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STUDIES ON THE STABILITY OF THIAMINE HYDEROCHLORIDE IN THE LIQUID B-COMPLEX PREPARATIONS

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Abstract

Three formulations of vitamin B-complex syrups were designed in the laboratory and stored at room temperature, 37°C and 45°C for a period of 6-12 months.

Separation and quantitative estimation of thiamine hydrochloride from these B-complex preparations was done by ascending one dimensional thinlayer chromatrogrpahy using a solvent system pyridine: acetic acid: n-propanol and water in the ratio 5:5:60:40. The detection is based upon oxidation of thimine hydrochloride spots to thiachrome on T.L.C. plates by spraying it with K_3FeCN_6 solution. Thiochrome spots were then eluted with isobutanol and measured fluorometrically. This experiment was repeated with the same B-complex preparations after four months and eight months to determine its stability.

Introduction

Liquid B-complex preparations are usually manufactured with the help of excipients, adjuvants, vehicles, and preservatives etc. Among all these varieties, the oral vitamin liquid preparations particularly exhibit the stability problem due to interaction of mixed substances, hydrolysis etc. Interaction between thiamine and riboflavin, thiamine and pantothenic acid, thiamine and cyanocobalamin, riboflavin and pantothenic acid etc, were found to occur often in all types of dosage forms, especially in liquid preparations containing water. Merck (1960) has found that even inert formulation additives and diluents may be involved in poorly stable products, if they supply moisture, alter pH, introduce trace metals or other reactive contaminants.

The therapeutic efficacy of thiamine in the B-complex liquid preparations is of very doubtful nature, since thiamine being compound of thiazole and pyrimidine type easily hydrolyses in a aqueous solution. On exposure to air of average humidity, the vitamin absorbs water forming a hydrate. Under high humidity it easily deliquesces. Despite its variable moisture content

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(upto 5%) it is stable as a solid at room temperature and at moderate high temperature even in contact with air. In aqueous solution it is stable at pH 2.5 to 4.5 and at low temperature (4°C) it is fairly stable at room temperature. Windheuser et al., (1962) had studied kinetics of thiamine hydrolysis at different pH values and suggested pH-2 as an optimum value for it stability which decreases with the increase of pH. Sabri, Mohar and Rao (8) reported that the stability of thiamine in water at pH-2 and 37°C was 100% for six months. They found that at pH-4, the best vehicle was 5 to 80% glycerine solution in water, or 10 to 80% glycerine syrup and cane sugar syrup was better vehicle than glycerine for Thiamine.

Merck (1960) has found that thiamine interacts with riboflavin, pantothethic acid and cyanocobalamin.

Another important factor of thiamine unstability in complex mixture is its oxidations. The oxidising agent converts thaimine to thiochrome which has no vitamin activity. Sometimes the atmospheric oxygen also forms small amount of thiochrome in alcoholic solution.

The sugar concentration of B-complex preparations have also critical values. It has been found out that components are only separated in formulations containing upto 58% sugar on a TLC chromatogram, while most of the formulations especially syrup preparations contain a high amount of sugar. Besides sugar, glycerine is the frequently used base in these preparations and there are evidences that a high proportion of glycerine in elixirs interferes with the drug absorption by delaying the emptying of stomach.

From above discussion it appears that the therapeutic efficacy of Thiamine in liquid multivitamin preparation is of doubtful nature. Though the manufacturers claim that they add overdosage in order to compensate the problem of stability, yet to satisfy each component of B-complex product which has quite different stability kinetics is suspecious and these factors might by responsible for the low blood absorption of Vitamin-B_1 in case of complex form when compared to that of in single vitamine-B_1 product.

Material and Methods

Thiamine hydrochloride was obtained from Merck and was found to be pure chromatographically. Sucrose was obtained form Sandoz Pharmaceuticals. It was of Phrmaceutical grade. All the other reagents and materials used were of A.R. grade and were used as such.

The method of separation of vitamins adopted in this work is ascending one dimentional thin layer chromatogrpahy. A new solvent system pyridine acetic acid: n-propanol: water in the ratio 5:5:60:40 was developed by trial and error method.This solvent system separated the B-vitamins from solutions containing upto 56% sugar. Thiamine hydrochloride spots on T.L.C. plate was then oxidized to thiochrome by spraying it with 2% [$(K_3$ Fe) CN_6] solution. Thiochrome was then eluted with isobutanol and measured fluorimetrically.

Preparation of the standard Cuve

Six spots of the standard thiamine hydrochloride solution were made with the help of micropipette adding 0.001, 0.002, 0.004 0.006, 0.008, 0.01 ml aliquots respectively. The spots were made 2 cm. apart from each other and 1.5 cm above the lower adge of the plate. The cocnentration of thiamine in these spots were 0.1, 0.2, 0.4, 0.6, 0.8 1.0 µg. respectively. A sample solution was also spotted in the same way. The plate was then dried for about two minutes in air and then developed with the solvent system and then re-dried for three minutes in a drier at 50C°.

