

The Value of Liquid Chromatography-Mass Spectrometry in the Detection of Chemical Constituents of Kidney Tonic and Aphrodisiac Chinese Herbal Preparations

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Abstract: *Objective:* To investigate the application of liquid chromatography-mass spectrometry (LC-MS) in the determination of chemical constituents of kidney tonic and aphrodisiac Chinese herbal preparations. *Methods:* In this study, 30 kidney tonic and aphrodisiac Chinese herbal preparations were selected as experimental materials, and chromatography and LC-MS were used to detect their chemical compositions in the control group and the experimental group, respectively. The excellent rate of detection method, identification time, and accuracy of the two methods were compared. *Results:* The excellent rate of the detection method in the experimental group (93.33%) was significantly higher than that in the control group (73.33%), and the difference was statistically significant ($P < 0.05$); compared with the control group, the detection time and retention time error in the experimental group were shorter, and the difference showed highly significant correlation ($P < 0.001$). *Conclusion:* LC-MS is of high value in the detection of chemical constituents of kidney tonic and aphrodisiac Chinese herbal preparations, which can significantly improve the excellent rate of the detection method and the success rate, shorten the detection time, and reduce the retention time error, and is worth to be popularized.

Keywords: Liquid chromatography-mass spectrometry; Kidney tonic and aphrodisiac Chinese herbal preparations; Chemical composition determination

Online publication: May 9, 2024

1. Introduction

Kidney tonic and aphrodisiac herbal preparations are a class of herbal formulas for male sexual function problems, usually treating symptoms such as impotence, premature ejaculation, and weakness of the waist and knees. Common herbs include ginseng, wolfberry, epimedium, and dulcimer herb, etc. These herbs are believed to be able to nourish the kidney and yang, benefit the essence and strengthen the body, and improve male sexual function. However, the manufacturing process of these drugs by certain manufacturers is substandard, and the illegally added ingredients vary greatly, resulting in serious overloading of chemical compositions in

many medicines, which poses serious health hazards ^[1]. Therefore, the use of efficient detection methods can improve the efficiency and accuracy of the detection of the chemical composition of drugs, so as to ensure the safety of medication ^[2]. Due to the relative complexity of the chemical system of traditional Chinese medicines, there is a wide variety of components and complex pharmacological structures, while there is a high degree of similarity among many traditional Chinese medicines ^[3,4]. Conventional chemical analysis methods can be limited by sample complexity and low concentration of components, whereas liquid-mass spectrometry can overcome these difficulties and provide highly sensitive, selective, and attributable chemical analysis results. Liquid chromatography-mass spectrometry (LC-MS) combines chromatographic separation and mass spectrometry detection technologies to achieve accurate identification and quantitative analysis of multiple chemical components in complex samples, with the advantages of high sensitivity, accuracy, and throughput ^[5]. In kidney tonic and aphrodisiac herbal preparations, LC-MS can help identify and quantify various active ingredients, such as phytosterols, alkaloids, flavonoids, polysaccharides, and peptides. Meanwhile, this method can also explore the metabolic pathways and metabolites of Chinese herbal preparations, providing important information for further studies on the pharmacodynamic mechanisms and drug-drug interactions of Chinese herbal medicines.

2. Materials and methods

2.1. Materials

2.1.1. Chinese herbal preparations

Kidney tonic and aphrodisiac Chinese herbal preparations were made from Chinese herbs with the effect of tonifying the kidney and aphrodisiac as the main raw materials, which were processed, extracted, and other processes. The Chinese medicinal preparations used in this experiment included the oral liquid of *Lysimachia nummularia*, oral liquid of antler velvet, oral liquid of Wuzi Yanzong prescription, oral liquid of saffron, Gongxiaoling oral liquid, and ginseng pill.

2.1.2. Experimental equipment

In the process of chemical composition determination of kidney tonic and aphrodisiac herbal preparations, the experimental equipment to be used included, but was not limited to, the following:

- (1) Liquid chromatography-mass spectrometry (LC-MS): Used for separating and detecting the chemical compositions in the medicines.
- (2) Chromatographic column: An important part of the liquid chromatography-mass spectrometry instrument, which is used to separate the chemical components in the sample.
- (3) Weighing instrument: Used for accurate weighing of samples and reagents.
- (4) Centrifuge: Used for centrifugal separation of solid particles and liquids in drugs and samples.
- (5) Oven: Used to dry the samples or reagents.
- (6) pH meter: Used to determine the acid-base properties of drugs or reagents.
- (7) Thermometer: Used to measure the temperature of the laboratory.
- (8) Ultraviolet-visible (UV) spectrophotometer: Used to determine the absorption spectrum, absorbance, etc. of drugs or reagents.
- (9) High-speed centrifuge: Used for rapid centrifugation of drugs or samples.
- (10) Magnetic stirrer: Used to stir the reagents or samples well.

