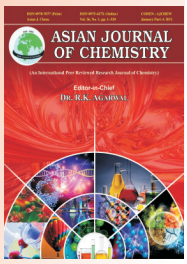
Asian Journal of Chemistry; Vol. 26, No. 10 (2014), 3025-3028

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.16531



Determination of Captopril by Using Phosphorus Molybdenum Blue as Spectral Probe Reagent

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Received: 16 October 2013; *Accepted*: 7 February 2014; *Published online*: 10 May 2014; AJC-15173

A novel method was established to determine captopril content by using phosphorus molybdenum blue as spectral probe reagent. The experiment indicated that PO_4^{3-} reacted with $[Mo_7O_{24}]^{6-}$ in 0.30 mol/L H_2SO_4 solution to form a product of phosphorus-molybdenum heteropoly acid ($[H_2PMo_{12}O_{40}]^-$) which was reduced to phosphorus molybdenum blue ($H_3PO_4 \cdot 10\ MoO_3 \cdot Mo_2O_5$) by captopril. The absorbance of phosphorus molybdenum blue was measured at the maximum absorption wavelength 730 nm and the amount of captopril could be determined based on this absorbance. A good linear relationship between absorbency and the concentration of captopril was in the range of 4.0-100.0 μg/mL and the regression equation was A = -0.01336 + 0.00504c (μg/mL) with a correlation coefficient 0.9992. The method had been successfully applied to the determination of captopril in pharmaceutical samples which the average recovery rate was in the range of 98.6-101 %.

Keywords: Phosphorus molybdenum blue, Captopril, Spectrophotometry.

INTRODUCTION

Captopril, 1-[(2*s*)-3-mercapto-2-methylpropionyl]-L-proline, an angiotensin-converting enzyme inhibitor, is used in the treatment of hypertension and congestive heart failure[1-3]. Up to now, several methods had already been reported for the determination of captopril in pharmaceutical formulations and biological matrices, including titrimetric method[4], electrochemistry[5,6], fluorimetry[7], electrochemistry[8], high-performance liquid chromatography[9-11], mass spectrometry[12] and capillary electrophoresis[13]. However, these methods had their own different disadvantages, such as consuming time[4], bad stability[5,6], higher relative standard deviation[7,8], relatively complicated and expensive apparatus[9-13].

In this paper, a novel method was established to determine captopril in pharmaceutical samples by using phosphorus molybdenum blue as spedtroscopic probe reagent. Phosphorus molybdenum blue, had been applying to quantificationally detect phosphorus in various samples[14-16]. The experiment indicated 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 was reduced to phosphorus molybdenum blue ($H_3PO_4 \cdot 10\ MoO_3 \cdot Mo_2O_5$) by captopril. The absorbance of phosphorus molybdenum blue was measured at the absorption maximum of 730 nm and the amount of captopril can be calculated based on this absorbance. This method was simple, sensitive, economical, environmentally friendly and does not require expensive equipment and facilities. In order to demonstrate the performance of the described method, the determination of captopril had been carried out in pharmaceutical and simple method equivalence assessment had also been performed. Analytical results obtained were satisfactory.

EXPERIMENTAL

Unless specially stated, all reagents used were of analytical grade and all solutions were prepared with distilled water. The main solutions were prepared as follows. A 500 mg/L captopril standard solution was prepared by dissolving 0.5 g captopril (A.R., Shanghai Xudong Haipu Pharmaceutical Co., Ltd. Shanghai, China) in distilled water. Then the captopril standard solution was transferred into 1000 mL volumetric flask and was diluted to the mark. The solution was kept at 4 °C without light. A 14.13 mg/mL stock Mo(VI) solution was prepared by dissolving 13.00 g of ammonium molybdate $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, A.R., Shanghai Colloid Chemical Plant, Shanghai, China) and mixing with 190 mL of 1:1 (v/v) H_2SO_4 (Luoyang Haohua

Chemical Reagent Co., Ltd. Luoyang, China), then which was transferred into a 500 mL standard flask and was diluted to the mark by using distilled water. The concentration of H_2SO_4 is 3.42 mol/L in the stock solution. A 1.17 mg/mL stock PO_4^{3-} solution was obtained by dissolving 0.8410 g of potassium dihydrogen phosphate (A.R., Beijing red star Chemical Plant, Beijing, China) in 500 mL standard flask with distilled water.