Then each individual spot of thiochrome was scratched off the plate cmpletely along with silica gel by a sharp razor into oil papers and then transferred to a 1.5 ml. centrifuge tubes marked with respective standard concentration. The spot due to assay solution was also transferred in a centrifuge tube in the same way. An amount of silica gel equivalent to a thiochrome spot was scratched off form the same plate and put into another centrifuge tube which served as blank.

To all the nine tubes 10 ml. of distilled isobutanol was added and shaken well to dissolve the thiochrome spots. The tubes were then centrifuged for five minutes at 20000 r.p.m. The slica gel particles settled at the bottom of the tube. The clear supernatant isobutanol solution were then decaunted into clean and dry column cuvettes. The final concentration per ml. of isobutanol solution in standards were then 0.01, 0.02, 0.04, 0.06, 0.08, 0.1 Mcg respectively. The concentration of thiochrome in each cuvette was then determined fluorimetrically.

Calculations

$$\text{Amount of Thiamine} = \frac{S \times V \times 100 \times D \times C}{1000} \text{ mg/100 of the sample solution}$$

where $S = \dfrac{\text{1 ml. of diluted sample solution}}{\text{Vol. of diluted sample solutions spoted.}}$

V = Volume of isobutanol used to extract thiochrome in ml.

D = Initial dilution of 1 ml. of assay sample

C = Concentation in µg obtained from the standard curve.

Results and Discussion

The B-complex vitamins, added to the syrups, were THIAMINE hydrochloride (B_1) riboflavin-5-phosphate monosodium hydrate (B_2), pyridoxine hydrochloride (B_6), NIACINAMIDE, d-pantethenol and cyanocoblamin (B_{12}). All these vitamins are usually present in most of the commercial formulations of B-complex syrup available in the market.

In the three formulations prepared, most of the vitamins were used in the salt form instead of the pue one for reasons of solubility and stability. Riboflavin has a relatively low solubility in aqeous solutions. Riboflavin-5-phosphate monosodium hydrate, in equivalent amounts, was used. The later has a higher solubility than riboflavin(Merck, 1960). Pantothenic acid, being quite unstable in aqueous solution has been used in the alcoholic form in most of the multivitamin preparations, hence the more stable d-pantothenyl alcohol, in equivalent amounts, was preferred.

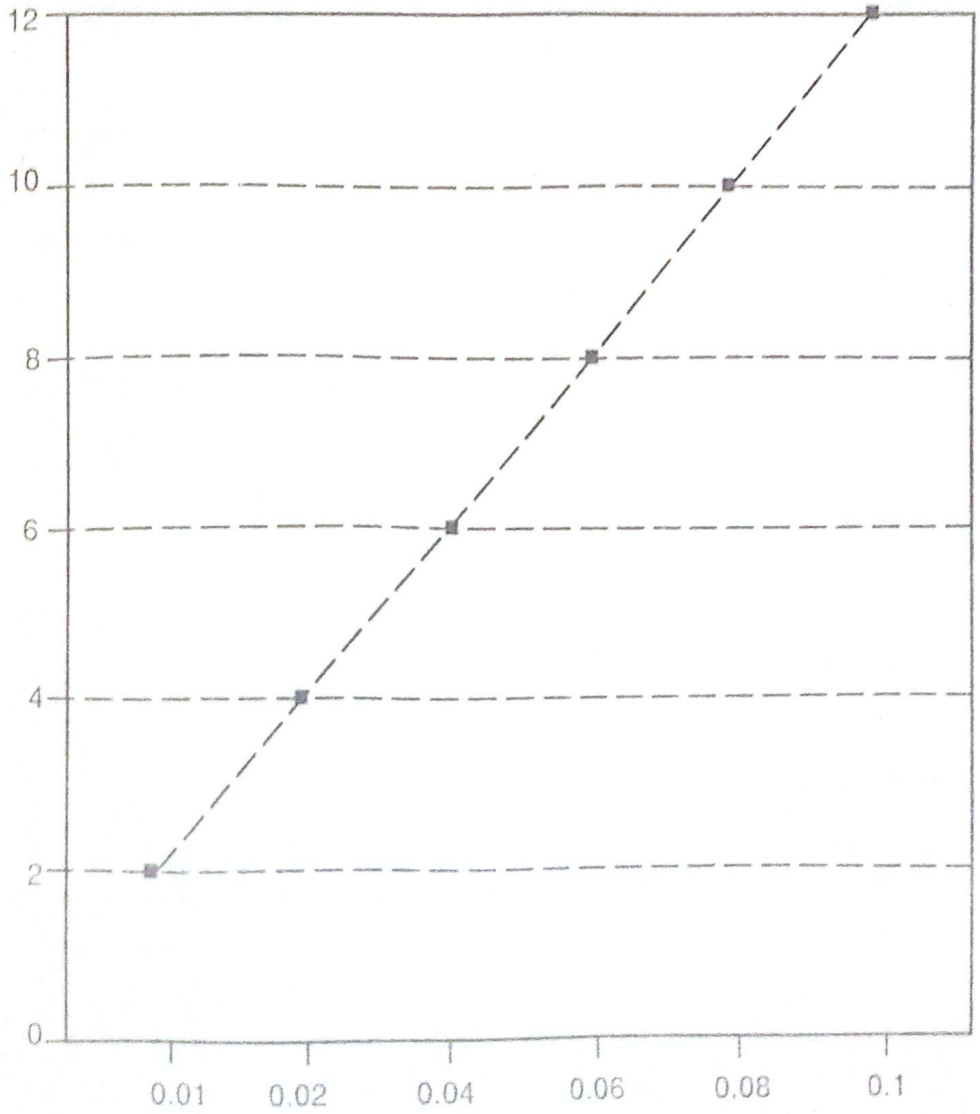
The pH of the syrups was maintained at 3.5 by the use of citric acid in appropriate amounts. Citric acid would also act, as a buffering agent. The normal range of pH for the maxium stability of B-complex vitamins is from 3.5 to 4.5 reported in the literature (Misawa, et al., 1966; Sabri and Mohan, 1965; Windheuser and Higuchi, 1962).

