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Spectrophotometric Determination of Tiopronin in Pharmaceutical Samples by Phosphorus Molybdenum Blue

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A novel method is established for spectrophotometric determination of tiopronin by phosphorus molybdenum blue. It is based on the fact that PO_4^{3-} reacts with $Mo_7O_{24}^{6-}$ in 0.47 mol/L H_2SO_4 solution to form a product of phosphorus-molybdenum heteropoly acid ($[H_2PMo_{12}O_{40}]^-$) which is reduced to phosphorus molybdenum blue ($H_3PO_4{\cdot}10MoO_3{\cdot}Mo_2O_5$) by tiopronin. The absorbance of phosphorus molybdenum blue is measured at the absorption maximum of 730 nm and the amount of tiopronin can be determined based on this absorbance. Absorbance is linear with the concentration of tiopronin in the range of 1.6-40 μg/mL and the regression equation is A = -0.1193 + 0.01927c (μg/mL) with a correlation coefficient of 0.9989 and the apparent molar absorption coefficient of 3.14×10^3 L/(mol cm). The detection limit and the RSD are 1 μg/mL and 0.92 %, respectively. Thus, the method is validated and commonly available to determine tiopronin in pharmaceutical samples.

Keywords: Phosphorus molybdenum blue, Tiopronin, Spectrophotometry.

INTRODUCTION

Tiopronin *i.e.*, N-(2-mercaptopropionyl)-glycine (TP), a synthetic thiol compound, has been used as a hepatoprotective agent, an antidote to heavy metal poisoning and a radioprotective agent. It has also been successfully applied to prevent kidney injury and to treat cystinuria and rheumatoid arthritis[1,2]. However, tiopronin has a relatively high frequency of side effects, such as the loss of taste or stomach upset.

The thiol group can be easily oxidized to disulfides either as a dimer or mixed forms with endogenous thiols. Thus, quantitative analysis of the tiopronin can be almost impossible, unless the thiol group can be stabilized in pharmaceutical sample. Up to now, several methods have been reported for the determination of tiopronin such as gas chromatography-mass spectrometry (GC-MS) method[3], chemiluminescent method[4], LC-MS-MS methods[2], HPLC-ESI-MS methods[1,5], fluorimetric methods[6,7] and electrochemical oxidation by amperometric flow injection analysis[8].

However, some laborious sample processing, expensive apparatus and complex derivation steps are involved in all of the above mentioned methods. So, it is necessary for laboratory to generate a commonly available method for the determination of tiopronin in pharmaceutical sample.

From the viewpoint of application, visible spectrophotometry has some advantages, for example, its equipment is simple and its operation is easy. In this paper, we have developed a novel method named phosphorus molybdenum blue spectrophotometry for the determination of tiopronin in pharmaceutical samples. Phosphorus molybdenum blue, mainly used for the determination of phosphorus in the sample[9-11], has not been reported for the determination of tiopronin. The experiment indicates that PO_4^{3-} reacts with $Mo_7O_{24}^{6-}$ in 0.30 mol/L H_2SO_4 solution to form a product with phosphorus-molybdenum heteropoly acid ($[H_2PMo_{12}O_{40}]^-$) which is reduced to phosphorus molybdenum blue ($H_3PO_4{\cdot}10MoO_3{\cdot}Mo_2O_5$) by tiopronin. The absorbance of phosphorus molybdenum blue is measured at the absorption maximum of 730 nm and the amount of tiopronin can be calculated based on this absorbance. In contrast with the above methods, this method is simple, fast and does not require expensive equipment and facilities. With the accredited specificity, lower limit of quantification, accuracy and precision, the method could be used to determine tiopronin in the pharmaceutical sample.

EXPERIMENTAL

The main solutions were prepared as follows. A standard solution of 1.000 g/L tiopronin (Xinyi Medicine Plant,

Xinxiang China) was prepared and preserved at 4 ºC without light. A 14.13 mg/mL stock Mo(VI) solution was prepared by dissolving 26 g of ammonium molybdate ($(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ A.R., Shanghai colloid chemical plant, Shanghai, China) and mixing with 460 mL of 1:1 (v/v) H_2SO_4 (Luoyang Haohua Chemical Reagent Co., Ltd. Luoyang, China), then transferred into a 1000 mL standard flask and was diluted to the mark by using distilled water. The concentration of H_2SO_4 is 4.23 mol/L in the stock solution. A 1 mg/mL stock PO_4^{3-} solution was obtained by dissolving 1.4329 g g of potassium dihydrogen phosphate (A.R., Beijing red star Chemical Plant, Beijing, China) in 1000 mL standard flask with distilled water. Unless specially stated, all reagents used were of analytical grade and all solutions were prepared with distilled water.

