Specificity and relative efficacy of different assay methods for thiamine hydrochloride in Vitamin B-Complex syrups.

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Abstract

Vitamin B-complex syrups were prepared using locally manufactured sucrose and liquid glucose vehicles, and stored at room temperature, 37°C and 45°C, for 6-12 months to evaluate the stability of thiamine hydrochloride. The stability testing of the syrups has been designed to determine quantitatively and qualitatively the changes which they undergo during storage.
The chemical stability has been studied using four different assay methods. The specificity and relative efficiency of the methods have been compared and the U.S.P. fluorimetric method has been found to be the most accurate and specific for the assay of the vitamin. (JPMA 35 : 179, 1985).

Introduction

In oral multivitamin preparations the usual vehicles consist of aqueous mixtures of propylene glycol, glycerine, ethanol, sucrose syrup and similar solvents, common to pharmaceutical elixirs and syrups adjusted to a suitable pH. Sherry wine, honey and cora syrup (liquid glucose) are sometimes found as constituents in the base formulations.[1] The correct choice of a vehicle often makes it possible to present a stable preparation of vitamins. The use of a vehicle which would dissolve the vitamin mixture and has a minimum amount of water content, helps to increase the stability of the vitamin preparations.[2]
Various factors such as the nature and the pH of the medium concentration of the vitamins, temperature, light, oxygen and catalysis by metals etc., significantly influence the stability of vitamins. Several excellent works on drug and vitamin stability and incompatibility are available.[3-6]
Thiamine hydrochloride, the vitamin B,, is highly thermolabile and one of the least stable of the B-complex vitamins in aqueous solutions. It may undergo oxidation and hydrolysis in liquid vitamin preparation unless appropriate stabilizing means are taken. In addition thiamine hydrochloride possesses reactive groups, which may enable it to interact with other vitamins and chemical agents. Therefore, use of a particular aqueous vehicle among other factors may greatly influence the stability of thiamine hydrochloride in B-complex syrups.

Materials

Thiamine Hydrochloride: (3-(4-amino-2-methyl-5 –pyrimidinyl-methyl)-5-(2-hydroxyethyl)-4-methyithiazolium chloride hydrochloride.)
Thiamine hydrochloride was obtained from Merck & Co. and was found to be pure chromato-graphically. It was dried before use at 105°C for 2 hours and stored in a tight, amber neutral glass bottle in desiccator over dried silica gel. The sample contained 100% of thiamine hydrochloride as per B.P. assay method.
Riboflavin: (6,7-dimethyl-9-(D-1-ribityl) isoalloxazine).
Riboflavin was obtained from Hoffmann La. Roche. It was dried before use at 105°C for 2 hours and stored in tight, amber bottle in a desiccator over silica gel.
Pyridoxine Hydrochloride : (3-\Hydroxy4, 5-di (hydroxymethyl)-2-methylpyridine hydrochloride.
Pyiidoxine hydrochloride was obtained from "Merck & Co." and dried before use under vacuum over

