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SPECTROPHOTOMETRIC DETERMINATION OF THIAMINE USING OXIDATION REACTION WITH 18-MOLYBDODIPHOSPHATE

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Abstract

A simple and highly sensitive method for the determination of thiamine in pharmaceuticals has been developed. It is based on the reaction between thiamine and 18-molybdodiphosphate heteropoly complex (18-MPC), which occurs at pH≈10, created by the addition of 10 % sodium carbonate. The reaction takes place at room temperature. 7-minutes are sufficient for the complete reduction of 18-MPC. The stability of heteropolyblue is limited and absorbance significantly decreases after 10 minutes of reaction. The optimal concentrations of 18-MPC and sodium carbonate were found to be 0.64 mM and 2.4 %, respectively. The ratio of 18-MPC to thiamine in the reaction is 2 : 1, which is shown by the method of continuous variations. Four electrons are accepted by the thiamine molecule during the oxidation of 18-MPC. In this case, a two-electron heteropolyblue is formed with an absorption maximum at 820 nm. The calibration dependence is linear in the range of thiamine concentrations from 2 to 80 μM with a detection limit of 0.8 μM using a cuvette with 0.5 cm path length. The proposed method has been successfully applied to the determination of vitamin B1 in three different pharmaceutical formulations containing other B vitamins, vitamin B6 and vitamin B12.

Keywords: spectrophotometry; thiamine; 18-molybdodiphosphate; pharmaceuticals.

СПЕКТРОФОТОМЕТРИЧНЕ ВИЗНАЧЕННЯ ТІАМІНУ ЗА РЕАКЦІЄЮ ОКИСНЕННЯ 18-МОЛІБДОДИФОСФАТОМ

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Анотація

Розроблено простий і високочутливий метод визначення тіаміну в фармацевтичних препаратах. Він ґрунтується на реакції між тіаміном і 18-молібдодифосфатним гетерополікомплексом (18-МФК), яка відбувається за pH ≈ 10, створеним додаванням 10 % карбонату натрію. Реакція відбувається за кімнатної температури. Для повного відновлення 18-МФК достатньо 7 хвилин. Стійкість гетерополісині є обмеженою, і світлопоглинання суттєво зменшується після 10 хвилин реакції. Знайдені оптимальні концентрації: 18-МФК – 0.64 мМ та концентрація натрій карбонату – 2.4 %. Співвідношення 18-МФК та тіаміну в реакції складає 2 : 1, що показано методом ізомолярних серій. Чотири електрони приймаються молекулою тіаміну під час окиснення 18-МФК. Водночас утворюється двохелектронна гетерополісинь із максимумом поглинання за 820 нм. Градуювальна залежність є лінійною в діапазоні концентрацій тіаміну від 2 до 80 мкМ з межею виявлення 0.8 мкМ за використання кювети з довжиною поглинаючого шару 0.5 см. Запропонований метод був успішно використаний для визначення вітаміну В1 в трьох різних фармацевтичних композиціях, що містять інші вітаміни групи В, такі як вітамін В6 та вітамін В12.

Ключові слова: спектрофотометрія; тіамін; 18-молібдодифосфат; фармацевтичні препарати.

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Introduction

The normal functioning of the human body requires organic and mineral substances, most of which the body can synthesize on its own. However, there is a group of essential substances that can only be obtained from the outside – these are vitamins. One of the B vitamins is thiamine (vitamin B1), which is found in yeast, soybeans, spinach, potatoes, carrots, cabbage, wheat and oat husks, as well as in bread made of unleavened flour. Thiamine deficiency causes diseases such as beriberi and Wernicke-Korsakoff syndrome, and hypervitaminosis leads to increased activity of acetylcholine, which plays an important role in the pathogenesis of allergy. It can cause anaphylactic shock, so its quantification is necessary [1]. For women and men, the recommended daily intake of thiamine is approximately 1.1 and 1.2 mg, respectively.

