The Simultaneous Determination of Six Components in Zadi-5 Powder by One Test and Multiple Evaluation Methods

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Abstract. Establish a one-test multi-evaluation method for simultaneous determination of piperine, Costunolide, Dehydrocostus Lactone, dehydrodiisoeugenol, Alantolactone and Isoalantolactone. Method: The drug was extracted with 70% ethanol, using Diamonsil C18 column (4.6mm × 250mm, 5μm), using acetonitrile-0.2% phosphoric acid as mobile phase, gradient elution, flow rate 1.0mL / min; column temperature 30 °C, detection Wavelength 220nm, 343nm. Using Alantolactone as the internal standard, the relative correction factors of the other five components were calculated to determine their content. Results: The six components have a good linear relationship (r> 0.9990) in their respective ranges, and the method precision and accuracy are good. The result of one test and multiple evaluation method for the content of each component in the three batches of samples is close to the standard curve method (RE≤2.23%). Conclusion: This method is accurate, reliable, simple and fast, and can be used for the quality control of Zadi-5 powder.

Keywords: One Test and Many Comments, Zadi-5 Disseminate, HPLC, Active Composition, Including Content

Zadi-5 is a traditional Mongolian medicine compound preparation. Its main ingredients include Nutmeg, Inulae radix, Radix aucklandiae ,Choerospondiatis fructus, Long piper etc. It has the clinical effect of treating upset and insomnia and uneasiness. It is particularly effective and is commonly used in Mongolian medicine [1]. Over the years, the majority of pharmaceutical analysts have conducted a lot of research work to improve the quality standards of the drug [2]-[4], but the content determination is mostly a single indicator, which cannot reflect the complexity and diversity of traditional Chinese medicine ingredients. At the same time, the quality control of the content of multiple ingredients can be more in line with the characteristics of traditional Chinese medicine. In 2016, Academician Liu Changxiao [5] proposed the concept of "quality marker". Researchers have increasingly realized that the quantification of only 1-2 active ingredients does not fully reflect the quality of traditional Chinese medicine and the effectiveness of clinical medicine. Sex and safety, quality control of traditional Chinese medicine tends to multi-index quality control, Wang Zhimin [6] et al. proposed a multi-index quality control mode with multiple tests and evaluations. In the case of insufficient reference products, only one cheap and easy to use The obtained reference substance
achieves the simultaneous quantitative determination of multiple components. This method has been further validated in many Chinese medicinal materials, Chinese herbal compound preparations and Mongolian medicine preparations [7-9]. In this experiment, taking the Alantolactone as a reference, the relative correction factors between piperine, Costunolide, Dehydrocostus Lactone, dehydrocosanolide, dehydrodiisoeugenol, and Isoantholactone were established. The relative correction factor is used to calculate the content of each ingredient, and the standard curve method is used to verify whether the results of the one-test multi-evaluation method are accurate and reliable, which provides a reference for the quality control of the preparation.

1. Material

1.1 Instruments

Agilent1200 high-performance liquid chromatograph (Agilent Corporation, USA); FA2104 electronic analytical balance (Shanghai Liangping Instrument Co., Ltd.); KQ-250E ultrasonic cleaner (Kunshan Ultrasonic Instrument Co., Ltd.).

1.2 Test

Drug Zadi-5 powder (batch numbers 20190611, 20190612, 20190613) was purchased from Aerkorqinqi Mongolian Medical Hospital, Chifeng City, Inner Mongolia. Piperine (181020), Costunolide (190321), Dehydrogenous lactone (1809026), dehydrodiisoeugenol (190520), Alantolactone (190517), Isoantholactone (190315) The reference product was purchased from Beijing Beina Chuanglian Biotechnology Research Institute. Acetonitrile is chromatographically pure, water is ultrapure water, and other reagents are analytically pure.

2. Methods and Results

2.1 Chromatographic Conditions

Diamonsil C18 column (4.6mm × 250mm, 5μm); mobile phase is acetonitrile (A) -0.2% phosphoric acid (B), gradient elution (0-7min, 65% B; 7-7.5min, 65% → 51% B; 7.5 ~ 23min, 51% B; 23 ~ 24min, 65% → 51% B; 24 ~ 35min, 65% B); flow rate: 1.0mL / min; segmented variable wavelength measurement, 0 ~ 10min is 343nm (piperine), 220nm from 10 to 35min (Costunolide, Dehydrogenous lactone, Dehydrodiisoeugenol, Alantolactone, Isoantholactone); column temperature 30 °C; Sample volume 20μL. The chromatogram shows that the chromatographic peaks of each component have good symmetry, the resolution is greater than 1.5, and the number of theoretical plates is greater than 5000.

