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A RAPID ASSAY PROCEDURE FOR THE DETERMINATION OF THIAMINE IN PHARMACEUTICAL PRODUCTS

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A fast and simple method of determining thiamine (vitamin B_1) in neat solution and in pharmaceutical products has been developed using titrimetric procedure. The method makes use of the reaction in which thiamine is oxidized [1] by potassium ferricyanide in thiochrome in an alkaline medium. In the present investigation the ferrocyanide ion produced in equivalent amount has been titrated in presence of the remaining excess potassium ferricyanide with zinc sulphate in an acid medium. The determination is interference free from the other vitamins, e.g., B_2, B_6 and B_{12}. Detection as low as 0.01 mg ml^{-1} has been obtained. The time required for analysis is short, being about 5 mins for a single determination.

Key words : Thiamine, Assay, Pharmaceutical products.

Introduction

It is well known that the determination of thiamine at small concentration levels is difficult, especially in food and pharmaceutical products. In order to remove the interference from the other B-vitamins, there has been applied numerous ways and means. So far it has not been possible to determine the vitamin B_1 without time-consuming separations [2-4], precipitation [5], or extraction based on either spectrophotometry or fluorimetry methods [6-10].

The interference from other soluble B-vitamins has been a major problem associated with all these methods. More recently developed techniques for the determination of vitamin B_1 in pharmaceutical preparations or a complex sample have included microbial [11], high performance liquid chromatography (HPLC) [12-14], spectrophotometry [15], spectrophotometry coupled with thin layer chromatography (TLC) [16]. Normally, however, these techniques have again demanded the separation of the samples as a preparatory treatment and it has involved considerable time and purchase of expensive apparatus. During our present study, we considered indirect titrimetric method, in which the vitamin is oxidized with potassium ferricyanide and the reduced form of oxidant in presence of potassium ferricyanide is titrated with zinc sulphate, to be an interesting alternative. Our main emphasis has been the applicability of the procedure to determine vitamin B_1 at very low concentration levels without involving separation process but being totally interference free from the other soluble B-vitamins. The sensitivity and simplicity of this method allow it to be useful for any routine analysis.

Experimental

(a). Materials and reagents. All reagents were of analytical - reagent grade and doubled distilled water was used to prepare all solutions. Vitamin B_1 (Fluka), Berin tablet and injection (Glaxo), Thianeuron capsule (Pfizer) and B-complex syrup Plexovit (Remington) were used in the present studies.

i. Potassium ferricyanide solution (0.4 mM). A suitable quantity of potassium ferricyanide was accurately weighed and dissolved in distilled water to obtain known volume of a 0.4mM solution.

ii. Zinc sulphate (0.6 mM). A known quantity of zinc sulphate ($ZnSO_4.\ 8H_2O$) was weighed accurately and dissolved in known volume of distilled water to obtain 0.6 mM solution.

iii. Sodium hydroxide solution (0.1N). 0.4 g of sodium hydroxide was dissolved in distilled water to make 100 ml solution.

iv. Diphenylamine. (0.16%). 0.16 g of Diphenylamine was dissolved in concentrated sulphuric acid and the volume was made upto 100 ml with the acid in a measuring flask.

(b). Vitamin(s) solution. i. *Vitamin B_1 neat solution.* 0.5g of vitamin B_1 was dissolved to make 500 ml solution in distilled water including a few millilitres of 0.1 N sodium hydroxide to maintain the solution at pH 8.5. By diluting this fifty times with distilled water a solution with 0.02 mg ml^{-1} vitamin was obtained.

ii. Mixture of vitamin B_1, B_2 and B_6. Equal quantities of vitamin B_1, B_2 and B_6 (0.5 g each) were dissolved in water to make 500 ml solution. 10 Millilitre of the solution was diluted to 500 ml using distilled water and a few millilitre of 0.1 N sodium hydroxide (to maintain pH 8.5). Each millilitre of the solution contains 0.02 mg each of B_1, B_2 and B_6.