Currently, a large number of methods have been proposed for the determination of thiamine, mostly chromatographic [2–6]. For routine analysis, the luminescent method based on the oxidation of thiamine to thiochrome by potassium ferrocyanide and extraction [7; 8] remains the main method. This method is the most reliable and selective. At the same time, it has a number of disadvantages. Excess ferrocyanide can destroy thiochrome, so extraction is used, but this complicates the method, and the use of toxic organic solvents is currently excluded in analytical chemistry. Hg(II) does not affect the fluorescence of thiochrome, but it is known to be highly toxic. Existing spectrophotometric methods are insensitive and non-selective [7–13].

Heteropoly anions (HPAs) are intensively used in chemical analysis as analytical forms for the determination of a limited number of elements. Only molybdenum HPAs with Keggin structure can be used as effective analytical forms because their formation reaction is selective and they can be reduced to form intensely colored heteropoly blues. There are only five elements that can form molybdenum HPAs with Keggin structure including P(V), Si(IV), Ge(IV), As(V) and Ga(III) [14; 15]. HPAs can be also used as analytical reagents, mainly for the determination of substances with reducing properties [16–18]. Typical analytes for which HPAs are used include important substances such as polyphenols or ascorbic acid. Recently, we proposed the use of 18-molybdodiphosphate heteropoly complex with Dawson structure (18-MPC) in the analysis as an efficient reagent for the determination of reducing agents, including polyphenols [19], ascorbic acid [20–22], p-aminophenol [23] and cysteine [20; 24]. 18-MPC has stronger oxidative properties than other HPAs. Its oxidizing capacity is strongly pH dependent, and it can be used in a wide pH range from 2 to 12. Therefore, by adjusting the pH, optimal conditions can be found for the determination of many reducing agents and high selectivity can be reached. Examples include the determination of ascorbic acid and cysteine [20] in a mixture. These reactions are easily automated by using flow methods of analysis [20–24].

The aim of this work is to develop a simple, selective and sensitive method for the determination of thiamine using its direct oxidation reaction with 18-MPC. The optimal conditions for obtaining the reduced highly colored form were found. Accuracy, precision and selectivity of the developed method were evaluated on the example of analysis of several pharmaceutical preparations.

Experimental part

Reagents and instrumentation.

The ammonium salt of the α-isomer of 18-MPC $(NH_4)_6P_2Mo_{18}O_{62}\times14H_2O$ was synthesized as described in the literature [21]. A 0.01 M solution of 18-MPC was prepared by dissolving 0.7855 g of the synthesized salt and diluting it to 25 mL with distilled water. A 4 mM solution of 18-MPC prepared by dissolving a 628.4 mg of the sample in a 50 mL flask was used as the analytical reagent. Thiamine chloride monophosphate dihydrate (> 99 % purity) was purchased from Sigma Aldrich, Germany. An 8 mM thiamine solution was prepared by dissolving a 67.46 mg of sample in distilled water in a 25 ml flask followed by a hundredfold dilution. A 10 % solution of sodium carbonate was used to create a strongly alkaline environment in which the reaction took place. The absorbance and absorption spectra were measured using a UV–vis spectrophotometer SF-46 (LOMO, St-Petersburg, Russia) with glass cells having a path length of 5 cm.

Procedure for the determination of thiamine with 18-MPC.

In a 25 ml volumetric flask, mix an aliquot of thiamine solution corresponding to its final concentration between 2 and 80 μM, 4 mL of 4 mM 18-MPC solution, 6 mL of 10% sodium carbonate solution, and bring to the mark with distilled water. After 7 minutes, the absorbance is measured in a 0.5 cm cuvette at a wavelength of 820 nm.

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Results and discussion

Investigation of the reaction between thiamine and 18-MPC and search of the optimal conditions for the determination of thiamine.

In a preliminary study, it was found that thiamine could be oxidized by 18-MPC in a strongly alkaline medium created by the addition of sodium carbonate or sodium hydroxide. Examination of the dependence of the absorbance on the order of addition of the reagents showed that the result obtained was little dependent on the mixing order, except when sodium carbonate and 18-MPC were mixed first. The maximum absorbance was obtained with the following mixing order – thiamine, 18-MPC and sodium carbonate, which was used in further experiments. From these results it is clear that if sodium carbonate and 18-MPC are mixed first, the rapid degradation of the heteropoly anion by the action of the base results in a significant portion of the reagent already being degraded by the time the analyte is added. This leads to the important conclusion that a more hydrolytically stable tungsten-substituted heteropoly complex, such as 16-molybdo-2-tungstophosphate, is preferable in this reaction.