silica gel for 4 hours. It was then stored in tight, amber bottle in a desiccator over silica gel.
Cyanocobalamin: (5,6-dimethylbenzimi-dazol-2-yl) cobamide Cyanide.
Cyanocobalamin was obtained from "Merck Sharp & Dohme (Pakistan) Ltd." It was dried at 105°C for 2 hours and stored in tight, amber bottle in a desiccator over silica gel before use.
Niacinamide: (Nicotinamide) Pyridine-3- carboxyamide.
Niacinamide was obtained from "Pak Bofors, (Wah) Pakistan." It was dried before use over silica gel for 4 hours and stored in a tight container in a desiccator.
Dexpanthenol: (d-Pantothenyl Alcohol;(D(+)-2,4-dihydroxy-N-(3-hydroxypropyl)-3,3-dimethylbutyramide).
It was obtained from "Roche Chemical Division Hoffmann La. Roche Inc. NewJJersey."
Citric Acid: (C6 H8 07 ,H2 0)
It was obtained from B.D.H. and used as such.
Methylparaben: (Nipagin M , Methyl p- hydroxybenzoate).
It was obtained from "Heyden Newport Chemical Corporation". It was dried over silica gel for 5 hours and stored in a tight bottle in a desiccator.
Sodium Cyclamate: (Sucaryl Sodium: Sodium Cyclohexanesulfamate).
Sodium cyclamate was obtained from Abbott Laboratories. It was dried before use at 105°C for 1 hour and stored in a tight bottle in a desiccator over silica gel
Postassium Sorbate: (2,4-hexadienoic acid Potassium salt).
It was obtained from Chas, Pfizer & Co. Inc. and used as such.
Glycerine: (Propane-1,2,3-triol)
It was obtained from Crescent (Pakistan) Industries and used as such.
Sucrose:(Ci 2 H2 2O11, sugar,caned sugar).
Sucrose was obtained from Crescent Sugar Mills. It was of Pharmaceutical grade and complied with the following U.S.P. specification.
(1)Description Colourless crystals, having
eign matter.
Specific + 65.9, determined in a
Totation solution containing 2.6 Gm. in each 10 ml.
The sample having been previously dried at 105°C for 2 hours.
(3)Residue on
ignition 0.11%
(4)Chloride To 10 ml. of a solution
in 10) add 1 ml. of silver nitrate
S: no opalescence is produced
with in 1 minute.
(5) 60p.p.m.
(6) CalciumTo 10 ml of solution
(1 in 10) add 1 ml of ammonium oxalate T.S: the solution remains clear for at least 1 minute.
7)Heavy metals 5 p.p.m.
(8)Invert sugar 0.3%
iquid Glucose: (Starch syrup, Corn syrup).
Liquid glucose (LF 56 and LF 59 grades) were obtained from Glaxo Laboratories (Pakistan) Ltd.
Water: Freshly boiled glass distilled water has been used through out the work.

Result and Discussion

Choice of the Assay Methods

The gravimetric method (B.P.) was used initially as a standard assay procedure. The reproducibility of the method was checked using pure thiamine hydrochloride (50 and 25 mg) and was found to be ± 0.10% (mean of 3 determinations). When this method was applied to samples, stored at room temperature, after two months period, the values were found to be higher in all cases to the extent of 25%. Since thiamine hydrochloride is an unstable vitamins and would normally degrade in the syrups on storage, it was thought advisable to use another assay method to check the accuracy of the gravimetric method. Hence the colorimetric method was applied to the assay of thiamine hydrochloride. Though the results obtained by this method appeared to be satisfactory in the case of formulation I, however the values obtained were unexpectedly higher to the extent of 60% in the case of formulations II and III. The reasons for such results are to be discussed later. At the end of the storage period the samples were also assayed by the fluorimetric method (U.S.P.) and the multicomponent spectrophotometric method. Thus, an attempt has been made to compare the efficiency of four different assay methods.

Acid-dye Colorimetric Method: The method is based on the salt formation of thiamine with an acid-dye, bromothymol blue, and measurement of the absorbance at 420 mu, after extraction of the yellow dye salt with chloroform at pH 6.6, against an appropriate reagent blank.

The calibration curve used for the assay of thiamine hydrochloride in B-complex syrup is given in Figure.