A model T6 UV/VIS spectrophotometer (Beijing purkinje general instrument Co., Beijing, China) was employed for scanning the absorption spectrum, in addition, a 722 grating spectrophotometer (Xiamen Analytical Instruments Plant, Xiamen, China) for photometric measurements and a model CS501 super constant temperature instrument (Chongqing Experiment Equipment Plant, Chongqing, China) for temperature control.

Procedure: At first, 3 mL of 1 mg/mL PO_4^{3-} was added into a 12.5 mL colour comparison tube and diluted to two-thirds of the mark by using distilled water. Secondly, it was transferred by 1.40 mL of 14.13 mg/mL Mo(VI) and 1 mL of 200 μg/mL tiopronin, respectively, then allow to shaking well after the solution was diluted to the mark with distilled water and the concentration of H_2SO_4 is 0.47 mol/L in the reaction solution. Finally, after standing for 40 min at 40 ºC in water bath, the absorbance was measured at 730 nm against a reagent blank prepared in the same way without tiopronin.

RESULTS AND DISCUSSION

Discussion of reaction mechanism: PO_4^{3-} reacts with $Mo_7O_{24}^{6-}$ to form a product with $[H_2PMo_{12}O_{40}]^-$. Subsequently, $[H_2PMo_{12}O_{40}]^-$ is reduced to phosphorus molybdenum blue ($H_3PO_4\cdot 10MoO_3\cdot Mo_2O_5$) by tiopronin due to the reducibility of the sulfhydryl group and tiopronin is oxidized to form disulfide[12].

The continuous variation method of equivalent mole was used to determine the reaction stoichiometric ratio of $[H_2PMo_{12}O_{40}]^-$ and tiopronin. Keeping the total amount of tiopronin (V_D) and $[H_2PMo_{12}O_{40}]^-$(V_R) constant ($V_R + V_D$ = 5 mL), whose concentration were both 1.23×10^{-3} mol/L, different amounts of tiopronin and $[H_2PMo_{12}O_{40}]^-$ were transferred into a 12.5 mL colour comparison tube and diluted to the mark with distilled water. Then the absorbance of every solution was measured. Absorbance had been plotted as function of the $V_D/(V_R + V_D)$ ratio (*i.e.*, mole fraction) (Fig. 1). According to the intersection of two tangents, 2:1 of the reaction stoichiometric ratio of tiopronin and $[H_2PMo_{12}O_{40}]^-$ was obtained.

Based on the continuous variation method of equivalent mole, the reaction stoichiometric ratio of tiopronin and $[H_2PMo_{12}O_{40}]^-$ is 2:1, which is consistent with the number of electronic transfer from the oxidation-reduction reaction of tiopronin and $[H_2PMo_{12}O_{40}]^-$. Therefore, it seems to be reasonable that reaction mechanism is as follow:

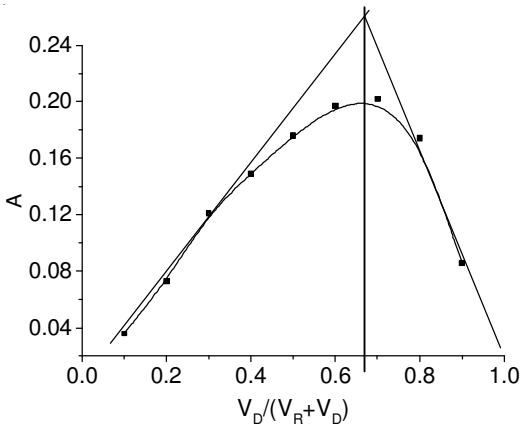
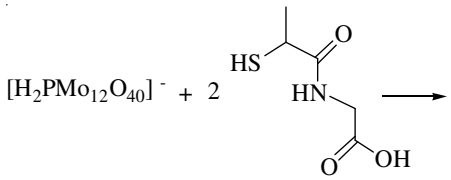
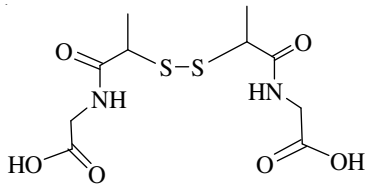


