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DETERMINATIONS OF PYRIDOXINE HYDROCHLORIDE (VITAMIN B_6) IN MULTIVITAMIN PREPARATIONS

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Determinations of pyridoxine hydrochloride in multivitamin preparations using colorimetric, spectrophotometric absorbance difference and multicomponent spectrophotometric methods have been carried out. The colorimetric method is found to be more accurate and precise than the other methods. Interference due to decomposition products can be eliminated with TLC separation prior to assay procedures.

INTRODUCTION

Vitamin B_6 is essential for growth and protein metabolism. Deficiency results in skin changes or nervous disorders and even epileptoid convulsions. Vitamin B_6 is widely used in vitamin B-complex and multivitamin preparations for pharmaceutical and dietary purposes in order to cure or avoid such disorders and deficiencies. It is imperative that control is necessary for B-complex and multivitamin preparations containing vitamin B_6.

The method reported in USP for the determination of vitamin B_6 in multivitamin preparations is the colorimetric method which consists of coupling vitamin B_6 with 2,6-dichloroquinone-chlorimide in ammonium chloride ammonium hydroxide buffer [1]. There are other methods for colorimetric determination of vitamine B_6, in which pyridoxine reacts with Folin–Denis reagent [2], it couples with diazotized sulphanilic acid [3] and diazotized *p*-aminoacetophenone [4,5] and a method based upon indophenol reaction [6].

Vitamin B_6 has also been determined spectrophotometrically by measurement of difference in absorbance values (ΔA) in two different solvents at 328 nm [7]. Other differential spectrophotometric procedures for the analysis of vitamin B_6 in multivitamin preparations are described [8–10]. The use of determinants in the calculation of multicomponent spectrophotometric analysis has been reported [11]. The assay methods of pyridoxine have been reviewed by Hashmi [14].

The present paper offers a comparative study of all the three methods utilized for the determination of vitamin B_6 in multivitamin preparations and consequently suggests their relative precision and accuracy. Interference in both types of analysis is caused by the presence of photodegradation and/or decomposition products. TLC separation prior to assay eliminates the interference and the method may then be employed for determining vitamin B_6 in multivitamin preparations.

EXPERIMENTAL

The sample preparations of the various dosage forms, the modified colorimetric [13] differential spectrophotometric [7] and multicomponent spectrophotometric assays [11] for vitamin B_6 in multivitamin preparations were performed as follows:

Colorimetric Method

Sample Preparation. Tablets and Capsules: A number of tablets or capsules were reduced to a fine powder and mixed well. A sample corresponding to about 100–500 μg of pyridoxine hydrochloride was placed in a 25-ml glass-stoppered cylinder and 15 ml 0.5*N* NaOH was added. The mixture was heated in boiling water-bath with occasional stirring for about 15 min, the solution was cooled and diluted to 20 ml with water, 200 mg MnO_2 was added and cylinder was stoppered and shaken for 15 min. An aliquot containing 10–50 μg of pyridoxine hydrochloride was transferred to a centrifuge tube containing sufficient isopropanol to make the total volume 25 ml. This mixture was centrifuged and filtered. Syrups: An aliquot of sample was pipetted into a volumetric flask and diluted with the proper volume of 95% ethanol to make the alcohol content at least 70%. The solution was filtered and aliquots of the solution were pipetted into test tubes for colour development. The treatment of sample with alcohol was carried out to prevent turbidity. Injections: An accurate amount of B-complex

injection equivalent to about 100 mg vitamin B_6 was measured and diluted stepwise to a concentration of about 10–50 μg of the vitamin in each ml, 1 ml solution was transferred to a tube containing sufficient isopropanol to make 25 ml, shaken and filtered.

Assay Procedure. The aliquots of the filtrate (5 ml) containing a known amount of vitamin B_6 were pipetted into each of the three tubes and treated as given in Table 1.

Chlorimide reagent (1 ml) was added to blank and the spectrophotometer was adjusted to read 100% transmittance at 650 nm, exactly one minute after the addition of the reagent. One ml portion of chlorimide reagents was added to the sample and the internal standard tubes, and the absorbances were read at 650 nm exactly one minute after the additon of chlorimide reagent. The contents of vitamin B_6 were calculated using the formula: $A/(As-A)$ μg/ml in standard) = μg/ml in sample solution where A is the absorbance of sample tube, and As is the absorbance of the internal standard tube.