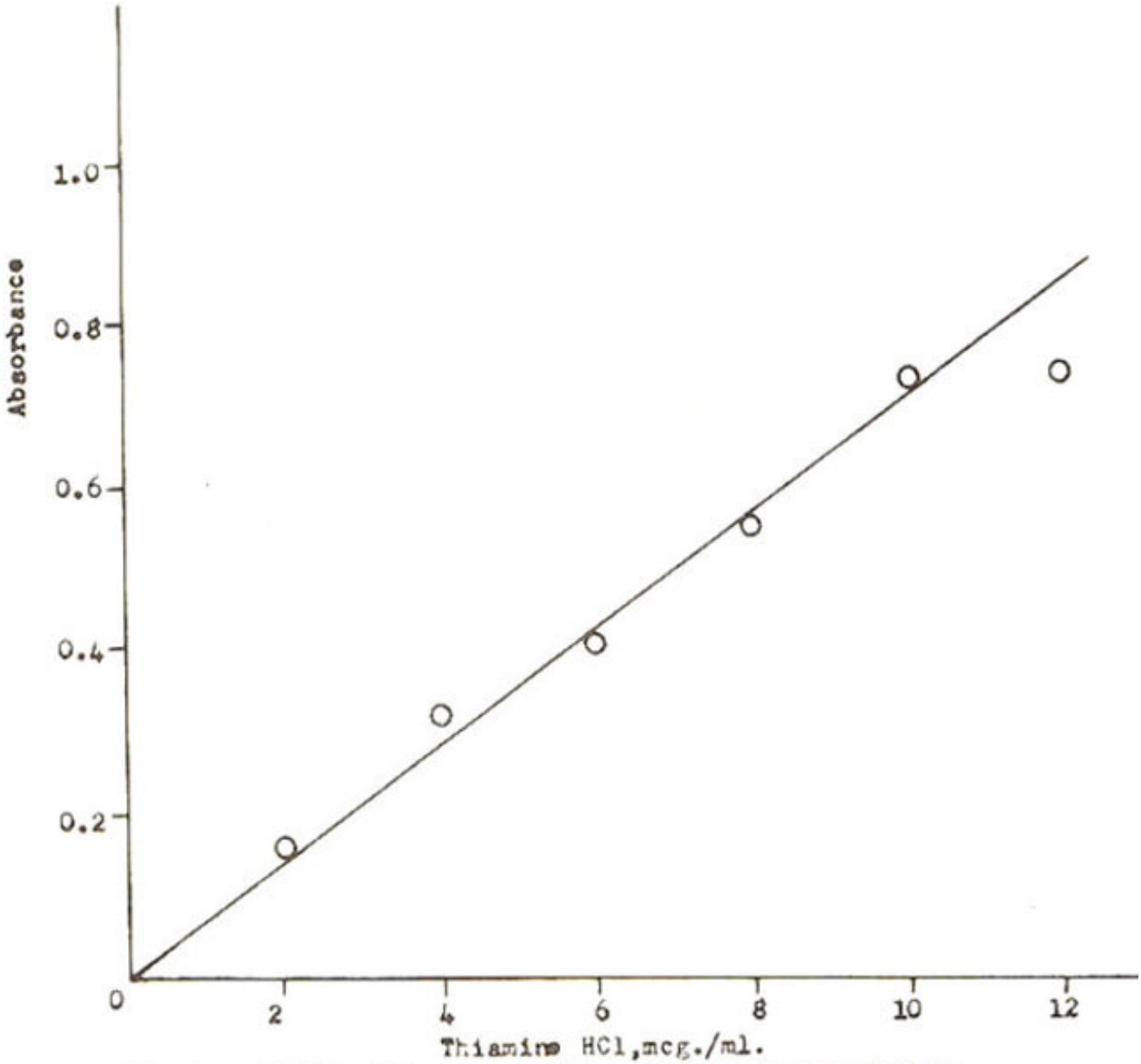

Fig.1 Calibration curve for thiamine hydrochloride.

The Beer's law relationship was found to follow within the concentration range of 4-10 mcg/ml of thiamine hydrochloride. This is in agreement with the observation by others7 :However, the method did not prove to be as precise as claimed by these authors. The reproducibility of the results based on 10 determinations (7.5 mcg/ml)has been found to be 7.40 ± 0.06 (average deviation). This value is of the same order as that reported by Gupta and Cadwallader (4.00 ± 0.04) versus U.S.P. method (4.00 ± 0.05). It appears that inspite of the experimental errors involved and the percentage difference (about the mean) in the absorbance values used for the preparation of the final calibration curve, the method works well for the assay of pure thiamine. When the method was applied to B-complex syrups, the results did hot agree with the U.S.P., method. The possible explanation for this is to be discussed later. The standard deviation (degree of spread of experimental data) and the coefficient of variation (ratio of standard deviation to mean) are reported in Table-II-IV, which give a measure of the precision of the acid-dye colorirnetric method.

Table –I

Precision of the Acid-dye Colorimetric Method.