SAMPLE TREATMENT

i. Berin tablet. Two tablets equivalent to 200 mg of

vitamin B_1 were crushed to powder using mortar and pestle and mixed thoroughly. To this was then added 60 ml distilled water; shaken and filtered. The residue was washed with small quantity of water and the washings collected with the filtrate. The filtrate was transferred to a 100 ml measuring flask and the volume made upto the mark with distilled water and 0.5 ml of 0.1 N sodium hydroxide so that the pH of the solution was 8.5. 1 Millilitre of this solution was diluted to 100 ml with distilled water. Each millilitre of the solution contained 0.02 mg B_1.

ii. Thianeuron capsule. One capsule (100 mg vitamin B_1) was emptied into a beaker. Added 50 ml distilled water, shaken, filtered and washed the residue with distilled water. The filtrate was treated with 12.8 ml of 9.1 N sodium hydroxide and finally diluted with distilled water making up the volume upto mark in a 500 ml measuring flask (pH 8.5). 10 ml of this solution was diluted to 100 ml with distilled water to give 0.02 mg ml^{-1} of vitamin B_1.

iii. B-complex syrup. 15 ml of the syrup containing 3 mg B_1 was diluted to 100 ml with distilled water including a few millilitre of 0.1 N sodium hydroxide so that the solution had pH 8.5 each ml of the syrup contained vitamin B_1 equivalent to 0.03 mg.

iv. Berin injection. One ampoule (100 mg vitamin B_1) was cut open into a beaker and washed out the vitamin with distilled water. This was then transferred to a 100 ml measuring flask containing a few millilitre of 0.1N sodium hydroxide and then volume was made up with distilled water. The solution had pH 8.5. 2 ml of this solution was diluted with distilled water to obtain 100 ml solution. Each millilitre of this solution was equal to 0.02 mg B_1.

PROCEDURE

(a) Determination of vitamin B_1 in a neat solution. 1 ml or a measured aliquot of the vitamin solution was pipetted out in a conical flask. 5 Millilitre of potassium ferricyanide solution (0.0004M) was added to it and allowed to stand for 3 mins to complete the reaction. After this period there were added 0.5 ml each of concentrated sulphuric acid and ortho phosphoric acid and 2 drops of diphenylamine indicator (0.16% in concentrated sulphuric acid). This was then titrated with 0.006M zinc sulphate solution to the appearance of violet-blue colouration at the end point.

Determination of vitamin B_1 in pharmaceutical products (tablets, syrup, injectables and capsules). A measured aliquot of the solution containing vitamin B_1 obtained after sample treatment was treated in the same way as the procedure described for neat solution of vitamin B_1. The location of end point was carried out in exactly the same as the standard procedure described before.

Determination of vitamin B_1 from a mixture containing B_1, B_2 and B_6. 1.0, 2.0, 3.0, 4.0 and 6.0 ml of the B-vitamins mixture were pipetted out into separate titration flasks. These were subjected to similar treatment as in the standard procedure and titrated to the end point using the specific titrant.

REACTIONS

1. Formation of thiochrome:

$$8K_3[Fe(CN)_6] + 8C_{12}H_{17}N_4OS\,Cl.HCl \xrightarrow{pH\ 8.0\text{-}8.5\ (Alkaline)}$$

↑ Potassium ferricyanide; ↑ Thiamine (Vitamin B_1)

$$\text{Thiochrome} + 6K_4[Fe(CN)_6] + 2Fe(CN)_2 + 8CN \quad \text{——— (i)}$$

2. Formation of Potassium zinc hexacyanoferrate (II).

$$9ZnSO_4 + 6K_4[Fe(CN)_6 \xrightarrow{\text{acidic}} 3K_2Zn_3[Fe(CN)_6]_2 + 9K_2SO_4$$

Hexacyanoferrate (II) → Potassium zinc hexacyanoferrate (II)

CALCULATION

9 moles $ZnSO_4$ = 8 moles vitamin B_1

1 mole " = 8/9 moles " "

