Determination of Tiopronin using Potassium Bichromate Discoloring Spectrophotometry

Xinrong Wen* and Changqing Tu
College of Chemistry and Environment, Jiaying University, Meizhou, Guangdong 514015, P. R. China
Corresponding author's e-mail: 198601012@jyu.edu.cn

Abstract. A new method for the determination of tiopronin by potassium dichromate discoloring spectrophotometry is established. The optimal conditions for the discoloring spectrophotometric determination of tiopronin by potassium bichromate are discussed. In the sulfuric acid medium, potassium bichromate can oxidize tiopronin, and its absorbance decreases with the increase of tiopronin concentration. The decrease value of absorbance is linearly related to the concentration of tiopronin, and the content of tiopronin can be indirectly determined by measuring the decrease value of absorbance. Within the scope of 0.02000-0.2000 mg/mL, the equation of linear regression is \(\Delta A = -0.005 + 1.96C\) (mg/mL), and linear correlation coefficient is 0.9991. This method is used to the determination of tiopronin in tiopronin tablets, and the results consistent with standard method.

1. Introduction
Tiopronin(Figure 1) is a glycine Derivative containing sulfhydryl group, and it is mainly used in the treatment of hepatic disorders, cystinuria, rheumatoid, arthritis and early senile cataract, etc. So, the determination of tiopronin is of great significance for life science. So far, flow injection analysis [1], liquid chromatography [2], HPLC[3], chemiluminescence method [4], Fluorometric assay [5], LC-ESI-MS [6] have been reported for the determination of tiopronin.

Figure 1. The molecular structure of tiopronin

In the sulfuric acid medium, the hydrosulfuryl (-SH) in tiopronin molecule can be oxidized by potassium bichromate, and it results in the absorbance decreases of potassium bichromate. Beer's law is obeyed between the mass concentration of tiopronin and the decrease value of absorbance in a
certain range of concentration, the content of tiopronin can be indirectly determined by measuring the decrease value of absorbance. The results show that when the dosage of 0.6404 mg/mL $K_2Cr_2O_7$ solution is 5.00 mL, the dosage of 3 mol/L $H_2SO_4$ solution is 2.20 mL, the dosage of 1.000 mg/mL tiopronin solution is 1.00 mL, reaction temperature is 85℃, reaction time is 25 min and placing time is 10 min, the decrease value of absorbance of potassium bichromate is linearly related to the concentration of tiopronin within the scope of 0.02000-0.2000 mg/mL, the equation of linear regression is $\Delta A = -0.005 + 1.96C$ (mg/mL), and linear correlation coefficient is 0.9991. This method is used to the determination of tiopronin in tiopronin tablets, and the results consistent with standard method. The recovery yields are 97.92%~99.50%.

2. Experimental

2.1 Equipment and reagents

UV-2401 UV-visible spectrophotometer; 723S spectrophotometer.

$K_2Cr_2O_7$ solution: 0.6404 mg/mL; Tiopronin standard solution: 1.000 mg/mL; $H_2SO_4$ solution: 3 mol/L.

2.2 Method

Take two 10 mL comparison tubes, 5.00 mL of 0.6404 mg/mL $K_2Cr_2O_7$ solution, 2.20 mL of 3 mol/L $H_2SO_4$ solution are transferred into the two 10 mL comparison tubes. Then 1.00 mL of 1.000 mg/mL tiopronin standard solution is added into one of the two 10 mL comparison tubes, the solution was diluted to 10.00 mL with distilled water and shaken up. After these solution reacted for 10 min at 80℃ in water bath and cooled back to room temperature, the absorbance($A_1$) of the blank solution ($K_2Cr_2O_7$+$H_2SO_4$) and the absorbance($A_2$) of the determination solution($K_2Cr_2O_7$+$H_2SO_4$+tiopronin) are measured at 437.5 nm against water after placing 10 min, the $\triangle A(A_1-A_2)$ is calculated.