Fig. 1. Determination of the complex formation by the continuous variation method of equivalent mole

$$12MO_7O_{24}^{6-} + 7H_2PO_4^- + 72H^+ \longrightarrow 7[H_2PM_{012}O_{40}]^- + 36H_2O$$





$$+ H_3PO_4\cdot 10MoO_3\cdot Mo_2O_5 + OH^-$$

Absorption spectrum: According to the procedure, the absorption spectrum of phosphorus molybdenum blues formed from the reaction of PO_4^{3-} and Mo(VI) against the reagent blank and the reagent blank and tiopronin against distilled water are shown in Fig. 2. It can be seen that the product of phosphorus molybdenum blue has an absorption peak at 730 nm (a). In comparison, the absorbance of tiopronin (b) and the reagent blank (c) are almost zero in the range of 450-800 nm. In order to obtain higher sensitivity, all the following measurements were carried out at 730 nm against the reagent blank.

Interference of PO_4^{3-} and Mo(VI): In order to study the influence of PO_4^{3-} on absorbance, 14.13 mg/mL Mo(VI) and 200 μg/mL tiopronin were kept as 1.4 mL and 1 mL, respectively. The amount of 1.00 mg/mL PO_4^{3-} ranging from 1 to 5 mL was studied. It can be seen from Fig. 3 that the absorbance increased with the amount of PO_4^{3-} until reaching maximum at 3 mL. When the amount of PO_4^{3-} exceeded 3 mL, the absorbance almost did not change. This result clearly showed that the formed $[H_2PMo_{12}O_{40}]^-$ in the solution reached a maximum and all tiopronin was completely oxidized. Meanwhile, the formed phosphorus molybdenum blue reached its maximum. So 3 mL of 1 mg/mL PO_4^{3-} was selected for further work.

Keeping the amount of 1 mg/mL PO_4^{3-} at 3 mL and 200 μg/mL tiopronin at 1 mL, the influence of Mo(VI) on the absorbance is presented in Fig. 4. The amount of 14.13 mg/mL Mo(VI) ranging from 0.4-2 mL was studied. It can be seen