The formation of heteropoly blue is influenced by a number of factors, including the concentration and nature of the substance required to create the optimal alkalinity of the solution, the concentration of 18-MPC, and the reduction time.

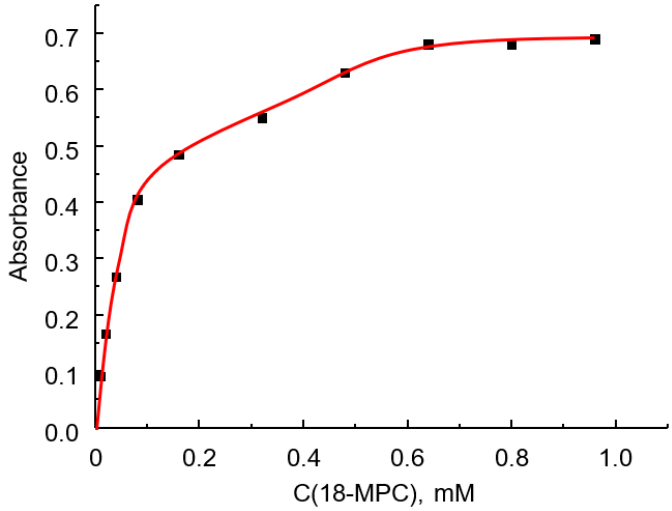

Fig. 1. Effect of 18-MPC concentration on the absorbance of heteropoly blue. C(thiamine) = 0.032 mM, C(Na_2CO_3) = 2.4 %, reduction time = 7 min, l = 1 cm, λ = 820 nm

As can be seen from Fig. 1, after a concentration of 18-MPC ≈ 0.6 mM, the graph of the dependence of absorbance on the reagent concentration is leveled. In other words, even when a twenty-fold excess of the reagent is reached, complete oxidation of thiamine does not occur, but the growth of absorbance practically ceases. The concentration of 18-MPC 0.64 mM was subsequently chosen as optimal.

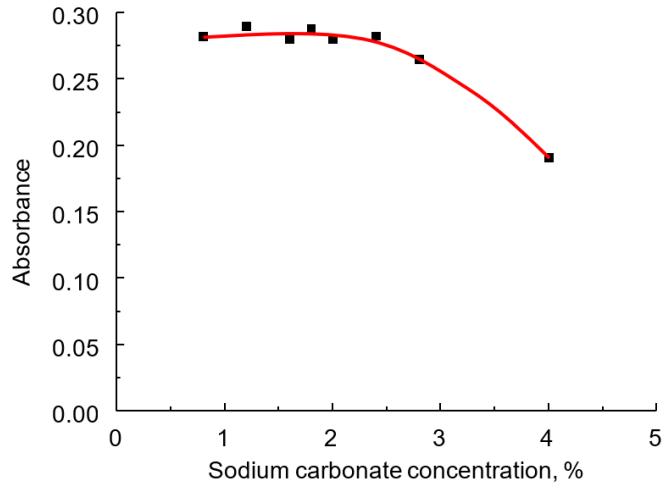

Fig. 2. Dependence of heteropoly blue absorbance on the concentration of sodium carbonate. C(thiamine) = 0.032 mM, C(18-MPC) = 0.64 mM, l = 0.5 cm, λ = 820 nm

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One of the disadvantages of the proposed analytical reaction is that the degree of heteropoly blue formation depends significantly on the reaction time, as well as on the nature and concentration of the substance used to create the required acidity. Using sodium hydroxide, it is possible to achieve a deeper reduction of 18-MPC. However, the results in this case become much less reproducible. Therefore, we, like other authors, recommend using sodium carbonate to create the required acidity. The addition of sodium carbonate leads to pH values of about 10.0, while the use of sodium hydroxide is accompanied by higher basicity near pH 11.0. As can be seen from Fig. 2, in the range of sodium carbonate concentrations from 1 to 2.5 %, the absorption of heteropolyblue remains almost constant and unchanged. A further increase in the soda concentration from 2.5 to 4 % leads to a decrease in absorption. This can be explained by the destruction of heteropolyblue in more basic solutions.