	Calculation of Standard Deviation and Coefficient of Variation					
N (detmn.)	X (conc. in/ug/ml)*	(mean)	$(X-\bar{X})$	$(X-\bar{X})^2$	$6 = \frac{(X-\bar{X})^2}{N-1}$ (Standard Deviation)	$\frac{6}{\bar{X}} \times 100$ (Coefficient of variation)
1	7.45	7.40	+ 0.05	0.0025	0.07	0.95
2	7.39		– 0.01	0.0001		
3	7.48		+ 0.08	0.0064		
4	7.35		– 0.05	0.0025		
5	7.43		+ 0.03	0.0009		
6	7.36		– 0.04	0.0016		
7	7.44		+ 0.04	0.0016		
8	7.30		– 0.10	0.0100		
9	7.50		+ 0.10	0.0100		
10	7.30		– 0.10	0.0100		
	Σ = 74.00			Σ = 0.0456		

* The reproducibility of the results based on 10 determinations (7.5 mcg/ml); average deviation 7.40–0.06.

Table II

Stability of B–Complex Vitamins After 12 Months Storage at Room Temperature.

		Concentrations of Vitamins in B–Complex Syrups (Multicomponent Spectrophotometric Assay									
Formulation & Vehicle	Time in Months	Thiamine Hydrochloride. (Vitamin B_1)		Riboflavin (Vitamine B_2)		Niacinamide (Vitamin B_3)		Pyridoxine Hydrochloride. (Vitamin B_6)		Cyanocobalamin (Vitamin B_{12})	
		mg/ml	% Retention	mg/ml	% Retention	mg/ml	% Retention	mg/ml	% Retention	ug/ml	% Retention
I Sucrose	0	1.50	100.00	1.20	100.00	8.00	100.00	0.40	100.00	1.00	100.00
"	12	6.00	400.00	1.38	115.00	11.20	140.00	1.60	400.00	–76.80	
II Liq.gl. LF-56	0	1.50	100.00	1.20	100.00	8.00	100.00	0.40	100.00	1.00	100.00
"	12	4.80	320.00	1.50	125.00	13.60	170.00	2.00	500.00	–57.60	
III Liq.gl. LF–59	0	1.50	100.00	1.20	100.00	8.00	100.00	0.40	100.00	1.00	100.00
"	12	5.16	344.00	1.50	125.00	11.60	145.00	1.12	280.00	–68.80	

Table III Stability of B–Complex Vitamins After 6 Months Storage At 37°C.

Formulation & Vehicle	Time in Months	Concentrations of Vitamins in B–Complex Syrups (Multicomponent Spectrophotometric Assay)									
		Thiamine Hydrochloride. (Vitamin B_1)		Riboflavin (Vitamine B_2)		Niacinamide (Vitamin B_3)		Pyridoxine Hydrochloride. (Vitamin B_6)		Cyanocobalamin (Vitamin B_{12})	
		mg/ml	% Retention	mg/ml	% Retention	mg/ml	% Retention	mg/ml	% Retention	ug/ml	% Retention
Sucrose	0	1.50	100.00	1.20	100.00	8.00	100.00	0.40	100.00	1.00	100.00
"	6	5.60	373.33	1.50	125.00	11.44	143.00	3.44	860.00	11.20	
II Liq.gl. LF-56	0	1.50	100.00	1.20	100.00	8.00	100.00	0.40	100.00	1.00	100.00
"	6	8.40	560.00	1.57	130.83	8.80	110.00	2.16	540.00	-69.60	
III Liq.gl. LF–59	0	1.50	100.00	1.20	100.00	8.00	100.00	0.40	100.00	1.00	100.00
"	6	6.80	453.33	1.38	115.00	7.60	95.00	1.48	370.00	-61.60	

Table III Stability of B–Complex Vitamins After 6 Months Storage At 45°C.