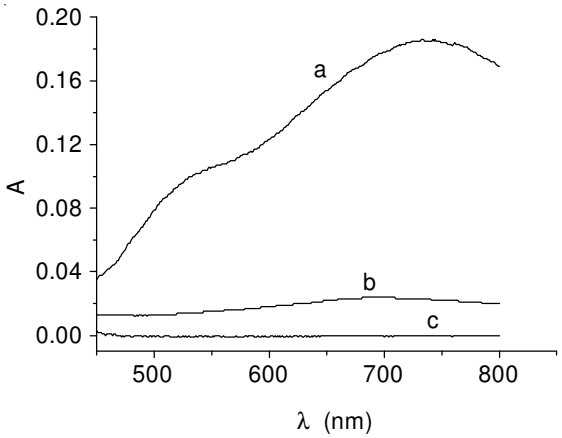


Fig. 2. Absorption spectrum of phosphorus molybdenum black

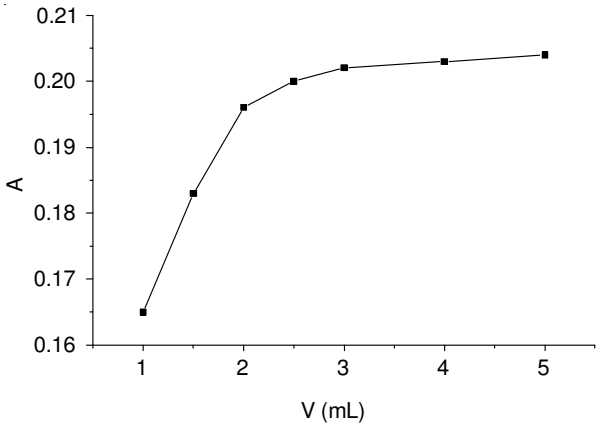


Fig. 3. Influence of PO_4^{3-} on absorbance

from Fig. 4 that the absorbance increased with the amount of Mo(VI) until reaching maximum at 1.4 mL. As the amount of Mo(VI) increased from 1.4 mL to 2 mL, the concentration of H_2SO_4 in the solution increased from 0.47-0.68 mol/L, increased by 0.21 mol/L, while the absorbance decreased from 0.206 to 0.148. According to "Discussion of reaction mechanism", increasing the concentration of H^+ and Mo(VI) was of greatly benefit to increasing the amount of $[H_2PMo_{12}O_{40}]^-$ and the oxidbillity of $[H_2PMo_{12}O_{40}]^-$ was enhanced at the same time, so the absorbance should increase with increasing the amount of Mo(VI). But the experiment had indicated that the absorbance significantly decreased when the amount of Mo(VI) was above 1.4 mL. This is attributed to the fact that oxidation potential of sulfhydryl-containing compounds increased with the increase of acidity, which made the reducibility of tiopronin reducing the $[H_2PMo_{12}O_{40}]^-$ in the solution decreased and the amount of phosphorus molybdenum blue reduced. Thus, 1.4 mL of 14.13 mg/mL Mo(VI) was selected for the rest of this work.

Interference of temperature and reaction time: Keeping other conditions constant, the effect of temperature on absorbance was studied. The absorbance of product was determined at different temperature (25, 30, 35, 40, 45 and 50 ºC). It was found that the absorbance of product was greatly affected by temperature and reached the top when the temperature was 40 ºC. So 40 ºC in water bath had been chosen for the optimal experimental conditions.

The absorbance of product was measured after standing for different times in minutes at 40 ºC water bath. It was found

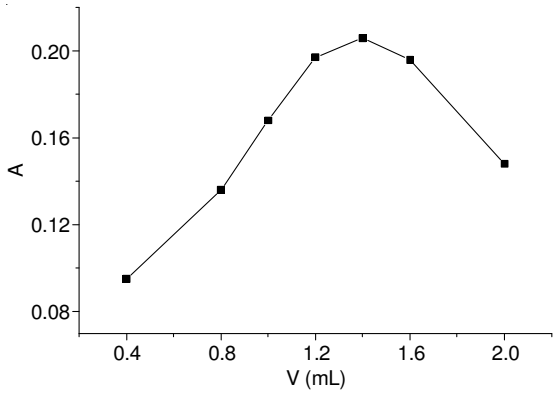


Fig. 4. Influence of Mo(VI) on absorbance

that the absorbance began to increase and became stable after 40 min. Furthermore, the absorbance can remain constant for at least 1 h. Therefore, 40 min of reaction time has been selected as the optimum.

Interference of the sulfuric acid: According to the proposed procedure, the influence of the additional H_2SO_4 (0.50 mol/L) on the determination of tiopronin was studied. It can be seen from Fig. 5 that the absorbance (A) decreased with increasing the amount (vol) of H_2SO_4. A maximum absorbance was obtained when H_2SO_4 was not added. The reason was that oxidation potential of tiopronin increased with the increase of acidity, which resulted in reducing the amount of phosphorus molybdenum blue. It agreed with the phenomenon in Fig. 4 that the absorbance significantly decreased, when the amount of Mo(VI) was above 1.4 mL. On the basis of the fact, a conclusion can be drawn that the acidity played an important role in the reducibility of tiopronin reducing the $[H_2PMo_{12}O_{40}]^-$ when the concentration of $[H_2PMo_{12}O_{40}]^-$ was constant in the solution.

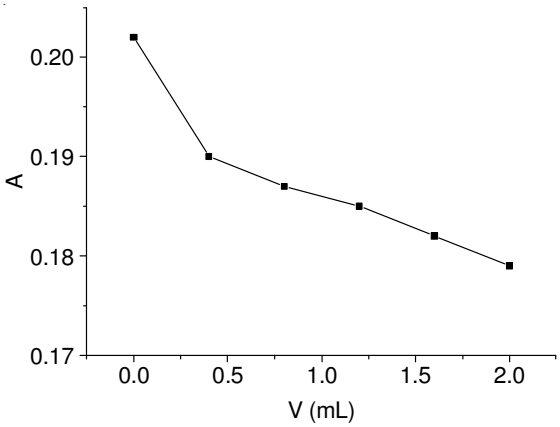


Fig. 5. Influence of H_2SO_4 on absorbance

Interference of coexisting components: A systematic study on the influence of excipients, carbohydrate, amino acids and minerals was carried out for the determination of tiopronin. The criterion for interference was a relative error of less than ± 5 % within analytical determination. The experimental results indicated that 450 μg/mL sucrose and dextrose, 350 μg/mL glutamic acid, 20.00 μg/mL Al^{3+}, 32.00 μg/mL Mn^{2+} and Ca^{2+}, 10.00 μg/mL Cd^{2+}, 6.00 μg/mL Zn^{2+}, 4.00 μg/mL Co^{2+}, 0.30 mg/mL Na^+, K^+ and Cl^-, 0.70 mg/mL Br^-, 0.72 mg/mL NO_3^- had no interference on the determination of tiopronin.

