

Spectrophotometric Determination of Penicillamine in Pharmaceutical Sample using Fe(III)-Tiron System

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Abstract—Hydrosulfuryl(-SH) in penicillamine molecule can reduce Fe(III) to Fe(II), then using tiron as chromogenic reagent of Fe(III), and the content of penicillamine is determined indirectly through determining the surplus content of Fe(III) in the system. An accurate fast spectrophotometric method for the determination of penicillamine by discoloration spectrophotometry using Fe(III)-tiron system has been established. The various effect factors on the determination of penicillamine using Fe(III)-tiron system have been investigated in detail. The results show that when the reaction temperature was 85°C, the reaction time was 20 min, the dosage of pH3.0 buffer solution was 15.00mL, the dosage of Fe(III) was 2.30mL, the dosage of tiron was 1.00mL, the maximum absorption wavelength of the complex of Fe(III)-tiron was 660 nm, good linear relationship was obtained between ΔA and the concentration of penicillamine in the range of 0.008000~0.04800 mg mL⁻¹, the equation of the linear regression was $\Delta A=0.0074+7.575C(\text{mg mL}^{-1})$, with a linear correlation coefficient was 0.9993. This proposed method had been successfully applied to determine of penicillamine in real pharmaceutical.

Keywords-penicillamine; Fe(III); tiron; discoloration spectrophotometry

I. INTRODUCTION

Penicillamine (PA, the molecular structure is shown in Fig. 1) is a sulfur-containing amino acid. Penicillamine is used in the treatment of rheumatoid arthritis, Wilson's disease, primary biliary cirrhosis, fibrotic lung disease, cystinuria and certain toxic metal poisoning. It is of great importance and significance for life science.

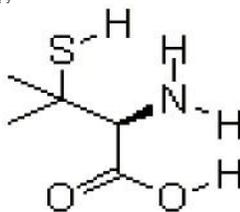


Figure 1. The molecular structure of penicillamine

Up till now, various different methods have already been applied for the determination of penicillamine, such as

spectrophotometry [1, 2], fluorescence spectrophotometry [3, 4], flow injection analysis [5, 6], voltammetry [7, 8], capillary electrophoresis [9], HPLC method [10], HPLC-UV method [11], LC-UV method [12], chemiluminescence-LC method [13], electrochemical analysis [14], etc. However, most of the methods mentioned above need either complicated and expensive equipment or tedious procedures. These problems limit the practical application of these methods, and make them inapplicable in common laboratory. Therefore, it is essential and significant to develop a simple, accurate, rapid and sensitive method for the determination of penicillamine in clinical analysis and drug quality control.

In this paper, a novel method for the indirect determination of penicillamine by discoloration spectrophotometry using Fe(III)-tiron system has been established. The various effect factors on the determination of penicillamine were investigated in detail. The results showed that by controlling pH=3.0, hydrosulfuryl (-SH) in penicillamine molecule can reduce Fe(III) to Fe(II), then using tiron as chromogenic reagent of Fe(III), and the content of penicillamine was determined indirectly through determining the surplus content of Fe(III) in the system. The maximum absorption wavelength of the complex of Fe(III)-tiron was 660 nm, good linear relationship was obtained between ΔA and the concentration of penicillamine in the range of 0.008000~0.04800 mg mL⁻¹, the equation of the linear regression was $\Delta A=0.0074+7.575C(\text{mg mL}^{-1})$, with a linear correlation coefficient was 0.9993. This proposed method had been applied to determine of penicillamine in real pharmaceutical, and the results agreed well with those obtained by pharmacopoeial method. This proposed method has the advantages of simply, rapidness, convenience, low analytical cost and so on.

II. EXPERIMENT

A. Apparatus and Reagents

A model 723S spectrophotometer (Shanghai Precision & Scientific Instrument Co., Ltd) was used for photometric measurements. A model UV-2401 UV-visible spectrophotometer (The Shimadzu Corporation, Japan) was used for scanning the absorption spectrum.

A stock of standard solution of 1.000 g L⁻¹ penicillamine

was prepared by dissolving 0.1000 g of penicillamine in 100mL with bidistilled water and stored at 4 °C in dark place, a working standard solution was prepared by appropriately diluting the stock standard solution. A stock of standard solution of 0.2500 g L⁻¹ Fe³⁺ was prepared by dissolving 1.0793 g NH₄Fe (SO₄)₂ · 12H₂O in 200mL bidistilled water, 4.0mL 3 mol L⁻¹ H₂SO₄ was added, and then diluting it to 500mL with bidistilled water. A stock of standard solution of 6.250 g L⁻¹ tiron was prepared by dissolving 0.6250 g tiron in 100mL with distilled water. Buffer solutions of different pH were prepared as references [15].

