

## Qualitative and Quantitative Study of Gukefuyao Preparation Using FT-IR and HPLC

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**Abstract.** Objective To establish methods for the quantitative and qualitative identify of Gukefuyao preparation. Methods Characteristic absorption peaks of Gukefuyao preparation powder and its different extracts were identified, compared and analyzed according to the peaks' shape, position and intensity in infrared spectra. The content of imperatorin was determined by HPLC. Results Fourier transform infrared spectroscopy (FT-IR) Fingerprint of 9 main common peaks was established preliminarily. The mobile phase was acetonitrile-water(62:38) with a flow rate at 1.0mL/min. Detection wavelength was 254nm. The linear ranges for imperatorin was 0.065 ~ 1.032 $\mu$ g. The average recovery was 99.3% (n =6) and RSD was 3.3%. In the meantime, the content of imperatorin in Gukefuyao should not less than 0.05mg/g. Conclusion The methods are simple, rapid and accurate, and can be used for the qualitative and quantitative analysis of Gukefuyao preparation.

### Introduction

Gukefuyao is a kind of traditional Chinese medicine (TCM) powders for external use. It can be well used for the treatment of traumatic injury in which the parts of the body becomes red, swelling, hot, pain and soft tissue adhesions. The preparation has been used for more than ten years in Jilin Longtang Chinese medicine hospital and unanimously approved by the patients. Gukefuyao is only an agreement prescription(home preparation).Its original quality standard is too simple and can not to show its real quality. Therefore, the paper was to carry out a series of studies on the identification of Gukefuyao.

Infrared spectroscopy is a kind of identification method, which has been reported in the research of traditional Chinese medicine and its preparation in recent years [1-4]. Therefore, infrared spectroscopy was used for the identification and analysis fo Gukefuyao and its different solvent extracts. Angelica dahurica is one of the main medicines in the preparation, and imperatorin is one of the main active ingredients of Angelica dahurica. By using imperatorin as screening index component, the high-performance liquid phase chromatography (HPLC) method was established for the content determination of Gukefuyao. It is very important for the study to improve the current quality standards of Gukefuyao and provided scientific basis for its quality control method.

### Instrument and reagent

**Instrument.** Burker TENSOR27 Fourier transform infrared spectrometer (Bruker, German), Agilent High-performance Liquid Chromatography (Agilent, America).

**Reference substance.** Imperatorin (batch lot: 11826-200410, specification: 20mg, for the content determination) provided by the National Institutes for Food and Drug Control.

**Reagent.** Acetonitrile and methanol are chromatographic pure reagent. Potassium bromide is spectrum pure reagent. Other reagents are the analysis pure. Water is Wahaha Pure water.

Gukefuyao is provided by Jilin Longtang Chinese medicine hospital.

## Methods and results of IR identification of Gukefuyao

**Preparation of the sample.** 3 samples of Gukefuyao (about 3g per sample) were weighted and then ultrasonic extracted with 30mL of ethanol, acetone and ethyl acetate for 20min, respectively. After extraction, the filtrate is concentrated and dried in the oven at 60 °C for 6h, then placed in the dryer for later use.

**IR spectroscopy determination of Gukefuyao.** Gukefuyao powder and 3 kinds of its extracts (2.1) were separately mixed with the amount of potassium bromide, grinded homogeneity and then tableting. The IR spectroscopy of these four samples were determined. The results are shown in figure 1 and table 1.

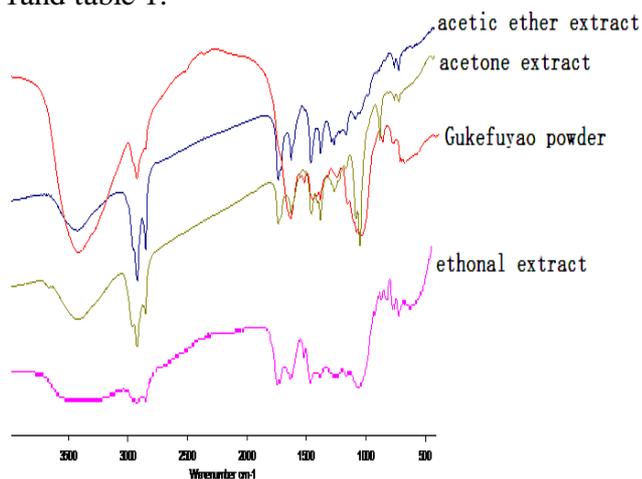


Fig. 1 FTIR fingerprints of Gukefuyao powder and their extract

Tab 1 FTIR fingerprint of characteristic absorption peaks of Gukefuyao powder and their extracts