A model T6 UV/visible 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.

Sample preparation: Ten captopril tablets (5 mg each, Shanghai Huanghai Pharmaceutical Plant, Shanghai, China) were already to grind well, 18.50 mg of the powder was accurately weighed and then dissolved in distilled water. The solution was filtered and the filtrate was transferred into a 100 mL volumetric flask, diluted to the mark, mixed well and preserved without light at 4 °C.

Procedures: At first, 1.20 mL of 1.17 mg/mL PO_4^{3-} was added into a 12.5 mL color comparison tube and diluted to two-thirds of the mark by using distilled water. Secondly, it was dropped by 1.10 mL of 14.13 mg/mL Mo(VI) and 1 mL of 500 μg/mL captopril, respectively, then allow to shaking well after the solution was diluted to the mark with distilled water and the concentration of H_2SO_4 of the reaction solution was 0.30 mol/L. Finally, after incubation for 40 min at 30 °C in water bath, the absorbance was measured at 730 nm against a reagent blank prepared in the same way without captopril.

RESULTS AND DISCUSSION

PO_4^{3-} reacted with $[Mo_7O_{24}]^{6-}$ to form a product with $[H_2PMo_{12}O_{40}]^{-}$. Subsequently, $[H_2PMo_{12}O_{40}]^{-}$ was reduced to phosphorus molybdenum blue ($H_3PO_4{\cdot}10\ MoO_3{\cdot}Mo_2O_5$) by captopril due to the reducibility of the sulfhydryl group and captopril was oxidized to form disulfide[17].

The continuous variation method of equivalent mole was used to determine the reaction stoichiometric ratio of $[H_2PMo_{12}O_{40}]^{-}$ and captopril. Keeping the total amount of captopril (V_D) and $[H_2PMo_{12}O_{40}]^{-}$ (V_R) constant ($V_R + V_D$ = 5 mL), whose concentration were both 2.30×10^{-3} mol/L, different amounts of captopril and $[H_2PMo_{12}O_{40}]^{-}$ were transferred into a 12.5 mL color 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 $V_R/(V_R + V_D)$ ratio (*i.e.* mole fraction) (Fig. 1). According to the intersection of two tangents, 2:1 of the reaction stoichiometric ratio of captopril and $[H_2PMo_{12}O_{40}]^{-}$ was obtained.

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

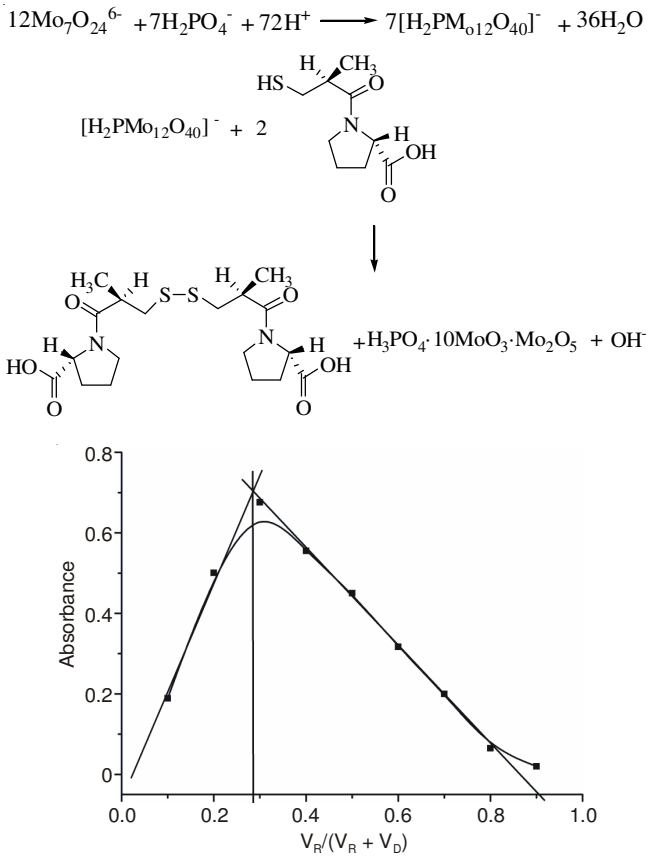

Fig. 1. Determination of the complex formation by the continuous variation method of equivalent mole

