European Journal of Medicinal Chemistry 44 (2009) 1452–1456

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

A novel spectrophotometric method for the determination of aminophylline with boric acid in pharmaceutical and mixed serum samples

Quanmin Li [a,*], Tiantian Zhang [a], Wenying Lv [b]

[a] College of Chemistry and Environmental Science, Henan Normal University, Henan Key Laboratory for Environmental Pollution Control, Xinxiang, Henan 453007, PR China
[b] Faculty of Environmental Science and Engineering, Guangdong University of Technology, Guangzhou, Guangdong 510000, PR China

ARTICLE INFO

Article history:
Received 25 May 2007
Received in revised form 28 August 2008
Accepted 22 September 2008
Available online 10 October 2008

Keywords:
Boric acid
Aminophylline
Spectrophotometry
Derivative reagent

ABSTRACT

This paper firstly describes a novel method to determine aminophylline (Ami) with boric acid (BA) by spectrophotometry. The study indicates that at pH 12.00 the absorbance of Ami decreases when BA is added. A simple, rapid, sensitive and reliable novel method based on the product of Ami and BA is obtained. Beer's law is obeyed in the range of Ami concentrations of 0.20–200 μg ml^{-1}. The equation of linear regression is $A = -2.57309 \times 10^{-4} - 0.00355\,C$ (μg ml^{-1}), with a linear correlation coefficient of 0.9969 and RSD 0.28%. The method is successfully applied to the determination of Ami in pharmaceutical samples and mixed serum samples, and average recoveries were in the range of 97.1–105.9%.

1. Introduction

Boron is an electron-lacking element, and has got strong ability of accepting electron. It is easy for boron to form the polymeric molecule or the steady complex with an electron donor. The complexation property of boron is of important meaning for the study of its biochemistry effects and nutrition physiology to plants in life science.

Up to the present, a number of specific reagents applied to determine boron by spectrophotometry have been developed based on the colorimetric reaction between boric acid (BA) and organic reagents, such as chromotropic acid [1], Azomethine-H [2–4], curcumin [5], crystal violet [6], methyl orange [7], and so on. Furthermore, several new derivatives of these organic dyes were synthesized and studied for spectrophotometric determination of boron [8–10].

However, there are no reports on the use of boron as the derivative reagent to determine organic pharmaceuticals. This paper firstly describes a novel method to determine Aminophylline (Ami, see Fig. 1) with BA by spectrophotometry.

Ami is the double salt of theophylline and ethylenediamine (en). Its pharmacological action lies on theophylline, and en enhances the water solubility of theophylline. Ami can relax the smooth muscle of respiratory passage and expand the bronchia, and is beneficial to respiration function. It is clinically applied to the treatment of bronchial asthma, panting bronchitis, blocked emphysema, and cardiogenic asthma. Chinese Pharmacopoeia (Part II, 2005) [11] describes a titrimetric method for determination of Ami in tablet and injection based on the detection of theophylline or en in Ami. However, this method is only suitable for the determination of high concentration Ami and incompetent for measuring low Ami content in biological samples. Additionally, Wang et al. [12] reported the determination of Ami by flow-injection chemiluminescence based on NBS–luminol system, with poor selectivity and narrow linear range (0.1–7.0 μg ml^{-1}). A HPLC method [13] and an FT-Raman spectroscopy [14] were also used for the determination of Ami. However, besides the high detection concentration range of 125–750 μg ml^{-1} by that HPLC method [13], the expensive apparatus is required in these two methods, which makes it not applicable in common laboratories.

When the direct measurement of Ami is carried out in aqueous solution in the UV region by spectrophotometry, the maximal absorption wavelength is 274 nm. However, many organic compounds have absorption of different degrees at the wavelength of 274 nm. So when the concentration of Ami in aqueous solution is determined by spectrophotometry directly, the results must be interfered with other organic compounds coexisting. The method proposed in our manuscript is to determine the concentration of Ami using BA at 297 nm, with an Einstein shift of 23 nm compared with the direct measurement of Ami. Consequently many organic compounds have less or even no ultraviolet absorption at 297 nm. For instance, the absorbance of Ami in aqueous solution is zero at 297 nm.

* Corresponding author. Tel./fax: +86 373 3326336.
E-mail address: mercury6068@hotmail.com (Q. Li).