Preparation of the test solution: Weigh about 1.0g of the sample of Zadi-5 powder, place it in a conical flask with a stopper, add 25mL of 70% ethanol, a tight stopper, weigh the mass, sonicate for 30min, take it out, and let it cool , Make up the lost quality with 70% ethanol, shake well, filter, and you're done.

2.2 Preparation of Reference

Substance solution Precisely weigh the appropriate amount of Piperine, Costunolide, Dehydrogenous lactone, dehydrodiisoeugenol, Alantolactone and Isoantholactone reference. The above-mentioned component control solutions with concentrations of 0.816 mg / ml, 2.89 mg / ml, 3.12 mg / ml, 0.53 mg / ml, 3.07 mg / ml, and 2.96 mg / ml, respectively, were kept under refrigeration at 4 °C until use.

2.3 Methodological Survey

2.3.1 Linearity and Range
Precisely draw 0.1, 0.2, 0.5, 1.0, and 2.0 mL of the reference solution under "2.3", place in a 10 mL volumetric flask, dilute to the mark with 70% ethanol, and shake well. According to the "2.1" item, the chromatographic conditions were used for injection measurement. The reference solution concentration was taken as the abscissa and the peak area integral value was taken as the ordinate. The linear regression was performed by the least square method. The linear equation of the Piperine is \( Y = 84.423X - 62.1 \) (\( r = 0.9996 \)); the linear equation of the Costunolide in the range of 28.9 ~ 578.0 μg · mL\(^{-1}\) is \( Y = 39.828X + 263.05 \) (\( r = 0.9993 \)); the linear equation of the Dehydrogenous lactone in the range of 31.2 ~ 624.0 μg · mL\(^{-1}\) is \( Y = 39.828X - 263.05 \) (\( r = 0.9993 \)); the linear equation of Dehydrodiisoeugenol in the range of 5.3 ~ 106.0 μg · mL\(^{-1}\) is \( Y = 92.762X + 146.85 \) (\( r = 0.9997 \)); the linear equation of Alantolactone in the range of 30.7 ~ 614.0 μg · mL\(^{-1}\) is \( Y = 30.953X - 85.95 \) (\( r = 0.9998 \)); the linear equation of Isoantholactone in the range of 29.6 ~ 592.0 μg · mL\(^{-1}\) is \( Y = 44.85X - 226.19 \) (\( r = 0.9998 \)). The results show that the components have a good linear relationship in the corresponding range.

2.3.2 Precision Test
Take the same batch of powders, prepare the test solution according to the method under "2.2", and inject the sample under the chromatographic conditions of "2.1", the RSD values of the peak areas of the components are 1.27%, 0.76%, 1.74%, 1.20%, 0.86%, 0.70%. It shows that the precision of the method is good.

2.3.3 Repeatability Test
Take 6 parts of the same batch of powder, prepare the test solution in parallel according to the method under "2.2", and inject the sample according to the chromatographic conditions under "2.1". The RSD values of the content of each component are 1.35 %, 0.21%, 0.36%, 1.65%, 0.87%, 0.56%. It shows that the method is reproducible.

2.3.4 Stability Test
Take the same batch of powders, prepare the test solution according to the method under "2.2", and inject samples at 0, 2, 4, 8, 12, 24, 48h according to the chromatographic conditions under "2.1". The peak area RSD of each component was measured as 0.65%, 0.89%, 1.02%, 0.24%, 0.71%, 0.46%, indicating that the solution had good stability within 48h.

2.3.5 Sample Recovery Rate Test
Weigh precisely 9 parts of the same batch of powder, about 0.5g each, divided into 3 groups, 3 parts each, add Piperine 0.756 mg / mL, Costunolide 3.412 mg / mL, Dehydrogenous lactone 3.574 mg / mL, Dehydrodiisoeugenol 0.311 mg / mL, Alantolactone 1.873 mg / mL, Isoantholactone 1.256 mg / mL control substance mixed solution 0.8, 1.0, 1.2mL, according to the method under "2.2" to prepare the test solution, under "2.1" under the chromatographic conditions of injection measurement, respectively calculate the recovery rate. Results The average sample recovery rates of each component were 97.3%, 98.2%, 96.5%, 97.7%, 95.8%, 97.1%, RSD were 0.73%, 1.41%, 0.99%, 1.64%, 1.63%, 1.37%. It shows that the method is accurate.