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 captopril against distilled water were shown in Fig. 2. It can be seen that the product of phosphorus molybdenum blue had an absorption peak at 730 nm (a). In comparison, the absorbance of captopril (b) and the reagent blank (c) were 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.

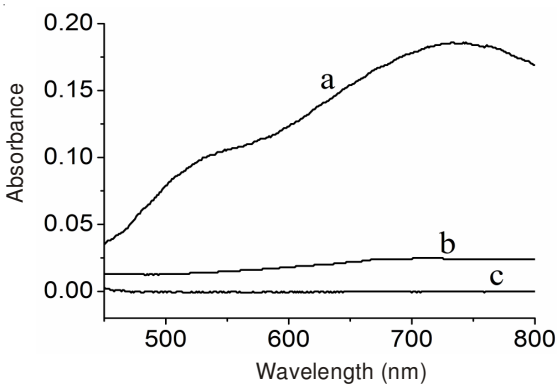

Fig. 2. Absorption spectrum

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 500 μg/mL captopril were kept as 1.10 and 1 mL, respectively. The amount of 1.17 mg/mL PO_4^{3-} ranging from 0.40 to 2.00

mL was studied. It could be seen from Fig. 3 that the absorbance increased with the amount of PO_4^{3-} until reaching maximum at 1.20 mL. When the amount of PO_4^{3-} exceeded 1.20 mL, the absorbance almost did not change. This result clearly demonstrated that the formed $[H_2PMo_{12}O_{40}]^-$ in the solution reached a maximum and all captopril was completely oxidized by it. Meanwhile, the formed phosphorus molybdenum blue reached its maximum. So 1.20 mL was selected as the optimum volume of 1.17 mg/mL PO_4^{3-} throughout the research.

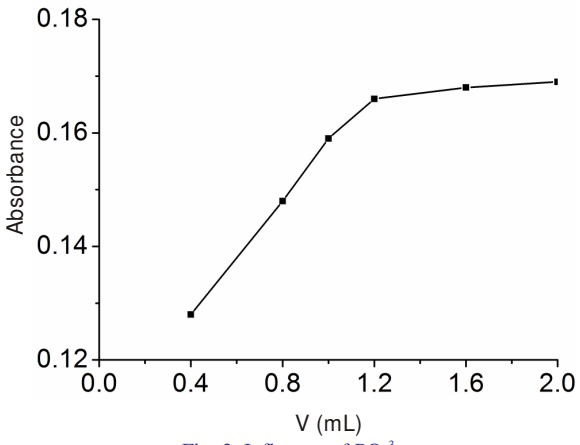

Fig. 3. Influence of PO_4^{3-}

Keeping the amount of 1.17 mg/mL PO_4^{3-} at 1.20 mL and 500 µg/mL captopril at 1 mL, the influence of Mo(VI) on the absorbance was presented in Fig. 4. As the amount of Mo(VI) increased from 1.10 to 1.20 mL, the concentration of H_2SO_4 of the solution was increased from 0.30 to 0.327 mol/L, increased by 0.027 mol/L, while the absorbance decreased from 0.190 to 0.163. 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 oxidability 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 1.10 mL. This was attributed to the fact that oxidation potential of sulfhydryl-containing compounds increased with the increase of acidity[18], which made the reducibility of captopril reducing the $[H_2PMo_{12}O_{40}]^-$ in the solution decreased and the amount of phosphorus molybdenum blue reduced. Thus, 1.10 mL of 14.13 mg/mL Mo(VI) was selected for further work.