1 mole " = 8/9 x 337g vitamin B_1

1000 ml (1M) = 8/9 x 337g vitamin B_1

$$1000\ ml\ (0.0006M) = \frac{8 \times 337 \times 0.0006}{9}\ g\ \text{vitamin } B_1$$

$$1ml\ (0.0006M) = \frac{8 \times 337 \times 0.0006}{9 \times 1000} = 0.0001797g\ \text{vit.}B_1$$

$$= 0.1797\ mg\ \text{vitamin } B_1$$

$$\text{Amount of thiamine mg/ ml of sample solution.} = \frac{\text{ml. } ZnSO_4,\ 0.0006M \times 0.1797 \times f}{\text{Volume of sample}}$$

Where f = 1.11, a constant which needs to be considered for obtaining accurate results.

Results and Discussion

The experimental condition to determine B_1 titrimetricaly was optimized for quantitative estimation of the vitamin from samples by studying such parameters as sample concentration, acid and titrant strength, pH of the reacting medium and the reaction time.

Reaction time and effect of pH on oxidation of vitamin. Potassium ferricyanide oxidizes thiamine chloride to thiochrome in an alkaline medium. The pH of the medium was varied from 7.5 to 9.0 for oxidation reaction. The equivalent amount of potassium ferrocyanide produced was titrated in an acidic medium with zinc sulphate in presence of the remaining potassium ferricyanide together with the reaction product. The effect of pH on the results is noticeable. The results in Table 1 showed that a pH range from 8.0 to 8.5 was suitable for the reaction to be quantitative with low percentage of error. However, the reaction failed to give desired results as the pH was either decreased to 7.5 or increased to 9.0.

TABLE 1. EFFECT OF pH ON THE DETERMINATION OF THIAMINE USING $ZnSO_4$, 0.0006M, AS TITRANT.

pH of the medium	Thiamine (mg)	$ZnSO_4$ 0.0006M (ml)	Corrected amount (Found, mg)	±Of Difference
7.5	0.02	0.1	0.0199	0.50
	0.04	0.2	0.0399	0.25
	0.08	0.3	0.0599	25.12
	0.12	0.4	0.0798	33.50
8.0	0.02	0.1	0.0199	0.50
	0.04	0.2	0.0399	0.25
	0.08	0.4	0.0798	0.25
	0.12	0.6	0.1196	0.33
8.5	0.02	0.1	0.0199	0.50
	0.04	0.2	0.0399	0.25
	0.08	0.4	0.0798	0.25
	0.12	0.6	0.1196	0.33
9.0	0.02	0.07	0.0139	30.50
	0.04	0.10	0.0199	50.25
	0.08	0.30	0.0598	25.25
	0.12	0.50	0.0997	16.91

Role of acid and oxidant strength for successful titration. The reaction is thought to involve titration of ferrocyanic acid in presence of potassium ferricyanide with zinc sulphate. The medium thus needed to be acidified. A quantiy 0.5 ml each of concentrated sulphuric acid and ortho-phosphoric acid was found to give sharper end point with 0.6 mM zinc sulphate using diphenylamine indicator. The use of concentrated sulphuric acid in larger quantity (1 ml or over) affected in the location of end point due to blackening of the titrating solution.

The strength of potassium ferricyanide was also found to affect the results. The procedure worked well with 0.4 mM potassium ferricyanide but as the strength was increased to 0.1M there was obtained a precipitate during titration with zinc sulphate. The procedure was thus restricted to the use of very dilute solution of the oxidant.

Concentration effect. In order to determine the range of concentration for which the procedure is applicable, experiments were carried out using pure vitamin B_1 solutions of 0.010, 0.020 and 0.05 mg ml^{-1} strengths. Table 2 illustrates interesting points regarding titration results of the vitamin at different concentrations. It was observed that scattering of the results was more associated with vitamins at higher concentration (0.05 mg ml^{-1}). The scattering of the results was completely absent for solutions studied at lower concentrations, e.g., 0.01-0.0 mg ml^{-1}. This suggests that the method is suitable for smaller quantities determination. However, concentration of 0.02 mg ml^{-1} of vitamin was selected for all further investigations.