3. Results and discussion

3.1. Selection of the optimal conditions

3.1.1. Absorption spectrum

When the dosage of 0.6404 mg/mL $K_2Cr_2O_7$ solution is 5.00 mL, 3 mol/L $H_2SO_4$ solution is 1.00 mL, 1.000 mg/mL tiopronin solution is 1.00 mL, according to the experimental method, the absorption spectrum of the blank solution($K_2Cr_2O_7$+$H_2SO_4$) and the determination solution($K_2Cr_2O_7$+$H_2SO_4$+tiopronin) in the range of 400～550nm are shown in Figure 2. It can be seen that the maximum absorption wavelength is at 437.5 nm. Therefore, 437.5 nm is used.
3.1.2. Effects of the reaction temperature, reaction time and placing time

0.6404 mg/mL K₂Cr₂O₇ solution (5.00 mL), 3 mol/L H₂SO₄ solution (1.00 mL), 1.000 mg/mL tiopronin solution (1.00 mL) are added. The absorbance (ΔA) of reaction temperature (25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 °C) is measured after the mixture react for 20 min. The results show that the ΔA reaches larger value and remains unchanged when the temperature is 80 ~ 90°C. Hence, 85°C is chosen.

The dosage of K₂Cr₂O₇ solution, H₂SO₄ solution and tiopronin solution remain unchanged, the absorbance (ΔA) of reaction time (5, 10, 15, 20, 25, 30, 35, 40, 45 min) is determinated at 85°C. It is found that the ΔA of solution reaches maximum value and no longer change when the reaction time is 25 ~ 45 min. So, 25 min is selected.

The dosage of K₂Cr₂O₇ solution, H₂SO₄ solution and tiopronin solution remain unchanged, when reaction temperature is 85 °C and reaction time is 25 min, the placing time (5, 10, 15, 20, 25, 30, 40, 50, 60, 90, 120 min) is studied. The absorbance (ΔA) remains constant when the placing time is 5 ~ 30 min. Hence, 10 min is employed.

3.1.3. The dosage of H₂SO₄ solution

The experimental results can be seen in Figure 3. The results show that the ΔA reaches its maximum and keep basically constant when H₂SO₄ solution is 2.20 mL ~ 2.40 mL. Therefore, 2.20 mL H₂SO₄ solution is chosen.

3.1.4. The dosage of K₂Cr₂O₇ solution

The effect of the dosage of K₂Cr₂O₇ solution on absorbance (ΔA) is investigated (Figure 4). Figure 4 show that the ΔA reaches its maximum and maintain a basic constant when K₂Cr₂O₇ solution is 4.50 mL ~ 5.00 mL. So, 5.00 mL K₂Cr₂O₇ solution is selected.

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Figure 2. Absorption spectrum
1-the blank solution (K₂Cr₂O₇+H₂SO₄);
2- the determination solution (K₂Cr₂O₇+H₂SO₄+tiopronin)

3.1.2. Effects of the reaction temperature, reaction time and placing time

0.6404 mg/mL K₂Cr₂O₇ solution (5.00 mL), 3 mol/L H₂SO₄ solution (1.00 mL), 1.000 mg/mL tiopronin solution (1.00 mL) are added. The absorbance (ΔA) of reaction temperature (25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 °C) is measured after the mixture react for 20 min. The results show that the ΔA reaches larger value and remains unchanged when the temperature is 80 ~ 90°C. Hence, 85°C is chosen.

The dosage of K₂Cr₂O₇ solution, H₂SO₄ solution and tiopronin solution remain unchanged, the absorbance (ΔA) of reaction time (5, 10, 15, 20, 25, 30, 35, 40, 45 min) is determinated at 85°C. It is found that the ΔA of solution reaches maximum value and no longer change when the reaction time is 25 ~ 45 min. So, 25 min is selected.

The dosage of K₂Cr₂O₇ solution, H₂SO₄ solution and tiopronin solution remain unchanged, when reaction temperature is 85 °C and reaction time is 25 min, the placing time (5, 10, 15, 20, 25, 30, 40, 50, 60, 90, 120 min) is studied. The absorbance (ΔA) remains constant when the placing time is 5 ~ 30 min. Hence, 10 min is employed.