Formulation & Vehicle	Time in Months	Concentrations of Vitamins in B–Complex Syrups (Multicomponent Spectrophotometric Assay)									
		Thiamine Hydrochloride. (Vitamin B_1)		Riboflavin (Vitamin B_2)		Niacinamide (Vitamin B_3)		Pyridoxine Hydrochloride. (Vitamin B_6)		Cyanocobalamin (Vitamin B_12)	
		mg/ml	% Retention	mg/ml	% Retention	mg/ml	% Retention	mg/ml	% Retention	ug/ml	% Retention
I Sucrose.	0	1.50	100.00	1.20	100.00	8.00	100.00	0.40	100.00	1.00	100.00
"	6	6.4	426.66	1.50	125.00	10.80	135.00	7.20	1800.00	13.12	
II Liq.gl. LF-56	0	1.50	100.00	1.20	100.00	8.00	100.00	0.40	100.00	1.00	100.00
"	6	4.80	320.00	1.50	125.00	11.60	145.00	1.60	400.00	-432.00	
III Liq.gl. LF–59	0	1.50	100.00	1.20	100.00	8.00	100.00	0.40	100.00	1.00	100.00
"	6	7.12	441.33	1.38	115.00	12.12	151.50	1.88	470.00	-160.00	

Multicomponent Spectrophotometric Method:-The method is based on the use of determinants in the calculation of multicomponents spectrophotometric analysis of vitamin preparations[8] The vitamin preparation contained (a) thiamine hydrochloride, riboflavin-5'-phosphate, niacinamide and pyridoxine hydrochloride, (b) thiamine hydrochloride, riboflavin-5 '-phosphate, pyridoxine hydrochloride and cyanocobalamine. The determination were carried out at pH 2.0 (phosphate buffer). Riboflavin was estimated independently at 446 rim., where there was no interference due to other component. For the rest of the analysis a correction factor was applied to subtract the absorbance. From the remaining total absorbance, the concentrations of thiamine hydrochloride, niacinamide and pyridoxine hydrochloride

were calculated. An attempt has been made to estimate cyanocobalamine at 361 rim., after the subtraction of absorbance due to riboflavin. The values obtained indicate that such low concentrations of vitaminB_{12} cannot be determined accurately in the presence of other vitamins.

Quantitative Determination of Thiamine Hydrochloride in Syrups:

The attempts made on the assay of thiamine hydrochloride in different formulations, in the initial stages of storage suggested that the B.P. gravimetric method is not suitable for such complex mixtures without some modification and hence the recently developed acid-dye colorimetric method, which is claimed to be specific for thiamine hydrochloride in pharmaceutical preparations[7] was applied. However, the concentration values obtained appeared to be lower or higher than those expected and hence two other methods i.e., the U.S.P., fluorimetric method and the multicomponent spectrophotometric method[8] were tried.

Since the fluorimetric method is considered to be the most specific of all the methods in general use and is the official assay for thiamine hydrochloride in U.S.P., it has been taken as an standard assay method. The concentration values obtained by other methods have been compared with those obtained by U.S.P., method and the reliability of these method in such assays is discussed in the light of U.S.P., method. As the B.P., method gave higher values, it was not used except in case of syrups stored at room temperature. This may be due to the interference of nitrogenous substances such as riboflavin, niaci-namidi, pyridoxine-HCI and cyanocobalamine which may give higher results. The gradual rise observed in assay values (Formulation II & III) by acid-dye method in all formulations may be due to interference of degradation products. The concentrations of vitamin B1, B2, 83, 86, and 812 determined by multicomponent analysis are reported in Table-II,IV.

Formulation I (Sucrose Vehicle): The percentage retention of thiamine hydrochloride obtained by U.S.P., method, after 12 months storage at room temperature, shows that the value has decreased slightly (4%). The B.P. method gives a 6% higher value which, though within the limits of the experimental error in such a formulation, is not in accordance with the expected behaviour of thiamine hydrochloride on storage for such a long period. The syrups showed some evidence of the degradation of thiamine hydrochloride by thinlayer chromatography. The acid-dye method gave 40% decrease in %age retention after 12 months, while the spectrophotometric method showed much higher value than the initial concentration. It appears that the acid-dye method is capable of determining thiamine hydrochloride in such formulations but the values are lower than those obtained by U.S.P., method. The higher values found by the spectrophotometric method are possibly due to the complexity of such a mixture and from the interference of the degradation products which may contribute significantly and for which no irrelavent absorption correction was applied. Moreover, a multicomponent spectrophotometric assay needs a very high degree of accuracy which is not possible with the spectrophotometer (Unicam SP800) used for the present work.