All reagents were of analytical reagent grade. Bidistilled water was used throughout.

B. Method

Take two 25mL volumetric flask, a suitable amount of 0.1000 g L⁻¹ penicillamine solution was transferred into one of the two 25mL volumetric flask. Then 2.30mL of 0.2500 g L⁻¹ Fe³⁺ solution, 1.00mL of 6.250 g L⁻¹ tiron solution and 15.00mL of pH=3.0 buffer solution were added into the two 25mL volumetric flask, the solution was diluted to the mark with bidistilled water and mixed well. After these mixture reacted for 20 min at 85 °C in water bath and cooled back to room temperature, the absorbance(Δ A) of the blank solution(Fe³⁺+tiron) was measured at 660 nm against the determination solution(Fe³⁺+ penicillamine +tiron).

III. RESULTS AND DISCUSSIONS

A. Absorption Spectrum

According to the experimental method, in the range of 500~750 nm, the absorption spectrum of the blank solution (Fe³⁺+tiron) and the determination solution (Fe³⁺+penicillamine+tiron) are shown in Fig. 2. It can be seen that the maximum absorption wavelength of the complex of Fe(III)-tiron is at 660 nm. Therefore, measurement wavelength was chosen as 660 nm.

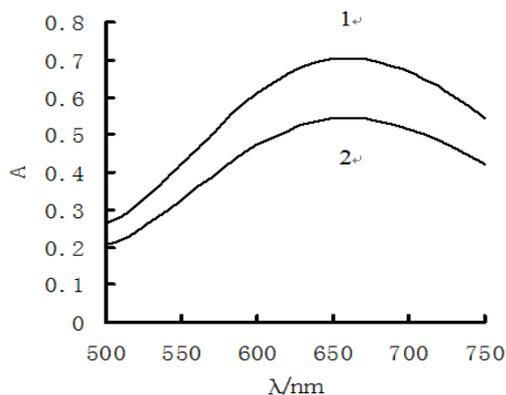


Figure 2. Absorption spectrum
1- Fe³⁺+tiron, 2- Fe³⁺+ penicillamine +tiron

B. Effects of Reaction Temperature and Time

The effect of temperature on absorbance (Δ A) was studied. The results showed that the absorbance (Δ A) reached its maximum and remained constant when the temperature was 85~90 °C. Hence, 85 °C was selected for all further studies.

The absorbance (Δ A) of different reaction time (5, 10, 15, 20, 25, 30, 35, 40, 45, 50 min) was measured at 85 °C. The results showed that the absorbance (Δ A) gets to its maximum and does not change when the time was 15~35min. Therefore, 20 min of reaction time was chosen for further experiments.

C. Effects of Ph and The Dosage of Buffer Solution

The effects of pH on absorbance (Δ A) were investigated. The results showed that the absorbance (Δ A) are maximal and remain constant when the pH was 2.8~3.0. Hence, pH3.0 buffer solutions was chosen and used for further studies.

The experimental results of the influence of the dosage of pH3.0 buffer solution showed that the absorbance (Δ A) reached maximal and does not change when the dosage of pH3.0 buffer solution is 12.50mL~15.00mL. Therefore, 15.00mL of pH3.0 buffer solution was chosen.

D. Effects of The Dosage of Fe(III)

The dosage of Fe(III) is regarded as an important factor. The effect of the dosage of Fe(III) on absorbance(Δ A) was studied(Fig. 3). As shown in Fig. 3, absorbance (Δ A) reached maximal and kept constant when the dosage of Fe(III) was 2.30~2.50mL. Hence, 2.30mL of the dosage of Fe(III) was chosen.

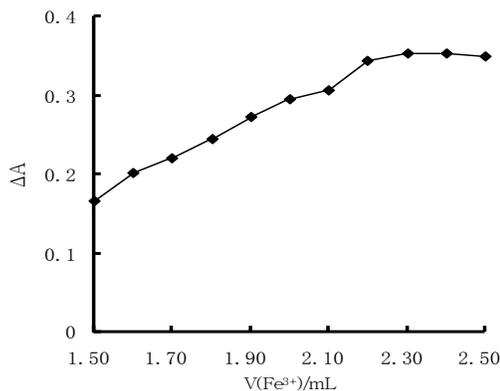


Figure 3. Effect of the dosage of Fe(III)

E. Effects of The Dosage of Tiron

The effect of the dosage of tiron on absorbance (Δ A) can be seen in Fig. 4. It is found from Fig. 4 that the absorbance (Δ A) reaches its maximum and keeps constant when the dosage of tiron was 0.80mL~1.60mL. Therefore, 1.00mL of the dosage of tiron was chosen in the subsequent studies.

