Indirect Determination of Penicillamine using Fe(II)-Phenanthroline Spectrophotometry

Xinrong Wen* and Changqing Tu
College of Chemistry and Environment, Jiaying University, Meizhou, Guangdong 514015, P. R. China
Corresponding author’s e-mail: wxrong5093@sohu.com

Abstract. Fe³⁺ can react with penicillamine to form Fe²⁺, the amount of Fe²⁺ is directly proportional to the addition of penicillamine in system, and then Fe²⁺ reacted with the chromogenic reagent of phenanthroline to form a stable complex which its maximum absorption wavelength is 511nm. The content of penicillamine is determinated by measuring the absorbance of the stable orange red complex. The method for the indirect determination of penicillamine using Fe(II)-phenanthroline spectrophotometry has been established. The results show that in 0.0008000~0.01040 mg/mL, the linear relationship between the absorbance and the mass concentration of penicillamine is A=0.0304+75.886C (mg/mL), and the linear correlation coefficient was r=0.9996. The method is used to the determination of penicillamine in tablets, and the results are satisfactory.

1. Introduction
Penicillamine (PA, Figure 1) is used in the treatment of rheumatoid arthritis, Wilson’s disease, cirrhosis, cystinuria and heavy metal poisoning. So far, the Chinese Pharmacopoeia used the potentiometric titration method to determinate the content of penicillamine. In addition, the other main methods reported in the literature are as follows, spectrophotometry, fluorescence spectrophotometry, flow injection analysis, HPLC method, electrochemical analysis, etc.

Figure 1. The molecular structure of PA
we find that the hydrosulfuryl(-SH) in penicillamine can reduce Fe³⁺ to Fe²⁺, and then Fe²⁺ reacted with phenanthroline to form a stable orange red complex which its maximum absorption wavelength is 511nm. The content of penicillamine is determinated by measuring the absorbance of the stable orange red complex. The method for the indirect determination of penicillamine using Fe(II)-phenanthroline spectrophotometry has been established. The results show that in 0.0008000~0.01040 mg/mL, the linear relationship between the absorbance and the mass concentration of penicillamine is A=0.0304+75.886C (mg/mL), and the linear correlation coefficient was r=0.9996. The method is used to the determination of penicillamine, and the results are satisfactory.

2. Experimental
2.1. Equipment and reagents
UV-2401 UV-visible spectrophotometer; 723S spectrophotometer.
Penicillamine solution: 0.2000 g·L⁻¹, stored at 4°C in dark place. Standard solution of Fe³⁺: 0.5000 g·L⁻¹, prepared by NH₄Fe(SO₄)₂·12H₂O. Phenanthroline(phen) solution: 0.0055 mol·L⁻¹. pH Buffer solutions are prepared as references[11].
All reagents are analytical reagent grade. Bidistilled water is used throughout.

2.2. Method
1.20 mL of phenanthroline solution, 2.50 mL of pH4.0 buffer solution, 0.30 mL of Fe³⁺ solution are added into a 25mL comparison tubes. Then appropriate amount standard solution of penicillamine or sample solution of penicillamine is added and the solution is diluted to 25 mL and shook up. This mixture reacted for 20 min at 70°C, and cooled back to RT, absorbance is determinated at 511 nm against the reagent blank aftering placing 5 min.

3. Results and discussion

3.1. Absorption spectrum
Fe³⁺ solution (0.50 mL), pH4.0 buffer solution (2.00 mL), penicillamine solution (0.50 mL), phenanthroline solution (1.20 mL) are added. The absorption spectrum of orange red complex formed by Fe²⁺ and phenanthroline is shown in Figure 2. Figure 2 show that the maximum absorption wavelength of orange red complex is at 511 nm. So, 511 nm is chosen for all the following measurements.

3.2. The reaction temperature, reaction time and placing time
Fe³⁺ solution (0.50 mL), pH4.0 buffer solution (2.00 mL), penicillamine solution (0.50 mL), phenanthroline solution (1.20 mL), reaction time (15 min), place time(5 min) are added. The absorbance of reaction temperature (30-90°C) are determined. The results show that the absorbance reaches maximum value and remains constant when the temperature is 65~80°C. Hence, 70°C is chosen.

The amount of Fe³⁺ solution, pH4.0 buffer solution, penicillamine solution and phenanthroline solution, place time remain unchanged, the absorbance of reaction time (5-40 min) are measured at 70°C. It find that the absorbance reaches maximum value and does not change when the reaction time is 20~25 min. So, 20 min is selected.