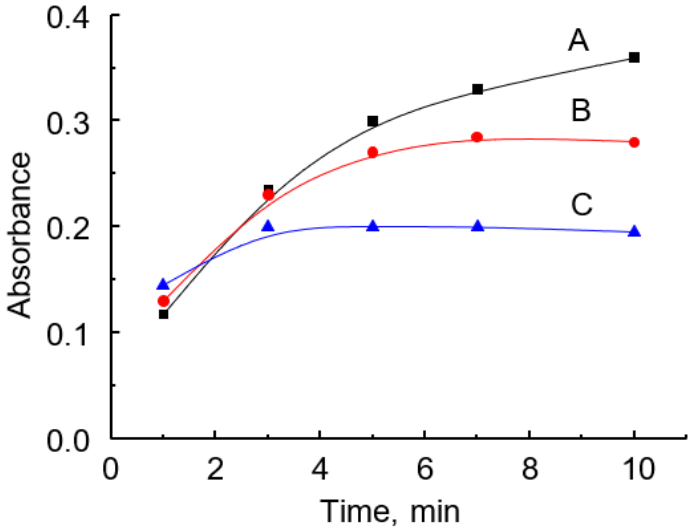

Fig. 3. Influence of absorbance of heteropoly blue on reaction time. C(18-MPC) = 0.64 mM, C(Na_2CO_3) = 1.2 (A); 2.4 % (B); 4 % (C), C(thiamine) = 0.032 mM, l = 0.5 cm, λ = 820 nm

The stability of the color for the obtained heteropoly blue over time was studied (Fig. 3). It turned out to be low. After 10 minutes, the absorbance drops by 15%, and after 30 minutes it decreases by half to the initial value. Therefore, absorbance measurements should be taken exactly 7 minutes after preparing the solutions and placing them in the cuvette. When using 1.2% sodium carbonate, the absorbance is higher than with higher concentrations of soda, but there is no place where the absorbance is constant. With a soda concentration of 2.4%, the absorbance weakly depends on time in the range from five to ten minutes (Fig. 3) and is significantly higher than with a soda concentration of 4%. We chose a soda concentration of 2.4%, since stable results could not be achieved with a lower concentration. The reaction time of seven minutes was chosen as optimal.

Determination of the molar ratio of reactants using the method of continuous variations.

One of the goals of the study of the oxidation reaction of thiamine by 18-MPC was to establish the ratio in moles in which the substances react. The molar ratio method failed to achieve this, since the equilibrium constant of the reaction was not very high and we did not observe a clearly defined inflection point on the saturation curve of thiamine by 18-MPC. For such cases, it is recommended to use the continuous variation method, in which a clear inflection point is observed regardless of the value of the equilibrium constant.

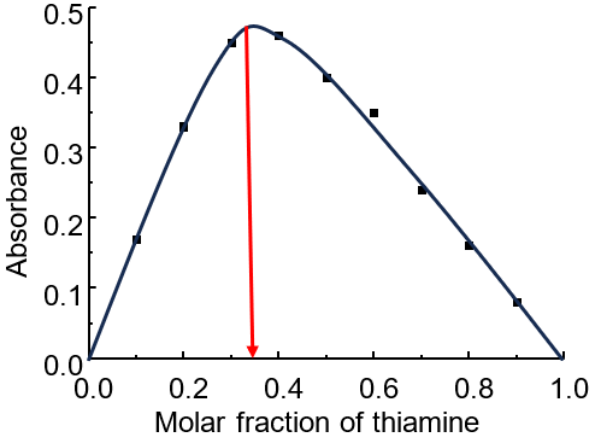

Fig. 4. Curve of the method of continuous variations for the reaction between 18-MPC and thiamine. C(thiamine) + C(18-MPC) = 0.32 mM, l = 1 cm, λ = 820 nm

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It follows from Fig. 4 that during the interaction of thiamine and 18-MPC, the highest absorbance value is achieved at a ratio of thiamine concentration to the sum of the reactant concentrations of ~0.33 : 1. A molar fraction of 0.33 corresponds to a ratio of thiamine to 18-MPC = 1 : 2. That is, for a chemical reaction between these substances to oxidize 1 mole of thiamine, 2 moles of 18-MPC are required. As is known, in oxidation reactions, 18-MPC behaves as a two-electron oxidizer [19–21]. When used in excess, 1 molecule of 18-MPC accepts two electrons in the oxidation reaction. Thus, a thiamine molecule gives up 4 electrons in a reaction with 18-MPA. Two electrons are involved in the oxidation reaction of thiamine to thiochrome (Fig. 5).