No	Peaks[cm <sup>-1</sup> ]			
	powder	EtOH	Acetone	EtOAc
1	3418	3393	3418	3424
2	2926	2926	2926	2924
3	2855	2853	2855	2853
4	1632	1628	1628	1628
5	1450	1464	1456	1464
6	1385	1379	1383	1381
7	1080	1072	1080	1088
8	768	760	760	760
9	712	721	721	721

Figure 1 and Table 1 shows that 9 common peaks were existed both in the IR spectra of Gukefuyao powder and in the IR spectra of its 3 kinds of extracts. This means that the 9 peaks are related to the main active components and can not change with the different solvent, and then determined to be the characteristic peaks of Gukefuyao. Among of them, peak of 3410 cm<sup>-1</sup> is hydroxyl absorption peak, peaks of 2925 cm<sup>-1</sup> and 1380 cm<sup>-1</sup> are -CH<sub>2</sub>- and -CH<sub>3</sub>[5].

## Content Determination Methods and Results of Gukefuyao

**Chromatographic Conditions.** The chromatographic conditions were a column C18 (Diamonsil 250 mm×4.6 mm, 5μm Analytical Column, America). The mobile phase was acetonitrile- water (62:38) and the flow rate was 1.0mL/min. The operating temperatures were maintained at room temperature but the oven was heated at 25°C to favour the mobile phase flow through the column. The detection wavelength UV was 254 nm and the injection volume was 10μL. The number of theory plates was not less than 3000 determined by the peaks of imperatorin.

**Reference solution.** To accurately weigh 1.29 mg of imperatorin to 10 ml volumetric flask and add 5ml of methanol. After that, the volumetric flask were shaken vigorously to promote the solubilization of imperatorin, then made up to the mark with methanol and were stored at -4°C for measurement.

**Sample solution.** About 1g Gukefuyao powder was accurately weighted and soaked in 45 ml methanol, then extracted by means of ultrasonic extraction (300 W, 50 kHz) for 1 hour. After cool to the room temperature, add methanol to the mark and then filtrated with 0.22μm millipore membrane for HPLC analysis.

**Negative sample solution.** The negative sample was prepared according to the proportion of Gukefuyao prescriptions except Radix Angelicae dahuricae. The preparation of the negative sample was the same as “sample preparation”.

**Standard curve.** The peak areas of 0.5 $\mu$ l, 1 $\mu$ l, 2 $\mu$ l, 4 $\mu$ l and 8 $\mu$ l imperatorin reference solution were measured, respectively. The regression equation and the correlation coefficient were established as:  $Y=64559X-5.9709$ ,  $r = 0.9997$ , where Y is the peak area and X is the injection volume of imperatorin( $\mu$ l). The experimental results show that the peak area had a good linear relationship with the amount of imperatorin in the range 0.065 ~ 1.032  $\mu$ g.

**Specific experiments.** The specificity of the method was verified by comparing the chromatograms of standard imperatorin, Gukefuyao sample and its negative sample with no Radix Angelicae dahuricae. The chromatograms obtained in HPLC for the negative sample did not show the peak with a similar retention time to that of the imperatorin(9.770 min  $\pm$  10%) (Figure 2). So, it was observed the absence of interferences of the other pharmaceuticals for Gukefuyao preparation, because none of the peaks appears at the same retention time as imperatorin peak. The results shows that the developed method is selective in relation to the other pharmaceuticals in the Gukefuyao preparation.