0223-5234/$ – see front matter © 2008 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2008.09.046

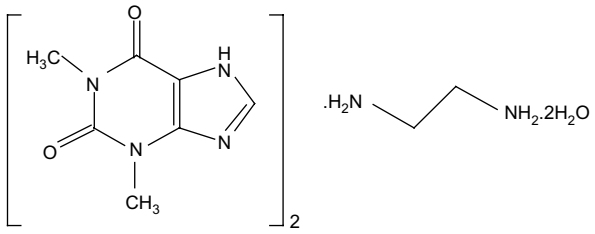


Fig. 1. Molecule structure of Aminophylline (Ami).

Compared with the direct UV spectrophotometry, the novel method proposed in our manuscript is very simple, economic and applicable. What is more, when other organic compounds (except for the amine compounds) coexist with Ami, they will not interfere with the determination of Ami. In other words, this novel method has high selectivity. Besides a novel method for the determination of Ami, we find that the primary amine derivative can be determined based on the reaction with BA. Therefore, we think that the application of BA to the determination of the primary amine derivative will have significant value for research and manufacture.

2. Material and methods

2.1. Apparatus

A model 752 UV–vis spectrophotometer (Xiamen Analytical Instrument Plant, Xiamen, China) was employed for photometric measurements. A TU–1900 UV–vis spectrophotometer (General Instrument Plant, Beijing, China) was used for scanning the absorption spectrum. All pH measurements were performed with a pH-3C digital pH meter (Shanghai Leici Device Works, Shanghai, China). A model CS-501 super constant temperature instrument (Chongqing Experimental Equipment Plant, Chongqing, China) was used for temperature measurements. A BS 110s electro-analytical balance (Beijing Sartorius Balance Ltd., Beijing, China) was used to weigh the materials.

2.2. Reagents and materials

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 stock of standard solution of 2.0 mg ml^{-1} of aminophylline (Ami) was prepared by dissolving 0.2000 g in 100 ml distilled water (the solution was preserved at 4 °C without light) and used to prepare the working Ami standards by suitable dilutions. A standard boric acid (BA) (Beijing Xinguang Chemical Reagent Plant, Beijing, China) stock solution (0.20 mol l^{-1}) was prepared by dissolving 6.2025 g of BA and 7.4560 g of KCl (Taishan Chemical Reagent Plant, Guangdong, China) in distilled water heated for a few minutes. This solution was stored in a 500 ml standard flask [15]. Buffered solution of pH 12.00 was obtained by mixing 6.00 ml solution of 0.20 mol l^{-1} NaOH and 25.00 ml solution of 0.20 mol l^{-1} KCl in 100 ml standard flask [16], and adjusted by a pH-3C digital pH meter.

Prior to analysis, 2.0 ml of the certified sample of Ami injection (Hainan pharmaceutical factory Co. Ltd.) was accurately taken into a 250 ml standard flask and then diluted to the mark with distilled water. Subsequently, the solution was mixed well and preserved without light at 4 °C.

2.3. General procedures

One milliliter of 2.0 mg ml^{-1} of Ami was taken to a 25.0 ml comparison tube. Sequentially, 2.00 ml NaOH–KCl buffer solution of pH 12.00 was added, and then 2.00 ml of 0.20 mol l^{-1} BA was added and diluting the solution to 12.5 ml with distilled water. The mixture was shaken well and the pH of solution was measured with a pH-3C digital pH meter. This solution was made at room temperature, and the absorbance value was measured immediately at 297 nm (297 nm proved to be the minimal absorption wavelength of the system measured by TU-1900 UV spectrophotometer, see Fig. 2) against a reagent blank prepared in the same way, but no BA.