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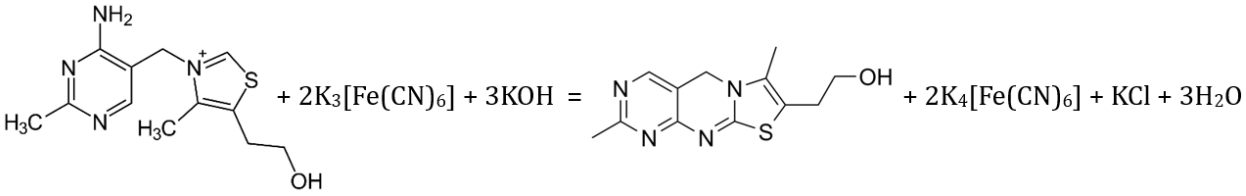

Fig. 5. Scheme of the reaction between thiamine and oxidizing agent

Therefore, it can be concluded that the oxidation of thiamine by 18-MPC occurs more deeply. The fact that thiamine gives up more than two electrons is also evidenced by another experimental fact that we discovered. For the method we developed, the apparent molar coefficient of thiamine is approximately 20 000 mol^{-1} L cm^{-1} under optimal conditions. If the stoichiometry of the reaction were 1 : 2, then 2 mole of heteropoly blue would be formed per 1 mole of thiamine. The molar absorptivity of heteropoly blue is approximately 11 000 mol^{-1} L cm^{-1}, and the value obtained for thiamine is higher. Therefore, the stoichiometry of the reaction differs from the classical one. If the ratio of 18-MPC : thiamine were 2 : 1 (Fig. 4), the molar absorptivity would be 11.000 × 2 = 22.000 mol^{-1} L cm^{-1}. That is, the reaction occurs according to a more complex mechanism, possibly with a partial rupture of the chemical bonds in the aromatic rings of thiamine.

However, another explanation is more plausible. The ratio of thiochrome to thiamine in chemical reaction depends on the pH, solvent, and nature of the oxidizing agent [7]. In addition to potassium hexacyanoferrate, many oxidizing agents have been proposed, including $KMnO_4$ and MnO_2, cyanogen bromide, H_2O_2, I_2, and Hg(II). Oxidizing agents such as $KMnO_4$, H_2O_2, and I_2 predominantly yield a nonfluorescent product. The oxidation of thiamine to fluorescent thiochrome is always accompanied by the simultaneous formation of thiamine disulfide, a condensation product of two thiamine molecules. Therefore, after the formation of thiochrome, which requires 2e, another 2e is required for further condensation to disulfide, yielding a non-fluorescent product.

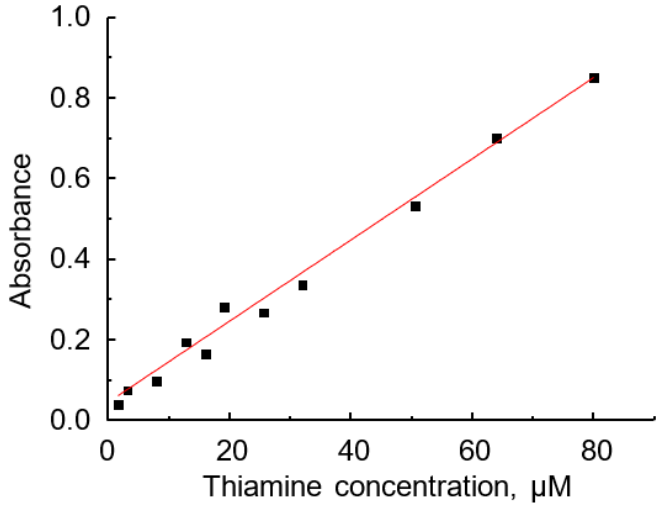

Fig. 6. Calibration graph for the spectrophotometric determination of thiamine with 18-MPC. C(18-MPC) = 0.64 mM, C(Na_2CO_3) = 2.4%, reaction time = 7 min, l = 0.5 cm, λ = 820 nm