The concentrations of the B-complex vitamins added to the laboratory prepared syrup formulations approximate the minimum dosage requirements per unit volume (0.6 cc) and approximate the amount present in the literature. Thus, representative formulations of uniform composition were prepared to provide a guide line, as a comparative basis, to the stability of commercial preparations under similar storage conditions.

The therapeutically inactive ingredients, i.e., the sweetening agents, buffering agents and the preservative were used in appropriate amounts sufficient to produce the desired effect or quality in the syrups.

The overall analysis shows that most of the B-complex preparations have a low potency than the labelled amount after a few months from four to eight (Table 4). It indictes a serious lack of control of potency and marketing of such products. Most of the commerical preparations contain sugar in the range of 60-70% where as separation is quantitative only upto

56% sugar. Other than the interactions between different analogues of vit-B, sugar may be one important factor. In various aqueous vehicles used for B-complex syrups some of the vitamins may change to less active or inactive compounds which may again influence the destruction of the others. Macleod and compell (1955) have shown that a high percentage of the multivitamin products are substandard in relation to the labelled potency. Hence the problem of vitamin combinations and their usefulness should be approached in a rational way, with due consideration of the conditions udner which simultaneous administration of vitamins is justified.



Standard curve for the assay of thiamine
Fig. 1

Table-1

Following Three B-complex Formulatons Were Prepared for the Study

Ingredients	Formulatoin	Formulation	Formulation
Vitamin B_1 (Thiamine hydrochloride)	30 mg	66.50 mg	83.33
Vitamin B_2 (Riboflavin)	120 mg	120 mg	120 mg
Vitamin B_6 (Pyridoxin Hydrochloride)	40 mg	40 mg	40 mg
Niacinamide	800 mg	800 mg	800 mg
D-panthenol	100 mg	100 mg	100 mg
Vitamin B_{12} (Cyanecebalamin)	100 m.c.g.	100 m.c.g.	100 m.c.g.
Citric Acid	600 mg	800 mg	800 mg
Methyl paraben	150 mg	150 mg	150 mg
Potassium Sorbate	50 mg	45 mg	50 mg
Sugar crystalline	55 mg	45 mg	70 mg
Distilled water q.s. to make		100 ml	100 ml

Table-2

Data Showing the Relation between the Fluorimetric Reading and the Concentration of Thiamine in the Standard Series

Conc. No ug/M1	Fluorimetric Readings	Average Readings	+ Standard - Deviation
0.01	2.3 2.1 2.4	2.26	± .125
0.2	3.4 3.5 3.3	3.40	± .10
0.04	5.5 5.4 5.2	5.36	± .125
0.06	7.4 7.5 7.3	7.40	± .10
0.08	9.7 9.5 9.3	9.50	± .20
0.1	11.4 11.3 11.4	11.36	+ 0.38

Table-3

Data and Result of Thiamine Determination in Liquid Preparations Sample Amount Spotted = 0.01 ml.

Sample No.	Vitamin	No.Of Detn. Soln.	Diln.Made ML Sample STD. Curve After TLC, Separation In ug.	Av.Amount Detnd.Form ML After TLC Separation.	Stated Amt./100 Tlc Separation.	Av.Ant Detnd/ 100 ML After TLC Separation	% Stated Amt.After TLC Separation
1.	B_1	2	15	0.187	30	33.4	111.3
2.	B_1	2	6	0.11	66.6	65.93	98.9
3.	B_1	2	7	0.129	83.33	90.27	108.32

Table-4

Stability of Thiamine in B-complex Preparations

Sample No	Vitamin	No.Of Detn.	Aver: Amt. Detd./100 ML TLC Separation In mgm.	Average Amt. Determined/ 100 ml by the same Method After 4 Months In mgm.	% Reduction of Vitamin.
1.	B_1	2	33.4	28.3	15.2
2.	B_1	2	65.93	60.05	8.9
3.	B_1	2	90.27	55.14	38.9

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