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 captopril was studied. The absorbance (A) linearly decreased with increasing the amount (V) of H_2SO_4, the linear regression equation was A = 0.158-0.0295 V (mL), with a linear correlation coefficient of 0.9812. A maximum absorbance was obtained when H_2SO_4 was not added. The reason was that oxidation potential of captopril increased with the increase of acidity[18], which resulted in reducing the amount of phosphorus molybdenum blue. It agreed with the phenomenon in Fig. 4 that the absorbance signifi cantly decreased, when the amount of Mo(VI) was above 1.10 mL. On the basis of the fact, a conclusion could be drawn that the acidity played an important role in the reducibility of captopril reducing the

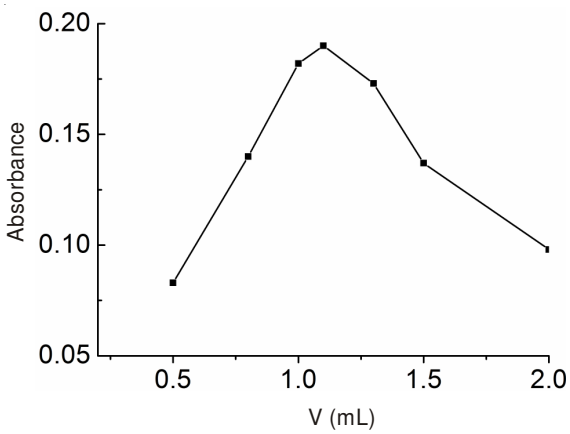

Fig. 4. Influence of Mo(VI)

$[H_2PMo_{12}O_{40}]^-$ when the concentration of $[H_2PMo_{12}O_{40}]^-$ was constant in the solution.

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, 50 °C). It was shown that the absorbance of product was greatly affected by temperature and got to the top when the temperature was 30 °C (Fig. 5). So 30 °C in water bath had been chosen for the optimal experimental conditions.

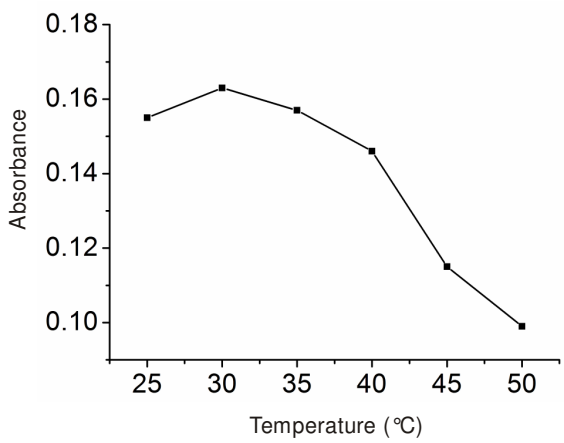

Fig. 5. Influence of temperature

The absorbance of product was measured after standing for different minutes at 30 °C water bath. It was found that the absorbance began to increase and became stable after 40 min. Furthermore, the absorbance could remain constant for at least 2 h. Therefore, 40 min of reaction time has been selected as the optimum.

Interference of coexisting components: A systematic study on the influence of excipients, carbohydrate, amino acids and minerals was carried out for the determination of captopril. The criterion for interference was a relative error of less than ± 5 % within analytical determination. The experimental results indicated that 350 µg/mL scurose and dextrose, 300 µg/mL glutamic acid, 20 µg/mL Al^{3+}, 18 µg/mL Mn^{2+} and Ca^{2+}, 5 µg/mL Cd^{2+}, 8 µg/mL Zn^{2+}, 2 µg/mL Co^{2+}, 0.20 mg/mL Na^+, K^+ and Cl^-, 0.80 mg/mL Br^-, 0.54 mg/mL NO_3^- had no interference on the determination of captopril.

