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Rapid Determination of Rutin Content in Chinese Patent Medicine Qi Ming Granules by RP-HPLC.

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Abstract

The aim of this study was to be the first to establish a method for determination of rutin content in Qi Ming Granules, providing a scientific basis for the development of its quality standards. Measurement was done directly by RP-HPLC using Agilent TC-C_{18} chromatographic column with a mobile phase of methanol-water-phosphoric acid (48.5:51.5:0.25, pH3.5), flow rate of 1.0 mL/min, and detection wavelength of 360 nm, ruggedness test was performed. The injection amount of rutin had a good linear relationship with the peak area within the range of 0.0300-1.498 μg, recovery rate was 99.8%, RSD = 1.23% (n = 6). Rutin contents in 10 different batch numbers of Qi Ming Granules were measured to be between 21.09-29.01 mg/g. The method established had the advantages of simplicity, rapidness and high precision, and was used for the first time as a quality inspection method for Qi Ming Granule.

Keywords: Qi Ming Granule, Rutin, RP-HPLC, Ruggedness test

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Introduction

Qi Ming Granule is a Traditional Chinese patent medicine which has gained wide clinical application in China. It mainly contains eight crude drugs, namely Radix Astragali, Radix Puerariae, Radix Rehmanniae, Fructus Lycii, Semen Cassiae, Semen Leonuri, Pollen Typhae and Hirudo. Clinical trials have shown that Qi Ming Granules can be effective in treating diseases such as diabetic retinopathy. As a main drug, Radix Astragali contains active constituents such as astragalosides and astragalus polysaccharides, which can nourish the liver, lower blood sugar, and enhance the body's immune function. Puerarin, the main constituent of Radix Puerariae, has the actions of improving blood rheology, enhancing insulin receptor sensitivity, and inhibiting protein glycosylation; as the main constituent of Radix Rehmanniae, catalpol has a hypoglycemic effect; Hirudo contains hirudin, antithrombin and a variety of amino acids, which have strong blood circulation promoting and stagnation removing actions [1-2]. Studies have reported that Qi Ming Granule also has a lipid regulatory action in diabetic patients [3], certain therapeutic effect on diabetic nephropathy-induced retinopathy [4-7], and favorable alleviatory actions on dry eye, and eye fatigue.

Although Qi Ming Granule has been widely used in clinical settings, so far it has not been recorded in the Chinese Pharmacopoeia, and its quality control has been relatively scarcely studied. With only one literature reference on relevant processing technology [8], there has been no study on quantification of its active constituents by HPLC so far. In the literature, its extraction process is optimized using only total nitrogen and flavonoids as indicators. Total flavonoid content in Qi Ming Granule has been determined by UV spectrophotometry using rutin as the reference substance, but never by HPLC. The present study prompts that as a representative flavonoid constituent, rutin is usually selected as an indicator constituent in the investigation of processing technology of flavonoid-based Traditional Chinese patent medicines, suggesting that the quality control of Qi Ming Granules can be achieved by controlling the content of rutin. Consequently, this work, for the first time, conducted a detailed study on the method of determination of rutin content in Qi Ming Granule. We chose rutin content as the quantitative indicator of quality control of the Granule. The use of HPLC enabled accurate, simple, and rapid quality control of Qi Ming Granule.

Experiments and Results

Instruments and Materials

Centra-208 UV-Vis spectrophotometer (Nanjing Scientific Instrument Co., Ltd.), 2695 HPLC (Waters, USA), Direct-QTM5 ultrapure water system (Millipore, USA), electronic balance (Katuopu, USA), DK2500WC ultrasonic instrument (Shanghai Tianpu Ultrasonic Instrument Co., Ltd.) have been used for this work. Ten different batch numbers of Qi Ming Granules were obtained from

Nanjing Jinbi Pharmaceutical Factory. The rutin reference substance, batch number 2014039-2365, was procured from National Institute for the Control of Pharmaceutical and Biological Products and HPLC grade methanol, analytical grade phosphoric acid and redistilled water were used to carry out the experiments.

Chromatography set-up

Chromatographic column: Agilent TC-C_{18} (4.6 mm × 250 mm, 5 μm); mobile phase: methanol-water-phosphoric acid (48.5:51.5:0.25, pH 3.5); flow rate: 1.0 mL/min; detection wavelength: 360 nm; temperature of column: 20°C. Number of theoretical plates was calculated to be not less than 2,000 based on the peak of rutin. Under these chromatographic conditions, the peak for the retention time of rutin in the test sample was consistent with the corresponding peak in the reference substance, see Fig. 1.