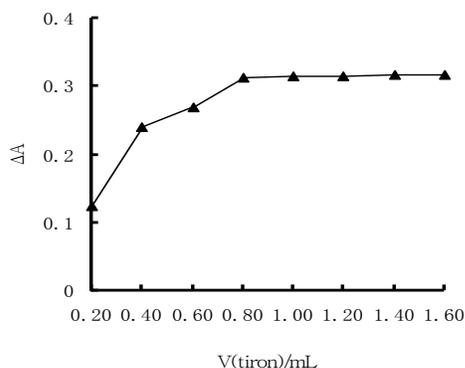


Figure 4. Effect of the dosage of tiron

F. Interference of Coexisting Components

A systematic study of the influence of excipients, carbohydrate and minerals on the determination of penicillamine was carried out. The tolerance levels were defined as standard deviation less than $\pm 5\%$ within analytical determination. The conclusion is drawn from the following: 12mg mL^{-1} glucose, sucrose, lactin, starch; 20mg mL^{-1} K^+ , Na^+ , Ca^{2+} , Mg^{2+} , Zn^{2+} , SO_4^{2-} , CO_3^{2-} , NO_3^- and Cl^- do not affect the determination.

G. Calibration Curve

Under the selected conditions, a linear relationship between absorbance (ΔA) and the concentration (C) of penicillamine was obtained in the range of $0.008000\sim 0.04800\text{ mg mL}^{-1}$ (Fig. 5). The linear regression equation is $\Delta A = 0.0074 + 7.575C$ (mg mL^{-1}), with a correlation coefficient of 0.9993.

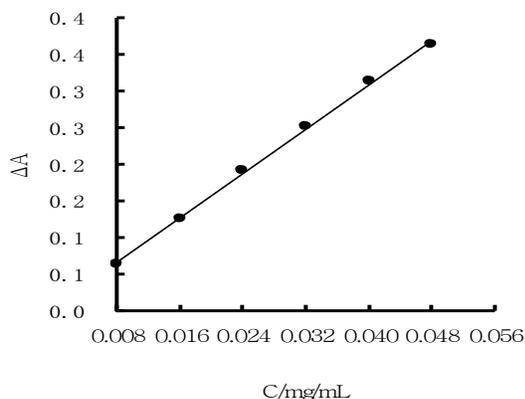


Figure 5. Calibration curve

H. Determination of Penicillamine in Pharmaceutical Sample

The proposed method was applied to the determination of penicillamine in penicillamine tablet. Meanwhile, the recovery tests of standard addition were performed. The

result obtained was compared with those obtained by pharmacopoeia method, as show in Table I.

TABLE I. THE DETERMINATION RESULT OF PENICILLAMINE IN PENICILLAMINE TABLET N = 5

Sample	Penicillamine tablet
Proposed method (mg tablet^{-1})	119.8
RSD (%)	0.3
Pharmacopoeia method[16] (mg tablet^{-1})	121.3
Added ($\mu\text{g}\cdot\text{mL}^{-1}$)	12.00
	20.00
Recovered ($\mu\text{g}\cdot\text{mL}^{-1}$)	11.96
	19.51
Recovery (%)	99.7
	97.6

Table I shows that the content of penicillamine in penicillamine tablet is $119.8\text{ mg tablet}^{-1}$ by this proposed method, and the content of penicillamine in penicillamine tablet is $121.3\text{ mg tablet}^{-1}$ by pharmacopoeial method. Obviously, the result of this proposed method agreed well with those obtained by pharmacopoeial method. It is indicated that the content of penicillamine in pharmaceutical sample can be accurately determined by discoloration spectrophotometry using Fe(III)-tiron system.

IV. CONCLUSION

In this paper, a novel method for the indirect determination of penicillamine by discoloration spectrophotometry using Fe(III)-tiron system was reported. The proposed method has been successfully used for the determination of penicillamine in penicillamine tablet, and the results agreed well with pharmacopoeia method, and the recoveries of standard addition were $97.6\%\sim 99.7\%$. It is obvious that the determination of penicillamine by discoloration spectrophotometry using Fe(III)-tiron system has certain practical significance and foreground of application.

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