2.2. Research methodology

2.2.1. Detection steps

- (1) Sample pretreatment: The composition and plant source of the herbal preparation were determined, and the relevant chemical composition structure, UV data, and mass spectrometry data were collected. The samples were crushed and screened to obtain uniform sample powder and extracted by adding appropriate amounts of methanol and other solvents according to certain ratios, so that the chemical components in the samples could be dissolved in the solvent.
- (2) Experimental group separation detection method: The extracted sample liquid was injected into a liquid chromatography-mass spectrometer and separated using a chromatographic column. Through mass spectrometry detection, the molecular weight and ion fragment of each component were determined, thus obtaining detailed information about the chemical components in the sample. Further mass spectrometry detection clarified the fragments present in the molecule by cleavage of the fragments and compared them with the molecular weight and the raw source for compound attribution analysis. Additional high-resolution mass spectrometry assays were performed to accurately record the molecular formula. Finally, stereoisomers were identified using reaction mass spectrometry in combination with standards.
- (3) Control group separation detection method: Chromatography was used for the chemical composition detection of the herbal preparations. The compounds in the samples were identified by separation and characterization, including separation using a chromatographic column and identification based on retention time. Meanwhile, data such as infrared spectra, miscibility points, and molecular weight were used for further confirmation and identification. Finally, the results were statistically and analytically analyzed to obtain the content and characteristics of the chemical components in the herbal preparations.
- (4) Data analysis: The mass spectra obtained were processed and analyzed using mass spectrometry data analysis software. Through processing and analysis, a mass spectrometry spectrum library was established for subsequent chemical composition identification and quantitative analysis.

2.2.2. Configuration of sample solution

The sample solution was configured to ensure that the solubility was appropriate; the solution type was methanol, which can make the chemical components in the sample fully dissolved and separated by liquid chromatography-mass spectrometry, it was chosen to configure the dosage unit of 1 g/L. In addition, there may be some impurities in the sample, which will interfere with the separation and detection of the results. To avoid this effect, appropriate pretreatment steps, such as sample screening, filtration, or dilution, were taken when configuring the sample solution to minimize the presence of impurities.

2.2.3. Establishment of a mass spectral library

The establishment of a mass spectral library was for comparing the mass spectra obtained from the assay with an existing database to determine the chemical composition of the sample. By analyzing the mass spectral profile of the pure product, the molecular weight and ionic fragments of each chemical component were determined and the corresponding mass spectra were established. First, a standard column was selected to accurately collect the plots of each standard, and the retention time and mass spectral information of each standard was extracted. Then the corresponding mode was used to build a standard mass spectral library, which was finally adjusted to the positive and negative ion mode to collect the standards separately for library building.

2.2.4. Identification of sample composition

The chemical components in the two groups of samples were separated and detected one by one, and the mass concentration and content of each chemical component in the samples were determined by comparing them with the mass spectra in the mass spectrometry library, so as to identify and determine the composition of the Chinese herbal preparations.

2.3. Observation indicators

2.3.1. Excellent rate of detection method

- (1) Excellent: The accuracy rate of chemical components of traditional Chinese medicine is significantly improved, the operation time is significantly shortened, the precision rate of purification operation is high, and the adaptive range of detection methods is significantly expanded.
- (2) Good: The accuracy of chemical components of traditional Chinese medicine is improved, the operation time is slightly shortened, the precision rate of purification operation is good, and the adaptive range of the detection method is expanded.
- (3) Poor: The accuracy of the chemical composition of each traditional Chinese medicine has decreased, the operation time has been extended, the precision rate of the purification operation has been reduced, and the adaptive range of the detection method has been narrowed.

Excellent rate = $100\% \times (\text{excellent} + \text{good}) / \text{total number of cases}$.

2.3.2. Detection duration and retention time error

The detection time length and retention time error of the two groups were recorded, compared, and analyzed.

2.4. Statistical methods

This study used SPSS20.0 statistical software for data analysis. Measurement data were expressed as mean \pm standard deviation (SD), and *t*-test was used; count data were expressed as number of cases (n) and rate (%), and χ^2 test was used. The difference was considered statistically significant at $P < 0.05$.

3. Results

3.1. Comparison of the excellent rate of detection method between the two groups

As shown in **Table 1**, the excellent rate of detection method in the experimental group (28/30, 93.33%) was significantly higher compared with that in the control group (22/30, 73.33%), and the difference was statistically significant ($P < 0.05$).

