

Quantitative Determination of Total Alkaloids in Piper Longum with Acid Dye Colorimetry

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Abstract

A method is established to quantitatively determine total alkaloids in Piper Longum by acid dye colorimetry. Piperine was used as a control standard and bromothymol blue as dye. The buffer solution of different pH value, the amount of buffer solution and the amount of acid dyes is investigated by acid dye colorimetry, the method for content determination of alkaloids of Piper longum is optimized. Optimum method of determination of alkaloids of Piper Longum is as following: the pH value of is 6.0, the amount of the buffer solution is 4.0mL, the volume of the acid dye is 6.0mL. It is simple, accurate and reliable, economic and fast.

Keywords

Piper longum, total alkaloids, acid dye colorimetry, content determination.

1. INTRODUCTION

Piper longum L. is a dry, nearly mature or mature fruit ear. It is used for abdominal cold pain, vomiting, diarrhea, cold congealing gas stagnation, chest pain, headache and toothache[1]. Piperine and other alkaloids, as the main chemical constituents of Piperine, have many pharmacological effects, such as anti-bacterial, anti-tumor, anti-oxidation, anti-inflammatory, analgesic and immune regulation[2,3]. Therefore, the establishment of a method for the determination of total alkaloids in Piper chinensis can provide a reference for the quality evaluation of Piper chinensis. Acidic dye colorimetry is based on the principle that alkaloids react with acid dyes in a certain pH medium to form chromogenic group complexes that can be detected by ultraviolet spectrophotometer. The method has the characteristics of high sensitivity, good reproducibility, simple operation and simple sample treatment[4-6]. Therefore, the content of total alkaloids in Piperine was determined by acid dye colorimetry with piperine as control substance in this study, which provided reference and theoretical basis for quality control and further study of Piperine.

2. INSTRUMENTS AND REAGENTS

2.1. Instruments

BS224S Electronic Balance (Cedoris Scientific Instruments Co., Ltd.), 510 Acidimeter (Beijing Zhongyuan Technology Co., Ltd.), TU-1901 Dual Beam Ultraviolet-Visible Spectrophotometer (Beijing General Instruments Co., Ltd.).

2.2. Reagents

Piperine reference substance (batch number: 16041311, manufacturer: Shanghai Yaji Biotechnology Co., Ltd.) was purchased from Zunyi Traditional Chinese Medicine Company and

identified by Director Yang Jianwen of Zunyi Medical University. The rest of the drugs or reagents were analytical pure.

3. METHODS

3.1. Preparation of Buffer solutions with Different pH Values

Reference [7], acetic acid-sodium acetate buffer solution with pH 4.47, phosphate buffer solution with pH 4.98, acetic acid-sodium acetate buffer solution with pH 5.97, phosphate buffer solution with pH 6.50, phosphate buffer solution with pH 6.96 and phosphate buffer solution with pH 7.59 were prepared respectively for reserve.

3.2. Preparation of Sample Solution

Take a certain amount of *Capsella chinensis*, sieve through 60 meshes, get fine powder, weigh 10.00g fine powder in a round-bottom flask, add 200 mL anhydrous ethanol and soak for 30 minutes, heat reflux extraction with an electric heating jacket for 1 hour, cool, filter, residue with anhydrous ethanol a small amount of wet washing, until the total filtrate is collected to 500 mL, stand by.

3.3. Preparation of Reference Solution

Precision weighing 0.010g standard piperine was placed in a 25 mL Brown volumetric flask, adding an appropriate amount of absolute ethanol, dissolving, volumetric setting, standby.

3.4. Preparation of Bromothymol Blue Solution

Precision weighing 0.100g bromothymol blue, adding 3.2mL concentration of 0.05mol/L sodium hydroxide solution, stirring dissolution, adding water dilution and set volume in 200 mL capacity bottle, stand by.

4. RESULTS AND DISCUSSIONS

4.1. Selection of Detection Wavelength

1.0mL piperine reference solution and sample solution were measured in the separating funnel respectively, then 5.0mL bromothymol blue solution and 5.0mL acetic acid-sodium acetate buffer solution were added to the two solutions, shaking well, adding 20mL dichloromethane, shaking, standing for 30 minutes, scanning under the ultraviolet spectrophotometer with wavelength of 200-600 nm. The results showed that the two solutions were scanned by ultraviolet spectrophotometer with wavelength of 200-600 nm. There are maximum absorption peaks at 418 nm, so the detection wavelength is 418 nm.