Calibration curve: According to the proposed procedure, a series of standard solutions of captopril was prepared. Absorbance had been plotted as function of the concentration of captopril. A linear relationship between the absorbance (A) and the concentration (c) of captopril was obtained in the range of 4-100 µg/mL. The equation of the linear regression was A = -0.01336 + 0.00504c (µg/mL) with a linear correlation coefficient of 0.9992 and the apparent molar absorption coefficient was 1.1×10^3 L/(mol cm).

Determination of reproducibility and limit of detection: According to the procedure, the product was determined 11 times (n = 11) with a R.S.D. of 0.87 %. Then, a reagent blank was measured 11 times (n = 11) and a detection limit had been obtained from three-time standard deviation of the reagent blank divided by the slope of the linear regression equation, the result was 3.23 mg/L.

Kinetic curve of reaction: Under the optimized experimental conditions, keeping the temperature at 30 °C 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 (t) and a line was obtained as $-\ln(A_{max}-A)/A_{max} = 0.1069 + 0.05198t$ (min), with a linear correlation coefficient of 0.9978 (Fig. 6). Since the quantity of PO_4^{3-} and Mo(VI) was much more excessive than that of captopril 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'·[captopril], the apparent rate constant ($k'_{30\,°C} = 5.2 \times 10^{-2}$ min^{-1}) was obtained. Similarly, under the selected conditions, keeping the temperature at 40 °C, the apparent rate constant ($k'_{40\,°C}$) was 7×10^{-2} min^{-1}.

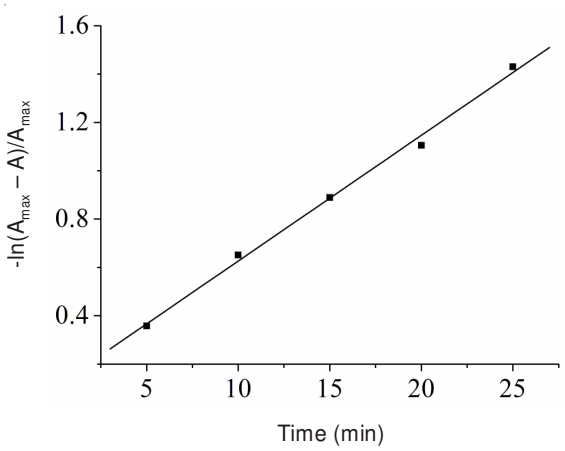

Fig. 6. Kinetic curve

By the Arrhenius formula ($E_a = RT_1T_2/(T_2-T_1)\cdot\ln k'_2/k'_1$) and apparent rate constants of 30 and 40 °C, the apparent activation energy (Ea) of the indirect determination of captopril was calculated to obtain the result of 23.29 kJ/mol, which was less than 40 kJ/mol. It indicated that the reaction could take place easily at 30 °C water bath[19].

Sample analysis: According to the procedure, different concentrations of pharmaceutical sample solutions were measured. The results were given in Table-1 and were in agreement with the reference method, with low R.S.D. and high recovery. As the sample in the experiment was real tablet, it also shows that other components of the sample do not affect the determination of captopril with $[H_2PMo_{12}O_{40}]^-$ and the results were satisfactory.

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

S.No.	Proposed method (µg/mL)	HPLC (µg/mL)	Added (µg/mL)	Found (µg/mL)	Recovery (%)	RSD (%) n = 5
1	15.08	14.97	5.00	20.13	101.0	0.73
2	14.98	14.96	10.00	24.89	99.1	0.45
3	15.08	15.05	15.00	29.87	98.6	0.75
4	15.09	15.07	30.00	45.03	99.8	0.54
5	14.98	15.03	50.00	65.14	100.3	0.40

Conclusion

A new method for the determination of captopril has been generated by using phosphorus molybdenum blue as spectroscopic probe reagent. This method has been success fully applied to the determination of captopril in pharmaceu tical samples and average recoveries were in the range of 98.6-101.0 % with satisfactory results. Especially, the method needs neither complicated protocol nor expensive apparatus, but simple, sensitive and environmentally friendly.

ACKNOWLEDGEMENTS

The authors are grateful for the financial support from the Basic and Front-line Technological Research Project of Henan Province (No. 122300410412, 132300410205).

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