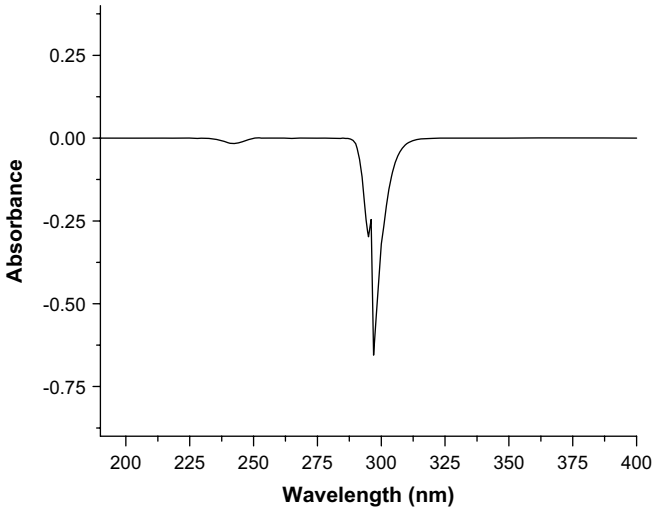


Fig. 2. UV absorption spectrum of Product I Ami (2.0 mg ml^{-1}): 1.00 ml; BA (0.20 mol l^{-1}): 2.00 ml; NaOH–KCl buffer solution (pH 12.00): 2.00 ml.

2.4. Calibration curve

According to the procedure, 0.20, 0.40, 0.60, 1.00, 2.00, 8.00, 14.00, 20.00, 60.00, 100.00, 160.00 and 200.00 μg ml^{-1}of Ami solution were prepared respectively, then the absorbance value of Product I was measured under the optimum conditions. Absorbance has been plotted as function of the concentration of Ami (see Fig. 3). A linear regression equation is attained as $A = -2.57309 \times 10^{-4} - 0.00355\ C$ (μg ml^{-1}) with a linear dependent

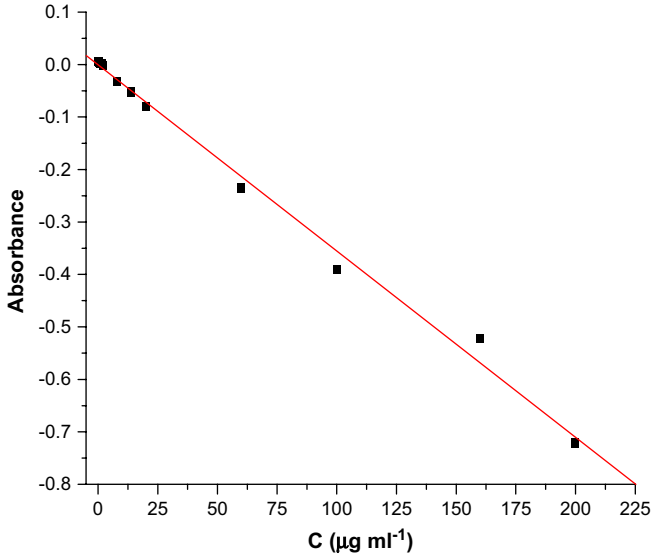


Fig. 3. Calibration curve BA (0.20 mol l^{-1}): 2.00 ml; NaOH–KCl buffer solution (pH 12.00): 2.00 ml.

coefficient of 0.9969, and ε_{297} is 2.2×10^3 l mol^{-1} cm^{-1}. The linear range of Ami is 0.20–200 μg ml^{-1}.

According to the procedure, the Ami concentration in solution of the Ami–BA system was determined 11 times ($n = 11$) with an RSD of 0.28%.

3. Results and discussion

3.1. UV absorption spectrum

The UV absorption spectrum of the Ami–BA system is shown in Fig. 2. It can be seen that the absorbance of Ami decreases to a great extent in the wavelength range of 286–320 nm when BA is added to the solution, and the system has a minimal absorption at 297 nm. In order to obtain the highest sensitivity, all the following measurements were carried out at 297 nm against reagent blank.

3.2. Effect of pH

The effect of pH on the Ami–BAsystem was examined by varying pH from 1.00 to 13.00 respectively. As shown in Fig. 4, at pH 1.00–7.00, the absorbance value of Product I (formed from Ami and BA) is almost 0, which indicates that under that condition, it is difficult for BA to react with Ami. The possible reason is that amino group (–NH_2) of Ami is protonated and turns into protonated amine salt (–NH_3^+). So the lone electron pair loses complexation capacity for the empty atomic orbital of boron. Therefore, the absorbance of the system nearly does not decrease. When pH is above 7.00, the absorbance value of Product I decreases distinctly corresponding to the growth of pH. It is assumable that when pH of the solution increases, the protonated amine salt (–NH_3^+) of Ami will turn back into amino group (–NH_2). When the pH value gets higher, more amino group (–NH_2) will be produced. In the light of the literature [17], boron centre can be chelated by N, and then boron center can also be chelated by nitrogen atoms of en. It is obvious that the absorbance value of the product from BA and Ami is smaller than that of Ami at pH 12.00 at 297 nm, which indicates the formation of the Ami–BA system. Fig. 4 shows that the absorbance value becomes minimal at pH 12.00. In other words, the degree of the reaction between BA and Ami is maximal. However, when pH increases to 13.00, the absorbance value almost gets to 0. It is likely that complex $[B(OH)_4]^-$ can be formed by BA and high concentration hydroxide ion (OH^-) (0.10 mol l^{-1}) [18], which holds back the complexation between BA and the lone electron pair of amino group (–NH_2) of Ami. In order to keep the high sensibility for determination of Ami, the experiment was carried out at pH 12.00.