The concentration of thiamine hydrochloride determined by the U.S.P., method in formulation Istored at 37°C (Table -II) indicates a 17% decrease in the percentage retention after 6 months. The acid(-)dye method showed gradual decrease in concentration and after six months 42% retention was recorded. The spectrophotometric method gave much higher value which may be due to the reasons mentioned above. At 45°C the U.S.P., method showed 30% decrease in the percentage retention and the acid-dye method showed 83% decrease in the %age retention, while the spectrophotometric method, as usual, produced much higher values. The gradual decrease in the concentration of thiamine hydrochloride, at an increase in temperature, is in accordance with the fact that the rise in temperature accelerates degradation.

Considering the U.S.P., method as standard, the acid-dye method has given relatively low values and the gradual decrease in %age retention, with time, would indicate that the method though specific for the assay of thiamine hydrochloride gives a constant error which probably depends on the nature of the interference. The spectrophotometric method, as mentioned earlier, would not give reliable results in

the presence of many interfering substances which are produced as a result of the degradation of the complex vitamin on prolonged storage. Such substances would probably absorb at one or more wavelengths chosen for the purpose of analysis and thus lead to an increase in the determined concentrations.

Formulation II (Liquid Glucose LF-56 Vehicle):

The percentage retention obtained by the U.S.P., method at the end of 12 months storage at room temperature was found to be 90%. The value recorded by the acid-dye method is 104% which is not in agreement with the general concept of the degradation of thiamine hydrochloride on storage for such a long period. The method shows the gradual rise in %age retention after 2 months, with time, indicating that it is not capable of distinguishing between thiamine hydrochloride and any related bases or other vitamin B-complex degradation products which might interfere with the assay. Since, the acid-dye method, showed a gradual decrease in %age retention in formulation I at all temperatures, it can be concluded that in this formulation the nature of the degradation product is different from those of the formulation II which certainly interfere with the specificity of the acid-dye method. This situation is more pronounced in the case of sample stored at 37°C. It is obvious that an increase in temperature would lead to an increase in the amount of degradation and hence a gradual increase in the assay values (instead of a gradual decrease). This would mean increased interference from the various vitamin degradation products or other formulation ingredients whose nature is not known. Since in such a complex preparation after prolonged storage, the qualitative examination of the nature of degradation products, by thin-layer chromatography or spectrophotometric method, is quite complicated and could not be made during the present work. The assay of thiamine hydrochloride in samples stored at 45°C by the acid-dye method also shows unexpectedly higher values with an excess of 64%. This would otherwise not be possible unless the degree of interference by the unknown substances is quite high. In the light of the limited qualitative information available on the nature of the degradation product it may be inferred that such assay values may result from contribution of the various substances present in relatively higher amounts in the syrups. Since it has not been possible to assess the effect, of a particular vitamin of the B-complex group or its degradation products, on the specificity of the acid-dye method, it is not possible to give any definite conclusions about the results obtained. However, Gupta and Cadawallader[7] have claimed on interference from a number of vitamins and other formulation ingredients. It may be possible that SO_2 present to the extent of 125 p.p.m., in the liquid glucose (used as vehicle for formulation II and considered to have better physical properties than the sucrose vehicle) causes the degradation of thiamine hydrochloride to a greater extent than that caused in its absence as observed in sucrose vehicle (formulation I). The suiphurdioxide is known to degrade thiamine hydrochloride and may also affect the stability of other vitamins, thus the nature and composition of the degradation products may be different from those present in formulation I and hence more interference with the acid-dye method, as observed from thç assay values, may be expected. The U.S.P., assay values (70% retention at 37 C and 62% retention at 45 C) after 6 months storage appear morrnal.

The multicomponent spectrophotometric assays, performed on all samples, are not satisfactory. Some of the reasons for obtaining such higher values have already been explained in the above section. Most probably these values are due to interference from the components of the complex mixture in which many ultraviolet absorbing species are present. The B.P., gravimetric method was used only for samples stored at room temperature, but the assay values were found to be 15% higher than the initial concentration after 6 months storage.