4.2. Investigation of Buffer Solutions with Different pH Values

Six samples of 1.0mL solution were taken in the separating funnel, 5.0mL bromothymol blue solution was added, and then 5.0mL buffer solution with pH values of 4.47, 4.98, 5.97, 6.50, 6.96 and 7.59 was added, followed by 20mL dichloromethane, shaking and standing for 30 minutes. The absorbance was measured at 418nm wavelength with dichloromethane as blank control. The results are shown in Table 1.

4.3. Investigation of Buffer Solution Dosage

Six 1.0 mL sample solutions were taken in the separating funnel, 5.0 mL bromothymol blue solution was added, and 1, 2, 3, 4, 5 and 6 mL of acetic acid-sodium acetate buffer solution with pH 5.97 were added in turn. Adding 20 mL dichloromethane, shaking, standing for 30 minutes, using dichloromethane as blank control, the absorbance was measured at 418 nm wavelength. The results are shown in Table 2.

Table 1. Results of different pH buffer solutions

pH	Absorbance	Concentration (mg/mL)	Content (%)
4.5	0.3801	0.0081	8.08
5.0	0.6403	0.0133	13.31
6.0	0.8537	0.0176	17.61
6.5	0.1442	0.0033	3.33
7.0	0.0835	0.0021	2.11
7.6	0.0451	0.0013	1.34

Table 2. Results of pH6.0 buffer solution

Buffer volume (mL)	Absorbance	Concentration (mg/mL)	Content (%)
1.0	0.8244	0.0170	17.02
2.0	0.8905	0.0183	18.35
3.0	1.0032	0.0206	20.62
4.0	1.0968	0.0225	22.50
5.0	1.0160	0.0209	17.67
6.0	0.9342	0.0192	16.23

4.4. Investigation on the Dosage of Bromothymol Blue Solution

0.5mL samples of bromothymol blue solution were taken in the separating funnel, then 3, 4, 5, 6, 7 and 8 mL of bromothymol blue solution were added in turn, and 4.0mL of pH 5.97 buffer solution was added to shake them well. Adding 20 mL dichloromethane, shaking, standing for 30 minutes, using dichloromethane as blank control, the absorbance was measured at 418 nm wavelength. The results are shown in Table 3.

Table 3. Results of the dosage of Finland solution of thyme

Dosage of acid dyes (mL)	Absorbance	Concentration (mg/mL)	Content (%)
3.0	0.2557	0.0056	11.15
4.0	0.4011	0.0085	17.00
5.0	0.4598	0.0097	22.36
6.0	0.4690	0.0099	22.73
7.0	0.4054	0.0086	17.17
8.0	0.3142	0.0068	13.50

In summary, 4.0mL acetic acid-sodium acetate buffer solution at pH 6.0 and 6.0mL bromothymol blue solution were selected as the conditions for content determination.

4.5. Preparation of Standard Curve

Precision measurement of the above standard solutions is 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 mL, which are placed in six 100 mL volumetric flasks, diluted by anhydrous ethanol and fixed volume. Then take 0.5 ml of the above solution and place it in 6 clean funnels. Add 6.0 mL bromothymol blue solution and 4.0 mL acetic acid-sodium acetate buffer solution at pH 6.0 to shake well. Add 20 mL dichloromethane, shake gently, and place it for 30 minutes. Take dichloromethane as blank control. Measure its absorbance at 418 nm wavelength. Take absorbance as ordinate (y) and concentration as abscissa (x). Draw standard curve. The equation of standard curve is $y = 49.694x - 0.0213$, $R^2 = 0.999$. The linear relationship is good.

4.6. Methodological Investigation

The absorbance (n=6) of the reference substance and the test substance were determined by the above method, and the precision test was carried out. The results showed that the RSD of the absorbance of the reference solution was 0.12%, and that of the sample solution was 0.15%.

The absorbance of the sample solution was determined at 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 and 60 minutes, and the stability test was carried out. The results showed that the RSD of absorbance was 2.6% within 50 minutes, so the color reaction should be controlled at 35 minutes as far as possible.

The sample solution was prepared according to the above method. The precise quantity was 12 mL. The color was developed according to the above method (n=6), and the repeatability test was carried out. The results showed that the content of total alkaloids was 0.217 (+0.002%) and RSD was 1.1%. This indicated that the method had good reproducibility.

5. CONCLUSIONS

To sum up, acid dye colorimetry was used to determine the total alkaloids in *Piper mandshurica* under the conditions of pH 6.0 acetic acid-sodium acetate buffer solution 4.0 mL and bromothymol blue solution 6.0 mL. The method is simple, accurate and reliable. To provide reference for quality control of *Piper chinensis*.

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