**Stability experiments.** The peak areas of the same sample solution was determined by HPLC at different time (at 0h, 2h, 4h, 6h, 8h, 10h and 12 h, respectively) and the relative standard deviation was 1.52%. The results indicated that the tested sample solution was kept stable within 12 hours.

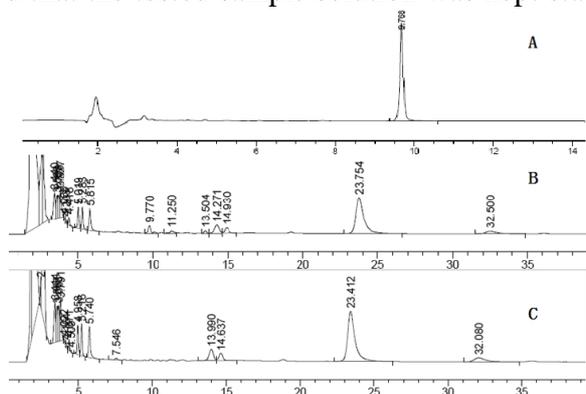


Fig 2 Chromatograms of imperatorin(A), sample (B) and blank sample (C)

**Precision experiment.** The precision of the method was assessed by testing the peak areas of one standard solutions 6 times. Then the average peak area and percentage of relative standard deviation were obtained as 148.56 and  $RSD=0.69\%$ , respectively. The results show that the HPLC had a good precision. In the case of the developed method, precision has been validated for various repeatability studies.

**Repeatability experiment.** 6 samples from one batch of Gukefuyao were prepared according to the method of “sample solution”. The imperatorin peak areas of the 6 samples were measured and then calculated the imperatorin content of each samples and  $RSD(1.3\%)$ . The results showed that the method has a favorable repeatability. Therefore, the study was considered validated.

**Recovery experiment.** The recovery experiment by adding standard samples was carried out for negative Gukefuyao samples with addition of three-level imperatorin. The average amount of imperatorin recovered in relation to the added amount was calculated as 99.3% ( $RSD 3.3\%$ ). The analytical results of six samples show that the method has a good recoveries.

**Determination of imperatorin in Gukefuyao.** The concentration levels of imperatorin in 3 lots of Gukefuyao (lot number: 0506, 0507, 0508) were measured and the results were 0.0605, 0.0613 and 0.0598 mg/g. For the analytical procedure of Gukefuyao sample, the methods were the same as described previously. According to the results, the content of imperatorin in Gukefuyao shall not be less than 0.05mg/g.

## Discussion

Traditional Chinese Medicine (TCM) is the precious wealth of Chinese nation, and has made a great contribution to the healthcare of Chinese people and the prosperity of the Chinese nation. TCM has attracted more and more attention for its significant biological activity and the world wide demand for the TCM has provided a broad space for its development. While the quality control becomes one of the most important bottlenecks in the development of TCM and its prescriptions. Among of the analysis and identification methods, infrared spectrometry has an important significance and practical values in the qualitative identification and content determination of TCM and preparation for its high efficiency, convenience and environmental protection[1].

FT-IR was used to identified Gukefuyao powder and its different solvent extracts in the paper. Little difference found in those infrared spectras showed that the main absorption peaks were consistent and could not be affected by extraction solvent. Therefore, 9 main peaks of Gukefuyao in these IR spectra were attributed as the infrared characteristic absorption peak.

Imperatorin from sovereign drug *Angelica dahurica* of Gukefuyao has many important functions such as antibacterial, anti-inflammatory, antipyretic and analgesic effects[6-7]. So the quantitatively experiment of Gukefuyao was designed with the content of imperatorin as index. The determination wavelength of imperatorin were selected as 300nm [8], 254nm [9-10] or 248nm [11] in some other papers. On the basis of above mentioned contents, UV wavelength scanning of imperatorin was obtained and the result showed that the maximum absorption wavelength of imperatorin was at about 249nm. As a result, the detection wavelength was set at 254 nm for facilitate the detection and popularization.

This paper develops the qualitative and quantitative analysis methods for evaluating the quality of Gukefuyao. These methods are simple, stable and accurate and can be used for the quality control of Gukefuyao.

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