TABLE-1
DETERMINATION RESULTS OF SAMPLES AND RECOVERY (n = 5, $t_{0.05,4}$ = 2.78)

Sample	Sample content (μg/mL)	Proposed method (μg/mL)	HPLC method (μg/mL)	Added (μg/mL)	Found (μg/mL)	Recovery (%)	RSD (%) (n = 5)
1	5.00	5.08	4.97	5.00	5.07	101.4	1.6
2	10.00	9.86	9.98	5.00	4.85	97.0	1.4
3	10.00	10.12	10.05	10.00	9.87	98.7	1.0
4	15.00	15.09	15.07	10.00	9.67	96.7	0.8
5	20.00	19.88	15.03	15.00	15.14	100.9	1.0

Calibration curve: According to the proposed procedure, a series of standard solutions of tiopronin was prepared. Absorbance has been plotted as function of the concentration of tiopronin. A linear relationship between the absorbance (*A*) and the concentration (c) of tiopronin is obtained in the range of 1.6-40 μg/mL. The equation of the linear regression is *A* = -0.1193 + 0.01927c (μg/mL) with a linear correlation coefficient of 0.9989 and the apparent molar absorption coefficient of indirect determination of tiopronin is 3.14×10^3 L/(mol cm).

Determination of reproducibility and limit of detection: According to the procedure, the product was determined 11 times (n = 11) with a RSD of 0.92 %. Then, a reagent blank is measured 11 times (n = 11) and a detection limit has been obtained from three-time standard deviation of the reagent blank divided by the slope of the linear regression equation, the result is 1 μg/mL.

Apparent rate constant and activation energy: Under the optimized experimental conditions, keeping the temperature at 40 ºC water bath, the absorbance of the solution was measured under different reaction time in initial rate method. $-\ln (A_{max} - A)/A_{max}$ was plotted as function of time (min) and a line was obtained as $-\ln (A_{max} - A)/A_{max} = 0.05519 + 0.07208t$ (min), with a linear correlation coefficient of 0.9969. Since the quantity of PO_4^{3-} and Mo(VI) was much more excessive than that of tiopronin in the solution, whose concentration variation was relatively small, the reaction could be regarded as a pseudo first-order reaction. Therefore, the reaction rate equation was d[product]/dt = k' [tiopronin], the apparent rate constant ($k'_{40\,^\circ C} = k'_1 = 7.208 \times 10^{-2}$ min) was obtained. Similarly, under the selected conditions, keeping the temperature at 40 ºC, the apparent rate constant ($k'_{35\,^\circ C}$) was 6.086×10^{-2} min^{-1}.

Then, in light of the Arrhenius formula ($E_a = RT_1T_2/(T_2-T_1)\cdot \ln k'_2/k'_1$) and different pairs of K-T data, the apparent activation energy (E_a) of the indirect determination of tiopronin was calculated to obtain the result of 27.13 kJ/mol, which was less than 40 kJ/mol. It indicated that the reaction could take place easily[13].

Sample analysis: In order to judge the quality of the elaborated method and determine tiopronin in pharmaceuticals, in accordance with the procedure, various concentrations of sample solutions were measured, so that their concentrations were in the linear range of tiopronin given in the Table-1. The results agreed well with those obtained by the high-performance liquid chromatography method (HPLC). At the same time, Low RSD for precision and high recoveries has been obtained. As the sample in the experiment is real tablet, it also shows that other components of the sample (starch, dextrin and calcium stearate) do not affect the determination of tiopronin with $[H_2PMo_{12}O_{40}]^-$ and the results are satisfactory.

Conclusion

A novel method for the spectrophotometric determination of tiopronin by phosphorus molybdenum blue has been proposed. The method has been successfully applied to the determination of tiopronin in pharmaceutical samples and average recoveries are in the range of 96.7-101.4 % with satisfactory results. In a comparison with previous methods, the presented analysis procedure does not require any sophisticated instruments and the method is simple, sensitive and rapid. These merits make it applicable for common laboratories.

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