopy (1H-NMR) to establish the NMR fingerprinting method of Colla Corii Asini based on the components of Colla Corii Asini's aqueous extracts and carried out the methodology validation; NMR hydrogen spectroscopy of samples from different sources was carried out to obtain the sample dataset, and the results showed that the NMR fingerprinting method of Colla Corii Asini had good precision, stability, and reproducibility, which indicated that Colla Corii Asini's NMR hydrogen spectrometry fingerprinting combined with the pattern recognition method can be used for the authentication of the traditional Chinese medicine Colla Corii Asini, which provides a reference for the quality control and evaluation of Colla Corii Asini.

2.4 NIR spectroscopy

NIR spectroscopy is a rapid and efficient modern analytical technique, which comprehensively utilizes the results of computer technology, spectroscopic technology and chemometrics, etc. It has the advantages of portable detection instruments, low detection cost and high sensitivity, and is widely used in various fields such as food and medicine. Dou Linlin[7] studied the rapid analysis method of NIR spectroscopy for amino acids in Colla Corii Asini, established a rapid analysis method for 13 amino acid-like components in Colla Corii Asini, and promoted the application of the established method for the rapid determination of amino acid-like compounds content in the hydrolyzed products of goat horn, on the basis of which, we summarized the general rule of the determination of the amino acid-like components content of hydrolyzed proteins by NIR spectroscopy, and provided a reference for the technology's The general rule of NIR spectroscopic analysis for the determination of amino acid components in hydrolyzed proteins was summarized on the basis of this technique to provide reference for the popularization and application of this technique. Chen Liyun et al found that the use of infrared spectroscopy can distinguish between Dong'a Gum and counterfeit Dong'a Gum, expanding the scope of infrared spectroscopy in the field of identification of traditional Chinese medicine.

2.5 Other methods

Chen Si-xiu et al[8] applied a DNA purification column to replace the more toxic organic solvents such as phenol and chloroform in the sodium dodecyl sulfate-proteinase K (SDS-PK) method to extract the donkey-derived genomic DNA in Colla Corii Asini, and optimized the SDS-PK method, which showed that the purity and concentration of the extracted DNA could satisfy the requirements for the molecular biology of Colla Corii Asini identification, and that the established PCR method could rapidly identify the The results showed that the purity and concentration of the extracted DNA could meet the requirements of molecular biology, and the established PCR method could rapidly identify the donkey-derived components

in Colla Corii Asini.

3. Agaricus preparations quality control methods

3.1 High performance liquid chromatography

High-performance liquid chromatography (HPLC) technique is used to react to the quality of Colla Corii Asini ingredients in compound preparations by determining the content of amino acids. Gao Zhuo Lin et al used HPLC to determine the content of four amino acids in compound Colla Corii Asini effervescent tablets, and their results showed that the method had good specificity and sample precision and stability. Wang Mingzhi et al[9] established a method for the determination of L-hydroxyproline, glycine, alanine and L-proline in Colla Corii Asini Blood Replenishing Oral Liquid by pre-column derivatization-reversed-phase high-performance liquid chromatography (HPLC). The experiments demonstrated that there were no interfering peaks of the derivatization reagents used in pre-column derivatization-reversed-phase HPLC, and the methodology validation was better in terms of precision, reproducibility, stability and recovery. Yin Ningning et al. established a high performance liquid chromatography (HPLC) method for the simultaneous determination of four amino acids in Compound Colla Corii Asini Blood Replenishing Granules, which is simple and reproducible, and provides a reliable method for controlling the quality of Compound Colla Corii Asini Blood Replenishing Granules.

3.2 Liquid chromatography-mass spectrometry (LC-MS/MS) tandem technique

Liquid chromatography-mass spectrometry tandem technique is the most effective means for content determination and authenticity identification of Colla Corii Asini preparations. Chen Yu et al established a high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method for the qualitative and quantitative determination of Colla Corii Asini in Fertility Preserving Spirit preparations, which is accurate, reliable and exclusive. Chen Wei et al[10] used sequence analysis with trypsin to enzymatically digest the gum components in Colla Corii Asini Strong Bone Oral Liquid and counterfeit products doped with different heterogeneous dermal gums, and then selected the molecular peaks of the characteristic molecules of Colla Corii Asini, m/z 539.8→612.4, and the characteristic peptide A of bovine origin, m/z 641.3→726.2, as the detected ion pairs, with ionization patterns of m/z 641.3→726.2, as the detection ion pairs. were used as the detection ion pairs, and the ionization mode was ESI+,for multiple reaction monitoring. Wang Chao et al used ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) for the simultaneous determination of the components of Colla Corii Asini and the components of cowhide source in 6 types and 67 batches of Colla Corii Asini health food products, and this method can discriminate the characteristic peaks of Colla Corii Asini and the characteristic peaks of cowhide source components rapidly and accurately at the same time. The combination of chromatography and mass spectrometry further improved the sensitivity and accuracy than single chromatographic technique, and the specificity was stronger.