The equation of the calibration graph, constructed under the optimal conditions of the developed method, has the form A = 0.046±0.023 + (0.0100±0.0006)×C(thiamine), where thiamine

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concentration is expressed in μmol L^{-1}. The calibration dependence is linear in the range of thiamine concentrations from 2 to 80 μM with a detection limit of 0.8 μM when using a 0.5 cm cuvette (Fig. 6).

Study of interference and analysis of pharmaceutical preparations.

No interferences were found for reducing sugars, salicylic acid and its derivatives, caffeine, oxyacids and common excipients (sodium chloride, EDTA, magnesium stearate, mannitol, cellulose, titanium dioxide, lactose, talc and starch) at [interferent]/[thiamine] ratios much higher than those found commonly in pharmaceuticals. Strong interference is expected from components of pharmaceutical formulations having reducing properties, such as ascorbic acid or polyphenols.

The proposed method was applied to the determination of thiamine in three pharmaceutical preparations. The results of thiamine determination in samples containing a large amount of other B vitamins indicate high selectivity of the proposed method. The results of analyses of pharmaceuticals in all cases coincided well with the values declared by the manufacturers, which confirms the accuracy and acceptable precision of the developed method (Table).

Table

Results for the determination of thiamine in multivitamin pharmaceutical compositions by the proposed method (mg/tablet ± Δ, n = 5, 95% confidence level)

Producer, weight of a tablet	Claimed value	Found by the proposed method	Relative standard deviation
Neurorubine Forte, Switzerland*	200.0	208.9±12.2	4.7
Neurobex, Teva, Israel**	15.0	15.0±3.5	2.9
Neuromultivit, GL Pharma, Austria***	100.0	98.4±3.8	3.1

* The drug contains 200 mg of thiamine nitrate (vitamin B1) in 1 tablet. The other ingredients are: pyridoxine hydrochloride (vitamin B6) 50 mg, cyanocobalamin (vitamin B12) 1000 μg, mannitol (E 421), powdered cellulose, microcrystalline cellulose, corn starch, magnesium stearate, colloidal silicon dioxide, hypromellose, macrogol 6000, talc, titanium dioxide (E 171), erythrosine (E 127)

** The drug contains 15 mg of thiamine nitrate (vitamin B1) in 1 tablet. The other ingredients are: pyridoxine hydrochloride (vitamin B6) 10 mg, cyanocobalamin (vitamin B12) 0.02 mg, wheat starch, lactose, talc, silicon dioxide, magnesium stearate, povidone, titanium dioxide E171, Kollicoat Protect, Ponso 4R E124

*** The drug contains 100 mg of thiamine nitrate (vitamin B1) in 1 tablet. The other ingredients are: pyridoxine hydrochloride (vitamin B6) 200 mg, cyanocobalamin (vitamin B12) 0.2 mg, microcrystalline cellulose, magnesium stearate, povidone, macrogol 6000, titanium dioxide, talc, methylcellulose, poly-(ethyl acrylate-methyl methacrylate) 30% of the variance

Conclusions

It has been shown that the oxidizing ability of 18-MPC is so high that it can be used to oxidize such a low-reactivity compound as thiamine. The reaction occurs at room temperature and does not require heating. This shows that the scope of application of this reagent can be significantly expanded to determine compounds that exhibit weak reducing properties. Moreover, the oxidation of thiamine does not stop at the stage of thiochrome formation and proceeds more deeply, requiring about 4e for the reaction to be complete. The downside of the proposed reaction is that the degree of heteropoly blue formation strongly depends on the reaction time and the concentration of sodium carbonate. During the optimization study, conditions were found under which this effect manifests itself to the least extent.

The proposed method has high sensitivity. The detection limit is 0.8 μM and can be significantly improved by using a cuvette with a longer path length. Other attractive features of the method are its simplicity and fairly high precision. The method has high selectivity suitable for determining thiamine in most pharmaceutical formulations, including those containing B vitamins.

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