TABLE 2. CONSUMPTION OF TITRANT ($ZnSO_4$, 0.0006M) AS A FUNCTION OF THE AMOUNT OF THIAMINE AT ITS DIFFERENT CONCENTRATIONS.

Thiamine mg ml^{-1}	Size of thiamine solution (ml)	$ZnSO_4$ 0.0006M (ml)	Thiamine (mg) Expected	Found	±% Difference
0.010	1.0	0.05	0.010	0.0099	1.00
	2.0	0.10	0.020	0.0199	0.50
	3.0	0.15	0.030	0.0299	0.33
	4.0	0.20	0.040	0.0399	0.25
	5.0	0.25	0.050	0.0499	0.20
	6.0	0.30	0.060	0.0599	0.16
	7.0	0.35	0.070	0.0699	0.14
	8.0	0.40	0.080	0.0799	0.12
0.020	1.0	0.10	0.020	0.0199	0.50
	2.0	0.20	0.040	0.0399	0.25
	3.0	0.30	0.060	0.0599	0.20
	4.0	0.40	0.080	0.0799	0.12
	5.0	0.50	0.100	0.0987	1.30
	6.0	0.60	0.120	0.1198	0.16
	7.0	0.70	0.140	0.1398	0.14
	8.0	0.80	0.160	0.1595	0.31
0.050	1.0	0.20	0.050	0.0399	20.20
	2.0	0.45	0.100	0.0899	10.10
	3.0	0.65	0.150	0.1298	13.40
	4.0	0.80	0.200	0.1590	20.50
	5.0	1.00	0.250	0.2109	15.60
	6.0	1.25	0.300	0.2497	7.60
	7.0	1.40	0.350	0.2797	20.00
	8.0	1.55	0.400	0.3096	22.60

Application. The procedure has been evaluated by applying it to the determination of vitamin B_1 in tablets, capsules, injectables, syrups and laboratory made mixtures. Detection in the lower concentration range 0.01 to 0.02 mg ml^{-1} was a practical preposition. Table 3 presents results of the vitamin B_1 in pharmaceutical products determined singly or in the presence of other B-vitamins, e.g., B_2, B_6, nicotinamide and B_{12}. The percentage difference in the determination is negligible, hardly being more than 0.01%. It is concluded that the procedure is reliable and free from interference not only by the B-vitamins but also the excipients and lubricants present in the preparations. Table 4 are the results of vitamin B_1 obtained from the laboratory made mixture. The results further illustrated that the presence of other vitamins did not interfere in the determination of vitamin B_1 and the procedure is quantitative, reproducible and interference free.

In conclusion the method is novel in the sense that the reaction is simple and free from any side reaction as far as the analysis of pharmaceutical products is concerned.

TABLE 3. QUANTITATIVE DETERMINATION OF VITAMIN B_1 IN PHARMACEUTICAL PRODUCTS.

Products	Label claim/ composition	Found quantity (mg)	Difference ±%
"Berin" Each tablet contains: 99.99	0.01		
Tablet (Glaxo)	Thiamine (100 mg)		
"Berin"	Each ampoule contains:	100.00	0.00
Injection	Thiamine (100 mg)		
(Glaxo)			
"Thiancuron"	Each capsule contains:	100.00	0.00
capsule	Thiamine mononitrate		
(Pfizer)	(100 mg) Pyridoxine hydrochloride (200 mg), Cyanocobalamine (200 µg)		
"Plexovit"	Each 15 ml contains:	2.99	0.0
Syrup	Thiamine hydrochloride		
(Remington)	(3mg), Riboflavin (3 mg) Psyridoxine hydrochloride (2 mg), and Nicotinamide (23 mg)		

TABLE 4. QUANTITY OF VITAMIN B_1 DETERMINED IN A LABORATORY MADE MIXTURE CONTAINING B_1, B_2 AND B_6.

Theoretical quantity (mg)	Found quantity (mg)	±% Difference
0.02	0.02	0.0
0.04	0.04	0.0
0.06	0.06	0.0
0.08	0.08	0.0
1.20	1.20	0.0

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