The dosage of Fe³⁺ solution, pH4.0 buffer solution, penicillamine solution and phenanthroline solution remain unchanged, when reaction temperature is 70°C and reaction time is 20 min, the effect
of the placing time (5-120 min) is studied. The results show that the absorbance remains constant after 5 min. Therefore, 5 min is employed.

3.3. pH buffer solution and the dosage of pH buffer solution

Fe$^{3+}$ solution (0.50 mL), penicillamine solution (0.50 mL), phenanthroline solution (1.20 mL), reaction temperature (70°C), reaction time (20 min), place time (5 min) are added. The effect of pH buffer solution are investigated. The results show that the absorbance achieves maximum and remain almost constant when the pH is 3.6~5.6. So, pH4.0 buffer solutions is used.

Keep the other dosage unchanged, in pH4.0 buffer solutions, the influence of pH4.0 buffer solution amount show that the absorbance is maximum and no more change when pH4.0 buffer solution amount is 2.00 mL~3.00 mL. Therefore, 2.50 mL pH4.0 buffer solution is ensured.

3.4. The dosage of phen solution

The effect of phen solution amount show in Figure 3. We can see that the absorbance reaches larger and basically unchanged when phen solution amount is 1.00 mL~1.40 mL. Hence, 1.20 mL phen solution is chosen.

3.5. The dosage of Fe$^{3+}$ solution

The effect of Fe$^{3+}$ solution amount is explored (Figure 4). Figure 4 show that when Fe$^{3+}$ solution amount is 0.20 mL~0.50 mL, the absorbance reaches maximum and remain essentially unchanged. Thus, 0.30 mL Fe$^{3+}$ solution has been selected.

![Figure 3](image1.png)  Effect of the dosage of Phen
Fe$^{3+}$:0.50 mL; pH4.0 buffer solution:2.50 mL; PA:0.50 mL; reaction temperature:85°C; reaction time:20 min; placing time:5 min.

![Figure 4](image2.png)  Effect of the dosage of Fe$^{3+}$
Phen:1.20mL; pH4.0 buffer solution:2.50 mL; PA:0.50 mL; reaction temperature:85°C; reaction time:20 min; placing time:5 min.

3.6. Calibration curve

Under the selected best conditions, a series of penicillamine solutions are prepared, the absorbance are measured at 511 nm according to the experimental method. In 0.000800~0.01040 mg/mL, the linear relationship between the absorbance and the mass concentration of penicillamine is $A=0.0304+75.886C$ (mg/mL), with a correlation coefficient of 0.9996(figure 5).
3.7. Sample determination

Twelve tablets of penicillamine tablet are taken and weigh 3.1777 g after removing the sugar coating, 2.1980 g penicillamine powder is weighed precisely after grinding carefully and mixing well, then dissolved in 100 mL pH4.6 HAc-NaAc buffer solution and is transferred into a 200 mL volumetric flask and diluted to the mark and mixed well and filtrated, this is a sample solution, standby.

According to 2.2, the content of penicillamine is determined using penicillamine sample solution, and the recovery tests of standard addition are performed. The determination results are corresponding to the results of the pharmacopoeia method, as shown in Table 1.

Table 1. The content of penicillamine in tablet n = 5

| Sample          | Proposed method (mg·tablet⁻¹) | RSD (%) | Pharmacopoeia method[1] (mg·tablet⁻¹) | Added (μg·mL⁻¹) | Recovered (μg·mL⁻¹) | Recovery (%) |
|-----------------|-------------------------------|---------|--------------------------------------|----------------|---------------------|--------------|
| Penicillamine   | 118.2                         | 0.3     | 120.8                                | 0.8160         | 0.7987              | 97.9         |

Table 1 shows that the penicillamine content in penicillamine tablet is 118.2 mg·tablet⁻¹, the recovery yields are 97.92%~99.50%, and the result is 120.8 mg·tablet⁻¹ by pharmacopoeial method.

4. Conclusion

A new method for the indirect determination of penicillamine using Fe(II)-phenanthroline spectrophotometry has been established. The method is applied to the determination of penicillamine in tablets, and the results agree well with pharmacopoeial method. It is indicated that penicillamine content in pharmaceutical sample can be accurately determined by Fe(II)-phenanthroline spectrophotometry.

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