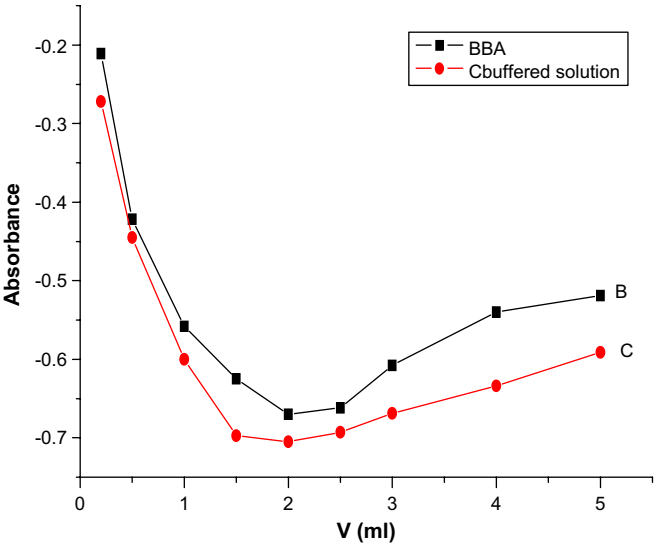


Fig. 5. Effect of amount of BA and buffered solution on the absorption of Product I Ami (2.0 mg ml^{-1}): 1.00 ml; B. NaOH–KCl buffer solution (pH 12.00): 2.00 ml. C. BA (0.20 mol l^{-1}): 2.00 ml.

3.3. Effect of amount of BA and buffered solution

3.3.1. Effect of amount of BA

In order to study the effect of amount of BA on the determination of Ami, the amount of BA ranging from 0.00 to 5.00 ml was submitted to the proposed procedure (see Curve B in Fig. 5). The absorbance value descends markedly with the rising amount of BA when the amount is less than 2.00 ml. It is likely that the degree of the reaction between BA and Ami is enhanced with the increasing amount of BA. The absorbance of the system decreases to the minimal when the amount of BA is 2.00 ml. However, when the amount of BA increases from 2.00 ml to 5.00 ml, the absorbance

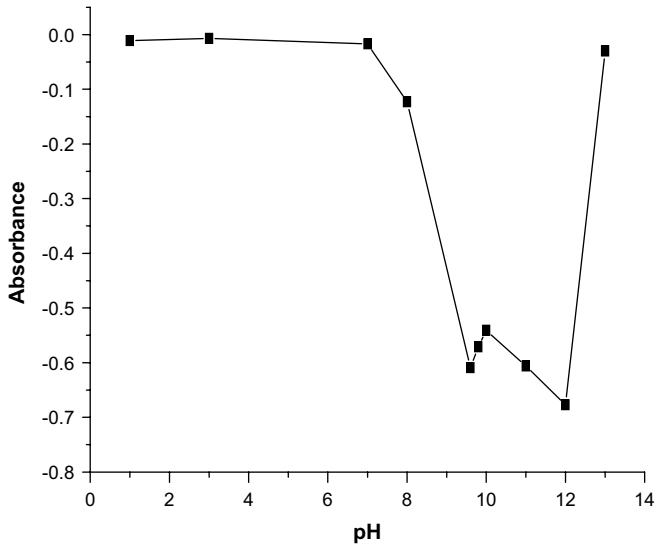


Fig. 4. Effect of pH on the absorption of Product I Ami (2.0 mg ml^{-1}): 1.00 ml; BA (0.20 mol l^{-1}): 2.00 ml.

