Quantitative Determination of Total Flavonoids from Polygonatum Sibiricum by Spectrophotometry

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Abstract. Objective: This study aims to establish a spectrophotometry method to determine the content of total flavones from Polygonatum sibiricum. Methods: Rutin was used as the control substance. After the color reaction of Al(NO₃)₃-NaNO₂-NaOH system, the maximum absorption wavelength was determined and the standard curve was drawn. Then the reliability of the method was verified by precision test, repeatability test, stability test and recovery test. Results: The maximum absorption wavelength of rutin is 510 nm, the standard curve is Y=10.232X-0.0047, R²=0.9991, and the linear fitting degree is high. Precision test Rsd = 1.02%, repeatability test Rsd = 2.79%, stability test Rsd = 2.25%, recovery test Rsd = 1.44%. It can meet experimental requirements.

1. Introduction

Polygonatum sibiricum, a traditional Chinese medicine, has the activity of anti-cancer [1], antioxidant, anti-immunosuppression [2], anti-inflammatory [3] and etc. It contains polysaccharides, amino acids, saponins, flavonoids and many trace elements [4, 5]. Flavonoids, a main component of Polygonatum sibiricum, is a powerful antioxidant that scavenges oxygen free radicals in the body [6], slows cell aging and prevents cancer. Flavonoids can improve blood circulation and cholesterol, inhibit the exudation of inflammatory enzymes, improve wound healing and pain relief, and can also be used in the treatment of various kinds of allergies [7]. Therefore, flavonoids have potential for further research and development.

Spectrophotometry is an effective method to determine the content of substances. It can produce different absorbance according to the continuous change of wavelength irradiation on the phase of the sample solution, and draw absorption spectrum curve according to the relationship between wavelength and absorbance. According to this curve, the maximum absorption peak of the sample can be measured, and the absorbance of the sample solution can be obtained by irradiating the sample solution at a specific wavelength. The method mentioned above is used to analyze the substances. It is called spectrophotometric method.
Therefore, this study intends to establish a spectrophotometric method for the determination of flavonoids in *Polygonatum sibiricum*, in order to provide a basis for the extraction, purification and in-depth study of flavonoids in *Polygonatum sibiricum*.

2. Experiment

The content of total flavonoids in *Polygonatum sibiricum* was determined by spectrophotometer after color reaction with rutin as control.

2.1. Preparation of standard solution

The rutin standard sample was weighed 100 mg and dissolved in 80% ethanol to obtain 0.4 mg/ml rutin standard solution.

2.2. Preparation of sample solution

Accurately weighing 2.0 g powder of *Polygonatum sibiricum*, dissolving it in 80% ethanol 200ml, the sample solution of *Polygonatum sibiricum* with concentration of 0.01g/ml can be obtained.

2.3. Standard curve

2.3.1. Determination of the maximum absorption wavelength

Using 0.4mg/ml of rutin as standard solution, the maximum absorption wavelength was determined by scanning in the visible light range of 400-800 nm.

2.3.2. Preparation of quantitative standard curve

6 different volumes of rutin standard solution and 5% NaNO₂ were added in a tube, respectively, shaking and placing at room temperature for 6 minutes. Then add 10% Al(NO₃)₃ in turn, shake well and placed at room temperature for 6 minutes, then add 1mol/l NaOH, stored at room temperature for 15 minutes. The absorbance of standard samples was measured at 510 nm. The linear regression equation was obtained by using concentration C (ug/ml) as abscissa and absorbance A as ordinate. The precision test, repeatability test, stability test and recovery test were also conducted in this experiment.

3. Results

3.1. Full wavelength scanning of rutin standard in visible light region

According to the full wavelength scanning of rutin reference substance at 400-800, it can be clearly seen that there is a maximum absorption peak at 510 nm (Figure 1). Therefore, 510 nm wavelength was selected as the wavelength of total flavonoids.

![Figure 1. Full wavelength scanning of rutin after color reaction](image)
3.2. Drawn of standard curve
Experiments were carried out according to the method described in "2.3.2". The initial volumes of rutin standard solution (0.4 mg/ml) in color reaction system were 0.0, 0.2, 0.6, 1.0, 1.4 and 1.8 ml, respectively. After the reaction, the absorbance was detected by a UV-Vis spectrophotometer (type: TU-1810, Beijing Puxi General Instrument Co., Ltd.). The results were shown in Figure 2.