Preparation of reference solution

Appropriate amount of rutin reference substance, which was dried under reduced pressure over phosphorus pentoxide for 12 h, was accurately weighed and added to 50% methanol to prepare a reference solution containing 50μg rutin/ mL.

Preparation of test solution

About 0.1 g of sample powder was accurately weighed, placed in a stoppered conical flask, and 50 mL of 50% methanol was added accurately to it. This mixture was then weighed, heated under reflux for 60 min, cooled, and weighed again. The lost weight was complemented with 50% methanol and shaken well. The supernatant was filtered with a microporous membrane (0.45 μm), and the subsequent filtrate was collected to serve as a test solution.

Reproducibility test

Six aliquots of 1 g of sample were accurately measured and subjected to experiment as per the method described above, chromatograms were recorded, and contents were calculated. The average content was calculated to be 23.20 mg/g, with a RSD of 1.21%, showing a good reproducibility.

Ruggedness test

Stability test

Reference and test solutions were injected into the liquid chromatograph at different times according to the above chromatographic conditions, and changes in peak area were examined, the results are shown in Table 1. As can be seen from Table 1, the reference and test solutions were stable within 24 h.

Validation of Methodology

Investigation of linear range

For preparing a reference solution, 29.95 mg of rutin reference substance was accurately weighed, placed in a 50 mL volumetric flask and dissolved by adding methanol, then diluted to the mark ***(diluted with what?reply: 50% methanol)***, and shaken well. One milliliter of the reference solution was accurately measured, placed in a 10 mL volumetric flask, diluted to the mark by addition of 50% methanol, and shaken well to serve as reference solution. Accurate aliquots of 0.5, 5, 10, 15, 20, 25 and 5 μL of reference solutions were aspirated and injected into the liquid chromatograph. Chromatograms were recorded, and peak areas were measured. Linear regression was performed with peak area (A) as the ordinate, and rutin amount (μg) as the abscissa, regression equation was Y = 3848.9X-0.485, r = 0.9999. The results showed that the rutin injection amount was in a good linear relationship with peak area between 0.0300-1.498 μg.

Injection precision test

To test the precision of injection, 5 μL of rutin reference solution was accurately measured, injected into the liquid chromatograph a total of five times, peak areas were recorded, and the average peak area was measured to be 1092.7, with a RSD of 0.78%, indicating a good injection precision.

Investigation of different columns

0.1 g of sample powder was accurately weighed, and subjected to experiment according to the method described above, sample was measured using different chromatographic columns and contents were calculated. The results are shown in Table 2. RSD was 2.95%, which indicated a good ruggedness of the method.

Table 2. *Investigation results of different columns*

Chromatographic column	**Content** (mg/g)
Kromasil C_{18}	23.15
Hypersil C_{18}	22.3
μBondapak C_{18}	22.16
Dupont C_{18}	23.56

Sample recovery test

Six aliquots of 2.0 mL of 0.560 mg/mL reference solutions were taken in 6 different 150 mL stoppered conical flasks, and placed in a water bath to evaporate methanol. Six aliquots of approximately 0.05 g of sample (content of 23.20 mg/g) powders were accurately weighed, placed in the above conical flasks, 50 mL of 50% methanol was added to each, followed by the preparation and determination of test solutions according to the planned method. Chromatograms were recorded, and recovery rates were calculated, the average recovery rate was calculated to be 98.9%, with a RSD of 1.25%, showing a good accuracy.