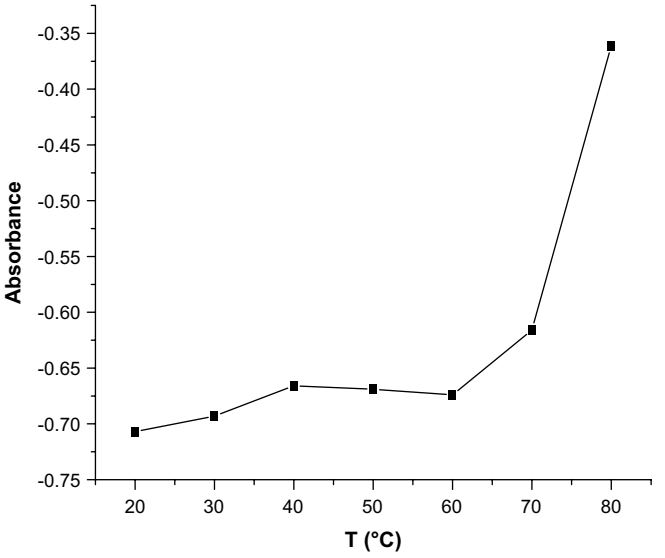


Fig. 6. Effect of temperature on the absorption of Product I Ami (2.0 mg ml^{-1}): 1.00 ml; BA (0.20 mol l^{-1}): 2.00 ml; NaOH–KCl buffer solution (pH 12.00): 2.00 ml.

Table 1
Effect of foreign ions on the determination of Ami with BA.

Foreign ions	Added as	Tolerance level ($\mu g\ ml^{-1}$)
K^+, Na^+, NO_3^-, Cl^-, SO_4^{2-}, urea	KCl, NaCl, $NaNO_3$, Na_2SO_4, $CO(NH_2)_2$	$\geq 5.77 \times 10^3$
HPO_4^{2-}	Na_2HPO_4	965
Mg^{2+}, Ca^{2+}, Ni^{2+}	Mg $(NO_3)_2$, $CaCl_2$, $NiSO_4$	400
Al^{3+}	$KAl(SO_4)_2$	136
CO_3^{2-}	Na_2CO_3	120
Cu^{2+}, Zn^{2+}	$CuSO_4$, $ZnSO_4$	16.80
Co^{2+}	$CoSO_4$	1.20
Fe^{3+}, Mn^{2+}	$NH_4Fe(SO_4)_2$, $MnSO_4$	0.80

value of Product I rises from −0.670 to −0.519. The possible reason is that when the amount of BA increases to some extent, the concentration of H^+ in the solution has a little increase. Then the protonation of amino group (–NH_2) of Ami enhances, and correspondingly the amount of Product I formed from the reaction between BA and Ami reduces. Therefore, the absorbance value of the solution ascends. So 2.00 ml of BA was selected in the later experiments.

3.3.2. Effect of amount of buffered solution

The effect of amount of buffered solution on the determination of Ami was studied according to the procedure, keeping pH at 12.00. Curve C in Fig. 5 shows that the absorbance value gets to the minimum when the amount of buffered solution is 2.00 ml. When the amount increases from 2.00 ml to 5.00 ml, there is a few increase in absorbance value, from −0.705 to −0.591. The possible reason is that when more than 2.00 ml buffered solution is added, the concentration of OH^- in the solution will have a little increase. Then more $[B(OH)_4]^-$ are formed from OH^- and BA [18], which results in some inhibition of the complexation of BA and Ami. In order to keep the high sensitivity of the determination, 2.00 ml of buffered solution was chosen.

3.4. Effect of temperature and standing time

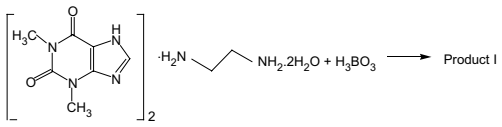
The absorbance value of Product I was measured at room temperature every 5 min. The absorbance value increases from −0.709 to −0.686 in an interval of 2 h. It is found that the reaction between BA and Ami takes place rapidly at room temperature, and the system is very stable.

The absorbance of Product I was determined at different temperatures with 5-min reaction time (see Fig. 6). It is shown that the absorbance value of the system is minimal (−0.707) when the temperature is 20 °C, and it increases to −0.361 when the temperature is 80 °C. The possible reason is that as the temperature ascends, boron polymeric forms will be produced between $B(OH)_3$ molecules after dehydration, such as $B_4O_7^{2-}$, which could interfere with the reaction between BA and Ami. Thus the stability of Product I reduces, and the concentration of Product I decreases obviously.