Formulation III (Liquid Glucose LF-59 Vehicle):

The U.S.P., method of the assay for the formulation shows 94% retention, while the acid-dye method shows 127% retention after 12 months storage. The latter method indicates also a gradual increase in %age retention which would not normally be expected. Assuming the U.S.P., method as standard, such an increase, in %age retention would perhaps mean some interference of different nature than that

occurring in formulation Il or an inherent defect in the assay method. Since the SO_2 contents of liquid glucose (LF-59) are relatively low, i.e., 50 p.p. m., relatively less degradation of thiamine hydrochloride, due to SO_2 would be expected and hence there will be less contribution from the interfering degradation products to the assay values. The only major difference, a part from SO_2 contents, in liquid glucose used as vehicle for formulation II (LF-56) and formulation III (LF-59) is that in the latter the gravity is slightly higher which would tend to stabilize the vitamin. This is obvious from the U.S.P., assay values. One of the possible explanation for such a behaviour particularly in formulation III could be that since the acid-dye method, when applied to such mixture, shows an increase in the %age retention, and if this is due to some inherent defect, then in the case of two truly degrading solution, the assay values will be higher for the solution showing less degradation. Though US.P., method gives 90% and 94% retention for the formulation II and III(LF56 & 59) respectively after 12 months, the acid-dye method gives 127% and 103 % retention respectively. The validity of the above assumption has not been varified during the present work.

The assay values obtained for samples stored at 37 C and 45 C respectively are also of the similar nature and in the presence of relatively low SO_2 content may be partially explained on the basis of the above assumption. The absorption spectra determined on the samples after 6-12 months storage provide some support in favour of the above assumption. In the presence of the experimental information available there does not appear to be any other possibility for such discrepancies in the assay values; The U.S.P., assay values for sample stored at 37 and 45 C are 80% and 66% respectively which are in accordance with the expected behaviour of thiamine hydrochloride on storage at elevated temperatures. The %age retention obtained by the B.P., and the spectrophotometric method, though higher than expected, are of the same magnitude as those obtained for the formulation II.

Assay of B-complex Vitamins in Syrups Using Multicomponent Spectrophotometric Method.

The assay values obtained for the B-complex vitamins, i.e., thiamine hydrochloride, riboflavin, niacinamide, pyridoxine hydrochloride and cyanocobalamin in formulation I-Ill stored at room temperature at 37°C and 45°C for a period of 6-12 months are reported in Tables-II-IV.

The assay results obtained by this method for the above vitamins stored at different temperatures show an increase in the percentage retention after 6-12 months period in all the formulations. This is apparently not in accordance with the stability behaviour of these vitamins since it is known that these vitamins degrade in the liquid media to an extent depending largely on the nature of the vehicle used and the vitamin interactions. Though such results showing quite high assay values would be not normally be presented, the idea is to show that if such a method is applied what type of results could be obtained. In addition if such an attempt be successful within the experimental error limitations, it will save time in an industrial quality control laboratory.

Conclusions

The above detailed discussion of the various aspects of the different assay methods used for the determination of thiamine hydrochloride in Bcomplex syrups lead to the conclusion that the accuracy and specificity of a certain assay method depends very much on the nature of the preparation containing, apart from the substance to be determined, a number of other substances which may or may not interfere. Since with the pure thiamine hydrochioride there has been found no such interference, the accuracy and specificity may be very high within the limitation of the experimental error.

In the development of a new assay method or when a newly developed method is applied to the assay of a certain product, it may be worth while to check, among other possible sources of errors, interference from or interaction with the various ingredients of the preparation with the assay reagents. This would insure that the specificity of the assay method in question is not affected by such factors.

There should also be a standard for comparison and to check the efficiency of the proposed method. The magnitude of errors in both must be similar. Otherwise in stability studies the results may be unreliable and may give misleading values such as those obtained in the acid-dye method for the assay of thiamine hydrochioride. The author hopes to study the factors affecting the accuracy and specificity of this method in detail in future.

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