Spectrophotometric Determination of Tiopronin using 2,2’-Bipyridyl as Chromogenic Reagent

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Abstract. The hydrosulfuryl(-SH) in tiopronin molecule has reducibility, and it can reduce Fe3+ to Fe2+. We use 2,2’-bipyridyl as chromogenic reagent of Fe2+. Fe2+ react with 2,2’-bipyridyl to form the orange red complex which the maximum absorption is at 522 nm. By measuring the absorbance of the orange red complex, the content of tiopronin can be determined. A new method for the spectrophotometric determination of tiopronin has been established. The content of tiopronin is determined using this method, and the results are satisfactory.

1. Introduction
Tiopronin(TPN, Figure 1) is a glycine derivative containing reductive sulfhydryl group and widely used in the clinical treatment of liver diseases. So far, the Chinese Pharmacopoeia used the titration method to determine the content of TPN. In addition, spectrophotometry, flow injection analysis, LC-UV, chemiluminescence, Electrochemical analysis have been used to determine the content of TPN.

Figure 1. The molecular structure of tiopronin

The hydrosulfuryl(-SH) in TPN molecule has reducibility, and Fe3+ can be reduced to Fe2+ by hydrosulfuryl(-SH), Fe2+ react with the chromogenic reagent of 2,2’-bipyridyl to form the orange red complex which the maximum absorption is at 522 nm. By measuring the absorbance of the orange red complex, the content of TPN can be determined. A new method for the spectrophotometric determination of TPN has been established. The results show that in 0.0004000-0.004000 mg/mL, the relationship between absorbance of the orange red complex and mass concentration of TPN is A=0.0057+44.652C (mg/mL). The content of TPN is determined using this method, and the results are in good agreement with the standard method.

2. Experimental
2.1. Equipment and reagents
UV-2401 UV-visible spectrophotometer; 723S spectrophotometer.

TPN standard solution: 0.1000 mg/mL, stored at 4°C in dark place; Fe³⁺ solution: 02000 mg/mL; 2,2-bipyridyl (BPY) solution: 0.02000 mg/mL. Buffer solutions of different pH was Prepared as references 8.

Analytical reagent grade reagents are used. Bidistilled water is used.

2.2. Method
Fe³⁺ solution (1.80 mL), bidistilled water (10.00 mL) and a given amounts of TPN solution or TPN sample solution are added into a 25 mL comparison tube, mixed well. Aftering this mixture reacted for 10 min at room temperature, 0.40 mL of BPY solution and 4.00 mL pH=5.0 buffer solution are added. The solution is diluted to 25 mL and shaked up, then the absorbance is measured at 522 nm against the reagent blank (prepared in the same way).

Figure 2. Absorption spectrum

3. Results and discussion

3.1. The optimal conditions

3.1.1. Absorption spectrum
The absorption spectrum of the orange red complex of Fe(II)-Byp is shown in Figure.1. It show that the orange red complex of Fe(II)-Byp has an absorption peak at 522 nm. So, 522 nm is selected.

3.1.2. Effects of the reaction temperature and reaction time
According to the experimental method, Fe³⁺ solution (1.50 mL), bidistilled water (10.00 mL), BPY solution(1.00 mL) and TPN solution (1.00 mL) are added. The effect of the reaction temperature is studied. The results show that the absorbance is not affected by the reaction temperature. So, room temperature is selected.

Keep other conditions unchanged and the reaction temperature is room temperature, the effect of the reaction time indicate that the absorbance remains constant when the reaction time is 5~80 min. So, 10 min is chosen.

Figure 2. Absorption spectrum

3.1.3. pH buffer solution
The experimental results from figure 3 show that the absorbance achieves maximum and no longer change when pH buffer solution is 4.8~5.6. Therefore, pH 5.0 buffer solution is used.
3.1.4. The amount of pH 5.0 buffer solution
The effect of the dosage of pH buffer solution on absorbance is investigated (Figure 4). The results show that the absorbance run up to maximum and basically unchanged when the amount of pH buffer solution is 3.00 mL ~ 8.00 mL. Hence, 4.00 mL pH 5.0 buffer solution is ensured.

![Figure 3](image1.png)  
**Figure 3** Effect of the pH buffer amount of solution Fe³⁺: 1.50 mL; TPN: 1.00 mL; BPY: 1.00 mL; reaction time: 5 min.

![Figure 4](image2.png)  
**Figure 4** Effect of the pH 5.0 buffer solution Fe³⁺: 1.50 mL; TPN: 1.00 mL; BPY: 1.00 mL; reaction time: 5 min.

3.1.5. The amount of BPY solution
Figure 5 show that in the range of 0.20 ~ 1.80 mL, the absorbance is basically constant, or the absorbance is not affected by the amount of the BPY solution. Thus, 0.40 mL is used.

3.1.6. The amount of Fe³⁺ solution
It can be seen form Figure 6 that the absorbance is basic stability when the amount of Fe³⁺ solution is 1.60 ~ 2.60 mL. So, 1.80 mL Fe³⁺ solution is selected.

![Figure 5](image3.png)  
**Figure 5** Effect of the amount of BPYFe³⁺: 1.50 mL; TPN: 1.00 mL; pH 5.0 buffer solution: 4.00 mL; reaction time: 5 min.

![Figure 6](image4.png)  
**Figure 6** Effect of the amount of Fe³⁺TPN: 1.00 mL; BPY: 0.40 mL; pH 5.0 buffer solution: 4.00 mL; reaction time: 5 min.
3.2. Calibration curve
According to the experimental method, take the mass concentration of tiopronin as the transverse coordinate and the absorbance as the longitudinal coordinate, the standard curve is drew (figure 7). In the range of 0.0004000-0.004000 mg/mL, the standard curve equation is $A=0.0057+44.652C$ (mg/mL) with linear correlation coefficient is 0.9993.

![Figure 7 Calibration curve Fe$^{3+}$:1.80 mL; BPY:0.40 mL; pH 5.0 buffer solution :4.00 mL; reaction time: 5 min.](image)

3.3. Determination the content of tiopronin
After removing the sugar coating, round and blended, 12 tablets of tiopronin tablet are weighed 3.1635g. 2.2505 g powder of tiopronin is weighed precisely and dissolved, then is transferred into a 250 mL volumetric flask and is diluted to 250.0 mL and mixed well, this is tiopronin sample solution.

The solution is preserved from light at 4℃.

According to the experimental method, the content of tiopronin is determined by tiopronin sample solution. Meanwhile, the standard addition recovery experiment and standard method experiment are performed. The results can be seen in Table 1.

| Sample          | By proposed method (mg·tablet$^{-1}$) | RSD (%) | By standard method[1] (mg·tablet$^{-1}$) | Added (μg·mL$^{-1}$) | Recovered (μg·mL$^{-1}$) | Recovery (%) |
|-----------------|--------------------------------------|---------|----------------------------------------|----------------------|--------------------------|--------------|
| Tiopronin tablet| 91.40                                 | 0.7     | 90.57                                  | 0.8000               | 0.7928                   | 99.1         |

It can be seen that the content of tiopronin is 91.40 mg·tablet$^{-1}$ by proposed method, and the result is 90.57 mg·tablet$^{-1}$ by standard method. The recovery yields are 99.1%~101.5%.

4. Conclusion
A new method for the determination of tiopronin has been established. The content of tiopronin is 91.40 mg·tablet$^{-1}$ by this method, and the result is 90.57 mg·tablet$^{-1}$ by standard method. It can be seen that the results are consistent. Obviously, this method has certain significance and application prospect.
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