In order to make the determination much simpler, the experiments were carried out at room temperature, and the absorbance was measured immediately after the solution was made.

3.5. Discussion of reaction mechanism

In acidic medium, the amino group (–NH_2) of Ami is protonized. So the lone electron pair of amino group (–NH_2) of Ami loses complexation capacity for the empty atomic orbital of boron. The protonated amine salt (–NH_3^+) of Ami which is formed in acidic solution turns back into amino group (–NH_2) with an electron pair at pH 12.00. Thereby in alkaline solution, the lone electron pair of amino group (–NH_2) of Ami can make complex with the empty atomic orbital of boron in the light of the previous literature [17]. Presumably it seems reasonable that the reaction equation is as follows:



It is obvious that under some certain conditions, when BA is added to the solution of Ami, Product I can be obtained from the reaction between BA and amino group (–NH_2) or N atom of Ami, which leads to the decrease of the absorbance value of the solution. What is more, the degree of the decrease of the absorbance value is linear with concentration of Ami. Thus the concentration of Ami can be determined according to this relation.

3.6. Study of potential interference

A systematic study of the potential influence of common ions and urea was carried out on the determination of Ami. The tolerance limits were defined with an error less than ±5% in the analysis (see Table 1).

As can be seen from Table 1, there is no effect when the determination of Ami is carried out with a certain amount of metal ion existent. When the concentrations of metal ions are beyond the tolerance limits, the absorbance of the solution increases to some extent. The possible reason is that the colloid hydroxide precipitated particulate of these metal ions can be formed at pH 12.00, which makes the absorbance value of the solution increase according to light dispersion of the precipitated hydroxide particulate. In addition, the tolerance limits of Co^{2+}, Fe^{3+} and Mn^{2+} are comparably lower. It is likely that their solubility product constant (K_{sp}) are smaller, 3×10^{-45} ($Co(OH)_3$), 3×10^{-39} ($Fe(OH)_3$) and 1.9×10^{-13} ($Mn(OH)_2$) [15], respectively, which indicates that it is much easier for the formation of their colloid hydroxide precipitated particulate. If the proposed method is applied to the determination of Ami in real samples with those metal ions mentioned above coexisting, they can be pre-separated from the solution by the formation of precipitated hydroxide in alkali solution. According to the K_{sp} of precipitate of these metal ions, it can be calculated that their concentration left in the solution is very far from their tolerance limits, which indicates that the determination of Ami is free from the interference of these metal ions. Therefore, the selectivity of this proposed method is enhanced greatly.

Table 2
Determination results and recoveries of Ami in injection ($n = 5$, $t_{0.05,4} = 2.78$).

Sample	The certified (mg/2 ml)	Present method (mg/2 ml)	Sample content ($\mu g\ ml^{-1}$)	Added ($\mu g\ ml^{-1}$)	Found ($\mu g\ ml^{-1}$)	RSD (%)	Recovery (%)
050207	253.25	250.94 ± 1.21	40	10	10.26 ± 0.15	1.17	102.6
050208	255.37	253.05 ± 1.07	40	20	20.20 ± 0.31	1.24	101.0
050209	254.55	255.89 ± 0.96	40	30	29.85 ± 0.31	0.83	99.5
050301	252.58	253.62 ± 0.51	20	20	20.26 ± 0.34	1.35	101.3
050302	251.90	253.31 ± 0.64	60	20	19.82 ± 0.24	0.96	99.1

Table 3
Recoveries of ACV in mixed serum sample ($n = 5$, $t_{0.05,4} = 2.78$).

Sample serum (0.1%) (ml)	Added ($\mu g\ ml^{-1}$)	Found ($\mu g\ ml^{-1}$)	RSD (%)	Recovery (%)
2	60	63.52 ± 2.32	2.94	105.9
4	80	81.75 ± 2.65	2.61	102.2
6	100	97.06 ± 1.08	0.90	97.1

4. Sample analysis

4.1. Analysis of Ami in pharmaceutical samples

According to the procedure, different concentrations of pharmaceutical sample solutions were measured, and the results agree well with the certified reference values (see Table 2). In addition, the satisfactory results with low RSD and high recoveries are obtained. As the sample in the experiment is the compound prescription, it also shows that other components in the pharmaceutical samples do not affect the determination of Ami.