3.1.3. The dosage of H₂SO₄ solution

The experimental results can be seen in Figure 3. The results show that the ΔA reaches its maximum and keep basically constant when H₂SO₄ solution is 2.20 mL ~ 2.40 mL. Therefore, 2.20 mL H₂SO₄ solution is chosen.

3.1.4. The dosage of K₂Cr₂O₇ solution

The effect of the dosage of K₂Cr₂O₇ solution on absorbance (ΔA) is investigated (Figure 4). Figure 4 show that the ΔA reaches its maximum and maintain a basic constant when K₂Cr₂O₇ solution is 4.50 mL ~ 5.00 mL. So, 5.00 mL K₂Cr₂O₇ solution is selected.
Figure 3  Effect of the dosage of sulfuric acid
K$_2$Cr$_2$O$_7$:5.00mL; tiopronin:1.00mL; reaction temperature:85 ℃; reaction time:25 min; placing time:10 min.

Figure 4  Effect of the dosage of potassium dichromate
H$_2$SO$_4$:2.20mL; tiopronin:1.00mL; reaction temperature:85 ℃; reaction time:25 min; placing time:10 min.

3.2. Calibration curve
Under the selected conditions, a series of standard solutions of tiopronin is prepared, then according to the experimental method, the absorbance(ΔA) is measured at 437.5 nm within the scope of 0.02000-0.2000 mg/mL (Figure 5). The equation of linear regression is ΔA=-0.005+1.96C (mg/mL), and linear correlation coefficient is 0.9991.

Figure 5  Calibration curve
K$_2$Cr$_2$O$_7$:5.00mL; H$_2$SO$_4$:2.20mL; tiopronin:1.00mL; reaction temperature:85 ℃; reaction time:25 min; placing time:10 min.
3.3. Determination of tiopronin in pharmaceutical sample

Forty tablets of tiopronin tablet are weighed 3.9595g after removing the sugar coating, round and blended. 2.4009 g powder of tiopronin is weighed precisely and dissolved and is transferred into a 250 mL volumetric flask, the solution is diluted to 250.0 mL with bidistilled water and mixed well, this is a sample solution, backup. The solution is preserved at 4 ℃, shielding from light.

When 0.6404 mg/mL K$_2$Cr$_2$O$_7$ solution is 5.00 mL, 3 mol/L H$_2$SO$_4$ solution is 2.20 mL, tiopronin sample solution is 0.30 mL, reaction temperature is 80 ℃, reaction time is 10min and placing time is 10min, the prepared solution of tiopronin sample is determined. The content of tiopronin in tiopronin tablet can be obtained. Meanwhile, the standard-adding recovery experiment and pharmacopoeia method experiment are performed. The results show in Table 1.

Table 1. The determination result of tiopronin in tiopronin tablet

| Sample     | Proposed method (mg·tablet$^{-1}$) | RSD (%) | Standard method[7] (mg·tablet$^{-1}$) | Added (μg·mL$^{-1}$) | Recovered (μg·mL$^{-1}$) | Recovery (%) |
|------------|------------------------------------|---------|--------------------------------------|----------------------|--------------------------|--------------|
| Tiopronin tablet | 98.46                             | 0.3     | 98.33                                | 8.00                 | 7.96                     | 99.50        |

Table 1 shows that the content of tiopronin in tiopronin tablet is 98.46 mg·tablet$^{-1}$ by this proposed method, agreed well with 98.33 mg·tablet$^{-1}$ obtain by pharmacopoeial method. The recovery yields are 97.92%~99.50%, and the RSD is 0.3%.

4. Conclusion

A new method for the determination of tiopronin by potassium dichromate discoloring spectrophotometry has been established. This method has been successfully used to the determination of tiopronin in pharmaceutical sample, and the results agree well with standard method. It is clear that the determination of tiopronin by potassium dichromate discoloring spectrophotometry has certain significance and application prospect.

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