Table 1. Comparison of the excellence rate of detection method in the two groups [n (%)]

Groups	Number of cases	Excellent	Good	Poor	Excellent rate
Control group	30	13 (43.33%)	9 (30.00%)	8 (26.67%)	22 (73.33%)
Experimental group	30	18 (60.00%)	10 (33.33%)	2 (6.67%)	28 (93.33%)
χ^2 value	-	-	-	-	4.320
<i>P</i> value	-	-	-	-	0.038

3.2. Comparison of detection time and retention time error of the two groups' detection methods

As shown in Table 2, compared with the control group, the detection time and retention time error of the experimental group were significantly shorter, and the differences showed a highly significant correlation ($P < 0.001$).

Table 2. Comparison of detection time and retention time error between the two groups (mean \pm SD, min)

Groups	Number of cases	Detection time	Retention time error
Control group	30	98.46 \pm 7.37	0.21 \pm 0.01
Experimental group	30	81.37 \pm 6.91	0.10 \pm 0.01
<i>t</i> value	-	9.265	42.603
<i>P</i> value	-	0.000	0.000

4. Discussion

Liquid chromatography-mass spectrometry (LC-MS) uses liquid chromatography as the separation system and mass spectrometry as the detection system. This technique combines the high separation ability of chromatography for complex samples with the high selectivity and sensitivity of mass spectrometry, as well as the ability to provide relative molecular mass and structural information. This study suggests that the LC-MS method demonstrates significant value in the detection of chemical constituents of kidney tonic and aphrodisiac Chinese herbal preparations, which is reflected in the following aspects:

- (1) High detection rate: Compared with the traditional chromatographic method, the LC-MS method has a higher detection rate of chemical components in Chinese herbal preparations.
- (2) High detection success rate: The success rate of the LC-MS method is significantly higher than that of the traditional chromatographic method in the detection of chemical constituents in Chinese herbal preparations for tonic kidney and aphrodisiac.
- (3) Short detection time: The detection time of the LC-MS method is significantly shorter than that of the chromatographic method, which makes the detection process more efficient.
- (4) Low retention time error: In the detection process, the retention time error generated by the LC-MS method is significantly lower than that of the chromatographic method, which further improves the accuracy of the detection.

Compared with the traditional chromatographic method, the LC-MS method usually has a higher rate of excellence in the determination of the chemical constituents of kidney tonic and aphrodisiac herbal preparations. Specifically, this method can provide higher sensitivity and selectivity, and reduce interfering substances in the sample, thus improving the accuracy and reliability of the detection results. Additionally, LC-MS is capable of efficient multi-component analysis, allowing for the simultaneous detection of multiple chemical components, improving analytical efficiency and cost-effectiveness. LC-MS can usually complete the separation and detection of samples in a shorter time, which is faster than traditional chromatographic methods. This is due to the fact that the liquid chromatography part allows for good separation of molecules using different columns and optimized methods as needed. The mass spectrometry (MS) part enables fast acquisition of mass spectrometry data for rapid identification and quantitative analysis. Therefore, LC-MS can save the experiment time and improve the analysis efficiency. In addition, the retention time error of the LC-MS method, i.e., the error in the retention time of separated peaks, is smaller. This helps to accurately determine the peak

shapes and peak heights of chemical components and improves the accuracy and reproducibility of the test results. Therefore, the LC-MS method is of high value in the determination of chemical constituents of kidney tonic and aphrodisiac Chinese herbal preparations, which can improve the accuracy, reliability, and analytical efficiency of the assay.

In addition to the application of LC-MS in the detection of the chemical composition of Chinese medicinal preparations, it is also widely used in other fields. For example, LC-MS technology can be used in vaccine research to analyze the composition, purity, impurities, and residues in vaccines, providing a powerful analytical tool for vaccine regulatory agencies to ensure the safety and reliability of vaccine products ^[6]. In food testing, the LC-MS method can be utilized to detect veterinary drug residues in food of animal origin to ensure food safety and compliance with regulatory requirements ^[7]. In clinical applications, tumor markers can be accurately measured by LC-MS, which can be used to study cancer biomarkers, monitor disease progression, assess treatment effects, and develop individualized medical treatment plans ^[8].

5. Conclusion

In summary, LC-MS has high value in the detection of the chemical composition of kidney tonic and aphrodisiac herbal preparations, which can significantly improve the excellent rate of detection method and detection success rate, shorten the detection time, and reduce the retention time error.

Disclosure statement

The author declares no conflict of interest.

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