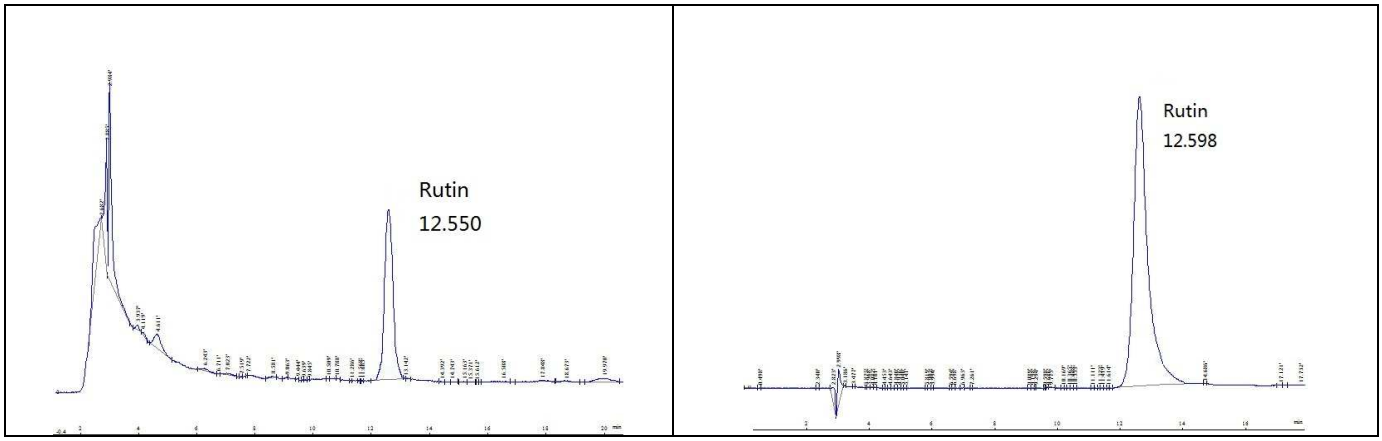

Figure 1. *HPLC chromatograms of rutin in Qi Ming Granule and rutin reference substance*

Table 1. *Stability test results*

	0h	2h	4h	8h	16h	24h	RSD
Reference solution	1093.5	1092.7	1090.3	1080.5	1084.4	1080.2	0.54
Test solution	825.7	836.3	834.6	851.2	839.0	842.2	1.01

Table 3. *Sample quantification results (mg/g)*

Sample batch No.	Rutin content	Sample batch No.	Rutin content
20131201	23.20	20140102	23.56
20131202	24.52	20140103	24.02
20131203	22.03	20140201	28.65
20131204	21.09	20140202	29.01
20140101	23.02	20140203	27.09

Quantification of sample

Ten microliters of reference solution as well as test solutions (prepared as per the "Preparation of test solution") of different batches of samples were accurately aspirated and separately injected into the liquid chromatograph, and subjected to measurement, followed by content calculation. The results are shown in Table 3.

Discussion

Selection of detection wavelength

Rutin reference solution was prepared using 50% methanol as the solvent and its spectrum was scanned at 200-400 nm wavelength. The results revealed a maximum absorption at 360 nm wavelength, which was consistent with the references [9-10], so the detection wavelength was selected to be 360 nm.

Selection of mobile phase

After referring to relevant literatures [11-15] on determination of rutin content in Traditional Chinese patent medicines and preliminary tests, a mobile phase of methanol-water-phosphoric acid (48.5:51.5:0.25, pH 3.5) was adopted finally for system suitability study. The results showed that the peak shape was symmetrical, number of theoretical plates was high, and resolution met the requirements. Therefore, methanol-water-phosphoric acid (48.5:51.5:0.25, pH 3.5) was selected as the measurement mobile phase.

Methodology comparison

The UV spectrophotometry applied currently has some problems in determination of total flavonoids, such as low accuracy, poor sensitivity, and poor specificity. This paper is the first to measure rutin content in Qi Ming Gran-

ules applying RP-HPLC. It was found by experimental study that rutin content in Qi Ming Granules was relatively high. HPLC determination of its content is characterized by high sensitivity, good resolution, simplicity and rapidness, so the use of rutin as a quality control indicator of Qi Ming Granule is relatively reasonable.

Conclusion

After determination of 10 batches of samples, rutin content was measured to be over 23 mg/g for 8 batches, over 24 mg/g for 5 batches, and over 27mg/g for 3 batches, indicating that the method established was simple, accurate and rapid. This paper is the first to use rutin content as a quantitative indicator of quality control of Qi Ming Granules, which can effectively control the quality of Qi Ming Granules, resolving the inability of quality control during its production process, and thus is of great significance and guidance to the industrial production of Qi Ming Granules. This article describes a rapid procedure for determining the rutin content in Qi Ming Granules by RP-HPLC method. RP-HPLC is a widely used technique for determining flavonoids in crude plant materials and extracts, as well as from oral dosage forms. Novelty of this work may convey the application of a common method for specifically complex matrix Qi Ming Granules.

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