2.4 Relative Correction Factor
Calculation taking carignone as the internal standard, calculate the relative correction factors of the other five components \( f_{k/s} \). The formula is \( f_{k/s} = f_{k} / f_{s} = (A_{k} / A_{s}) \times (C_{k} / C_{s}) \) in the formula \( A_{k} \) is the peak area of the internal standard; \( A_{s} \) Peak areas of other components; \( C_{k} \) is the internal standard mass concentration; \( C_{s} \) Mass concentration of other components. The relative correction factors calculated for each component at different concentrations are: piperine 2.857 (RSD = 1.26%), Costunolide 1.364 (RSD = 1.69%), Dehydrogenous lactone 1.186 (RSD = 1.07%) ,
Dehydrodiisoeugenol 3.434 (RSD = 0.84%), Isoanthenolactone (RSD = 1.20%).

2.5 Chromatographic Peak Positioning
In this experiment, the relative retention value method was used to locate the chromatographic peak of the measured component. The relative retention values of each component and Alantolactone under different instruments and different brand chromatography columns were piperine 0.2964 (RSD = 0.65%), Costunolide 0.6423 (RSD = 0.17%), Dehydrogenous lactone 0.8211 (RSD = 0.05%), Dehydrodiisoeugenol 0.9124 (RSD = 0.28%), Isoantholactone 1.1219 (RSD = 0.52%), the results show that the relative retention of each component has no significant difference between different instruments and columns.

2.6 Determination of Sample Content
Take three batches of Zadi-5 bulk samples, prepare the test solution according to the method under "2.2", inject samples according to the chromatographic conditions under "2.1", and determine the content of each component by one test and multiple evaluation method. The formula for calculating the mass concentration of the component to be measured is:

\[ C'_k = \frac{C_k \times A'_k}{A_{k,i} \times f_{k,i}} \]

where \( C'_k \) is the mass concentration of the component to be measured; \( C_k \) is the mass concentration of the internal standard; \( A'_k \) is the peak area of the component to be measured; \( A_{k,i} \) is the peak area of the internal standard peak; \( f_{k,i} \) is the relative correction factor; \( k \) is the number of the component under measurement.

Alantolactone was used as the internal standard substance to measure the piperine, Costunolide, Dehydrogenous lactone, Dehydrodiisoeugenol, and Isoantholactone in the Zadi-5 powder were 1.477 mg/g, 6.718 mg/g, 7.056 mg/g, 0.649 mg/g, 2.523 mg/g, respectively. Measured by standard curve method for the content of Alantolactone, piperine, Costunolide, Dehydrogenous lactone, Dehydrodiisoeugenol, and Isoantholactone contained in Zadi-5 powder Esters are 3.564mg/g, 1.460mg/g, 6.800mg/g, 6.981mg/g, 0.653mg/g, 2.493mg/g, according to the results, there is no significant difference between the content of each component obtained by the one-test multi-evaluation method and the standard curve method (RE≤2.23%). The application of one-test multi-evaluation method is feasible in the quality evaluation of Mongolian medicine preparation Zadi-5 powder.

3. Conclusion
In this experiment, one-test and multi-evaluation method was used. According to the inherent relationship and ratio between Alantolactone and various active ingredients in the compound preparation of Mongolian medicine Zadi-5 powder, by determining the stable and low-cost monomeric Alantolactone, the components can achieve the simultaneous determination of the content of 6 components of piperine, Costunolide, Dehydrogenous lactone, Dehydrodiisoeugenol, Alantolactone and Isoantholactone. The factor can accurately determine the content of the corresponding component. This method is simple and accurate, and can be used as a quality control evaluation method for Zadi-5 powder. The one-test multi-evaluation method focuses on quantitatively clarifying the interrelationship between the main components or the effective components, which is more in line with the overall view of the traditional Mongolian medicine theory, and makes up for the defects of the ambiguity of the fingerprint spectrum technology. Under the circumstances, the simultaneous quantitative determination of multiple index components greatly reduces the cost and time of testing, improves the practicality of the method, and can more effectively, more comprehensively and more accurately control the quality of Mongolian medicine preparations. The elucidation of the mechanism and the research and development of new drugs are of great significance.
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