The curve equation was $y=10.232x-0.0047$, in which $y$ was the absorbance and $x$ was the concentration. $R^2=0.9991$ was obtained. It showed that the linear relationship between the concentration of rutin ($C$) and the absorbance ($A$) was very good in the concentration range of 0-0.072 mg/ml, and could be used as a standard curve.

![Standard curve for determination of flavonoid](figure2.png)

3.3. Reliability test

3.3.1. Precision test. Four successive spectrophotometric tests were carried out on the same standard solution of rutin. The results showed that there was no obvious change in the absorbance values. As shown in Table 1, the $Rsd=1.02\%$ was calculated. The precision of the spectrometer was proved to be reliable.

| No. | Concentration (mg/ml) | Absorbance | Rsd |
|-----|-----------------------|------------|-----|
| 1   | 0.04                  | 0.415      |     |
| 2   | 0.04                  | 0.408      |     |
| 3   | 0.04                  | 0.418      |     |
| 4   | 0.04                  | 0.413      | 1.02\% |

3.3.2. Stability test. The same sample solution (0.01 g/ml) was measured three times in 240 minutes at different intervals. The results showed that the absorbance of the sample had no obvious change. The $Rsd$ was calculated to be 2.40\% (Table 2). It was proved that the sample after color reaction had good stability in 240 minutes.

| No. | Time (min) | Absorbance | Rsd  |
|-----|------------|------------|------|
| 1   | 60         | 0.238      |      |
| 2   | 120        | 0.227      | 2.40\% |
| 3   | 240        | 0.231      |      |
3.3.3. Repeatability test. According to "2.2" operation, three parallel tests were conducted. Three different sample solutions were prepared, and the absorbance at 510 nm was measured after color reaction, to verify the repeatability of this quantitative method. It can be seen in Table 3, the absorbance showed no significant change with three parallel tests.

Table 3. The absorbance of the same sample with three parallel tests

| No. | Weigh of *Polygonatum sibiricum* (g) | Absorbance | Rsd  |
|-----|-------------------------------------|------------|------|
| 1   | 2.02                                | 0.241      |      |
| 2   | 1.99                                | 0.224      | 3.66%|
| 3   | 2.01                                | 0.232      |      |

3.4. Reliability test

Sample solution was prepared with reference to "2.2", and 2 ml sample solution was added into 6 test tubes, in which the content of sample was 0.0454 mg. The weights of rutin standard 0.2, 0.2, 0.4, 0.4, 0.6, and 0.6 mg were added into No. 1-6 test tube respectively. The absorbance was measured in turn after color reaction. The recovery of sample was calculated according to formula (1).

\[
R\%=(M-I)*100%/A
\]  
(1)

Where R is the recovery rate; M is the contents in sample solution measured with standard curve, mg; I is the initial content of flavonoids in sample solution, mg; A is the content of rutin added in sample solution, mg. The results showed (Table 4) that the absorbance changed regularly with the increase of the standard addition of rutin. The Rsd=1.44% was calculated according to formula (1), which proved that the method had high accuracy.

Finally, according to the standard curve, the yield of flavonoids in *Polygonatum sibiricum* was 0.57%, which meant the content of total flavonoids in 1 g *Polygonatum sibiricum* was about 5.7 mg.

Table 4. The results of recovery test

| No. | Initial content in sample(mg) | Rutin added(mg) | Measured content(mg) | Recovery rate (%) | Average (%) | Rsd   |
|-----|------------------------------|----------------|----------------------|------------------|-------------|-------|
| 1   | 0.0454                       | 0.2            | 0.248                | 101.30           |             |       |
| 2   | 0.0454                       | 0.2            | 0.245                | 99.80            |             |       |
| 3   | 0.0454                       | 0.4            | 0.441                | 98.90            |             |       |
| 4   | 0.0454                       | 0.4            | 0.443                | 99.40            |             |       |
| 5   | 0.0454                       | 0.6            | 0.635                | 98.27            |             |       |
| 6   | 0.0454                       | 0.6            | 0.628                | 97.10            |             |       |

4. Conclusion

After the color reaction of aluminum nitrate-sodium nitrite-sodium hydroxide, several groups of absorbance values were measured according to the wavelength corresponding to the maximum absorption peak of rutin standard curve, and the standard curve of rutin was drawn. Similarly, the content of total flavonoids in *Polygonatum sibiricum* could be calculated according to the standard curve of rutin. After a series of precision test, repeatability test, stability test, as well as sample recovery test and calculated the RSD% of each group of experiments, the experimental data showed that the influence of this standard curve is reliable. Therefore, this spectrophotometry method can be used to determine the content of flavonoids in *Polygonatum sibiricum*.

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