4.2. Analysis of recovery of Ami from mixed serum samples

The mixed serum samples were prepared for the analysis of recovery of Ami with the proposed method, and the results are shown in Table 3. High accuracy and good recoveries are obtained, which indicates that the proposed method can be successfully applied to recover Ami in the mixed serum samples.

5. Conclusion and perspectives

It is the first time that the determination of Ami was carried out using BA as derivative reagent by spectrophotometry.

The experiment was carried out at pH 12.00 (NaOH–KCl buffer solution) at room temperature, and the amounts of BA and buffered solution are both 2.00 ml. The linear range of Ami is 0.20–200 $\mu g\ ml^{-1}$ with RSD 0.28%. When BA is used as derivative reagent, it is economic and easily obtained. A simple, sensitive, rapid and reliable method for the direct determination of Ami is developed. The determination of Ami with the proposed method is free from the interference of metal ions and urea. Neither the new reagent need to be synthesized nor the expensive apparatus are required to be adopted. The proposed method can be successfully applied to the determination of Ami in pharmaceutical samples with satisfactory results, and average recoveries from pharmaceutical and mixed serum samples are between 97.1 and 105.9%.

In this study, BA can make complex with the lone electron pair of amino group ($-NH_2$) or N atom of Ami. Presumably BA can react with other compounds which contain either of these two functional groups. Then the application of boron as the derivative reagent by spectrophotometry may be developed to a wider scope. Therefore, the determination of this kind of organic pharmaceuticals using boron as the derivative reagent by spectrophotometry has got a significant value and practical foreground.

References

[1] D.F. Kuemmel, M.G. Mellon, Anal. Chem. 29 (3) (1957) 378–382.
[2] P. Carrero, A. Malave, E. Rojas, C. Rondon, Y. Petit de Pena, J.L. Burguera, M. Burguera, Talanta 68 (2005) 374–381.
[3] J.F. Van Staden, T.A. Merwe, Analyst 125 (11) (2000) 2094–2099.
[4] P. Carrero, J.L. Burguera, M. Burguera, C. Rivas, Talanta 40 (12) (1993) 1967–1974.
[5] S. Thangavel, S.M. Dhavile, K. Dash, S.C. Chaurasia, Anal. Chim. Acta 502 (2004) 265–270.
[6] I.L. Garcia, M.H. Cordoba, S.P. Concepcion, Analyst 110 (1985) 1259–1262.
[7] J.F. Van Staden, M. Tsanwani, Talanta 58 (2002) 1103–1108.
[8] Z.J. Li, Z.W. Cui, J. Tang, Food Chem. 94 (2006) 310–314.
[9] S. Akhmad, O. Koji, O. Mitsuko, M. Shoji, Talanta 66 (2005) 136–144.
[10] Z.J. Li, Q.J. Song, Z.W. Cui, Q. Wei, J. Zheng, Talanta 65 (2005) 1307–1312.
[11] Committee of Chinese Pharmacopoeia, Chinese Pharmacopoeia (Part II), China Chemical Industry Press, Beijing, 2005, p. 616.
[12] Z.P. Wang, Z.J. Zhang, Z.F. Fu, W.Z. Luo, X. Zhang, Talanta 62 (2004) 611–617.
[13] C.H. Yin, C. Tang, X.Y. Wu, J. Pharm. Biomed. 33 (2003) 39–43.
[14] S. Mazurek, R. Szostak, J. Pharm. Biomed. 40 (2006) 1235–1242.
[15] W.B. Chang, K.A. Li, A Concise Analytical Chemistry Handbook, first ed. Peking University Press, Beijing, China, 1981, p. 263.
[16] Analytical Chemistry Studio of Zhongnan Mineral College, Handbook of Chemical Analysis, Science Press, Beijing, China, 1984, p. 383.
[17] H. Hagen, S. Reinoso, M. Albrecht, J. Boersma, A.L. Spek, G.V. Koten, J. Organomet. Chem. 608 (2000) 27–33.
[18] Inorganic Chemistry Studio of Beijing Normal University, Huazhong Normal University and Nanjing Normal University, Inorganic Chemistry, third ed. Higher Education Press, Beijing, China, 1992, p. 602.