4. Conclusion

The quality control methods for Colla Corii Asini and its preparations include a variety of technical means such as high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), nuclear magnetic resonance (NMR), and NIR spectroscopic analysis, which are mainly used for the detection of specific components (e.g., amino acids, peptides, and nucleic acids) in Colla Corii Asini. High performance liquid chromatography (HPLC) is stable and reliable, and can be used for the quality control of Colla Corii Asini, but it can not accurately distinguish the species of Colla Corii Asini skin source; gas chromatography-mass spectrometry (GC-MS) can identify and compare the chemical composition of Colla Corii Asini before and after the concoctions, and it can carry out accurate and quantitative analysis, but the quality and purity requirements for the analyzed samples are relatively high; NIR technology is suitable for distinguishing Colla Corii Asini raw materials with different origins, and it is better for the accuracy of the identification but the sensitivity of the detection is relatively low.

High-performance liquid chromatography (HPLC) is to react to the quality of the components of Colla Corii Asini in compound preparations by determining the content of amino acids, and its specificity, sample precision and stability are better; liquid chromatography-mass spectrometry tandem technology is accurate and reliable, with strong specificity, and can be used for the quality evaluation of Colla Corii Asini preparations, which is an effective means of identifying the authenticity of Colla Corii Asini. At present, there are not many methods that can be applied to the quality control of Colla Corii Asini preparations, which can't carry out comprehensive control of Colla Corii Asini preparations, so the focus of the next research should be put on the use of modern technology to jointly establish a method for the quality control of Colla Corii Asini preparations.

References

[1] National Pharmacopoeia Committee Pharmacopoeia of the People's Republic of China [S], 2020 edition One [M]. Beijing: China Medical Science and Technology Press, 2020.

[2] Li Jinyang, Hu Tingting, Yu Ying, etc Textual research on ass hide glue [J/OL]. Journal of Liaoning University of Traditional Chinese Medicine, 1-21 [2004-05-02] http://kns.cnki.net/kcms/detail/21.1543.r.20240110.1541.005.html.

[3] Fu Yingjie, Li Xinjian, Jia Yumin, etc Establishment of HPLC fingerprint of enzymatic hydrolysate of ass hide glue [J]. traditional Chinese patent medicines and simple preparations, 2021,43 (08): 2120-2125.

[4] Lin Xiaoyi, Li Gen, Wang Fengyu, etc Research progress on quality inspection methods of ass hide glue and its products [J]. Food and Machinery, 2021,37 (7): 207-212.

[5] Zhang Pengyun, Li Rong, Long Chunxia, etc Analysis of volatile components in ass hide glue by HS-SPME-GC-MS combined with automatic deconvolution technology [J]. Food and Machinery, 2019, 35 (03): 52-57+143.

[6] Deng Shuhong, Guo Chuanen, Jiang Hong, Nie Lei Nuclear magnetic resonance fingerprint for identification of ass hide glue [J]. Journal of Shandong University (Science Edition), 2021,56 (7): 103-110.

[7] Dou Linlin Study on the quality control method of Dong'e ass hide glue based on spectral technology [D]. Zhejiang University, 2022.

[8] Chen Sixiu, Zhang Xinfang, Liu Wenyan, Wang Tiantian, Zhao Yundong, Wang Yanshuang, Gao Lijun, Li Mingcheng, Sun Liyuan Improvement of extraction method of animal derived DNA from ass hide glue and identification of donkey derived components [J]. Chinese Journal of Pharmacy, 2019,54 (22): 1840-1845.

[9] Wang Mingzhi, Zhang Huafeng, Zhou Jian, etc Determination of main amino acids in ass hide glue Buxue Oral Liquid by precolumn derivatization RP-HPLC [J]. Journal of Northwest Pharmacy, 2020,35 (1): 42-45.

[10] Chen Wei, Fan Wenxi, Wang Xiaomin, etc UPLC-QqQ/MS method was used to detect the ingredients of ass hide glue Qianggu Oral Liquid ass hide glue and miscellaneous skin sources [J]. Chinese Folk Medicine, 2023,32 (24): 28-32.