Spectrophotometric Method

Tablets: Twenty tablets were weighed, finely powdered and mixed thoroughly. Two portions of this powder each containing 1 mg of vitamin B_6 were weighed separately and transferred into two 100-ml volumetric flasks. Glycerinated phosphate buffer (pH 7) was added to one flask, and glycerinated 0.1N HCl to the other. The flasks were shaken vigorously for about 10 min, then made up to volume with respective solvents. The solutions were filtered through oridinary filter paper, rejecting the first 10-ml of the filtrate, the clear filtrate was transferred immediately to silica cell and absorbance was measured at 328 nm, using 0.1N HCl solution as blank. In vitamin B-complex and multivitamin tablets, where the absorbance was high, the final concentration containing 10 μg/ml of vitamin B_6 was reduced to 5 μg/ml to obtain an accurate response from the instrument. When the vitamin B_6 content of each tablet was 1 mg or more, an aliquot of finely crushed powder, equivalent to 5 mg of vitamin B_6 was transferred to 50-ml volumetric flask. Water was added and the flask was shaken for 5 min. Volume was made up to the mark with distilled water and the contents mixed thoroughly. The mixture was filtered and 5-ml aliquots of the filtrate were transferred to two 50-ml volumetric flasks. The volumes were made up to the mark with 0.1N HCl and phosphate buffer, pH 7. Capsule: A number of capsules were crushed and to a portion equivalent to 5 mg of vitamin B_6, about 5 ml of polysorbate 80 was added and the content mixed thoroughly. The mixture was warmed on a water-bath for 3–4 min, mixing the contents during heating. 10 ml glycerine was added to this and mixed well, 10 ml water was then added and mixed until a clear solution was formed. The contents were transferred to a 50-ml volumetric flask and made up to volume with water. 5 ml portions of this solution were transferred to two 50-ml volumetric flasks and made up to volume with glycerinated phosphate buffer (pH 7, and 0.1N HCl), and shaken vigorously, the absorbance was read at 328 nm using 0.1N HCl solution as blank. Injections: An aliquot of the sample containing 1 mg of vitamin B_6 was transferred to two 100-ml volumetric flasks. Glycerinated 0.1N HCl and glycerinated phsophate buffer (pH 7) were added to each flask, shaken, and the volume made up to the mark with the respective solvents. After thorough shaking the solution was filtered and the absorbance was read at 328 nm, using 0.1N HCl solution as blank. Syrups: Aliquots of the liquid containing 1 mg of vitamin B_6 were transferred into two 100-ml volumetric flasks, made up to volume with glycerinated 0.1N HCl and glycerinated, phosphate buffer (pH 7), and shaken thoroughly. Haziness that was developed in phosphate buffer (pH 7) was removed by simple filtration. 50 ml of each solution was extracted four times, using 20 ml ether for each extraction, to remove the flavours. The absorbance of the aqueous solutons was read at 328 nm using 0.1N HCl as blank.

Table 1

	Blank (ml)	Sample (ml)	Internal standard (ml)
Sample	5	5	5
Ammonium chloride–ammonium hydroxide buffer	1	1	1
Sodium acetate	1	1	1
Boric acid	1	1 (water)	1 ml standard solution (4 μg vit B_6)

Multicomponent Spectrophotometric Method

A suitable aliquot of the syrup was serially diluted with pH 2.0 buffer (KCl–HCl) to give finally 100 μg/ml pyridoxine hydrochloride. Absorbance spectrum of the solution was recorded in the UV and visible region, using a Unicam SP 800 spectrophotometer. The concentration of pyridoxine hydrochloride was determined by the multicomponent spectrophotometric analysis. The method of calculation used for the three component assay is according to Rapson [12].

TLC of Vitamin B_6

TLC of photodegraded solutions of vitamin B_6 was carried on silica gel G layer using the solvent system (a)

Table 2. Details of products investigated and assay figures for vitamin B_6.

Description	Active ingredients	Colorimetric method (%)	Spectrophoto metric method (%)	Multicomponent spectrophotometric method (%)
*Laboratory Prepared Dosage Forms**				
B-complex tablets	Each tablet contains B_1, 10:B_2,5: B_6,2.5:Nic,5.	100	99	–
Multivitamin tablets	Each tablet contains B_1,30:B_2,15,B_6,5: Cal.pant, 10:Nic, 60:C,100.	101	100	–
B-complex capsules	Each capsules contains B_1,50;B_2,20; B_6,10;Cal.pant,20;Fol,0.5;Nic,100.	100	98	–
B-complex syrup	Each 5ml of syrup contains B_1,25;B_2,2.5; B_6,5;Nic,25.	100	100	109
B-complex syrup	Each 15 ml of syrup contains B_1,3;B_2,3; B_6,2;Nic,23.	102	101	111
B-complex injections	Each ampule of 3 ml. contains B_1,100; B_6,100;Lido,1.	98	98	–
*Commerical Dosage forms**				
Multivitamin film coated tablets	Each tablet contains A,7.5;B_1,10;B_2,5; B_6,5;B_{12},6; Cal.pant,10;C,200.	107	105	–
B-complex coated tablets	Each tablet contains B_1,20B_2,10;B_6,5; B_{12},12;Cal.pant,10;Nic,50, C,300	106	107	–
B-complex tablets	Each tablet contains B_1,10B_2,2.5;B_6,0.6; Cal.pant,3:Nic,25.	103	102	–
Multivitamin capsules with iron	Each capsule contains B_1,4:B_2,4:B_6,1.5: B_{12},10:Cal.pant,3:Nic,15;C,100: Fol,0.1.	108	110	–
B-complex syrup	Each 5 ml of syrup contains B_1,6;B_2,6: B_6,1:B_{12},2:Nic,30.	107	106	120
B-complex injections	Each ampoule of 6 ml contains B_1,166; B_6,8.3;Lid,1.	108	108	--

*Cal. pant, Nic, Fol, and Lido: Calcium pantothenate, nicotinamide, folic acid and lidocaine respectively. The figures denote quantity in mg.

acetone – dioxane – water – ammonia (9:2.5:7.5:2),(b) n-propanol – formic acid – water (20:1:4), (c) ammonia – methanol (1.5:100) and sprayed either with chlorimide, or $KMnO_4$ reagent [13]. Vitamin B_6 was removed from silica gel layers and assayed according to the above methods.

RESULTS AND DISCUSSION

The assay figures in Table 2 show the vitamin B_6 percentage recovery for the laboratory prepared and commercial formulations using the colorimetric, differential spectrophotometric and multicomponent spectrophotometric procedures. The assay result obtained by the differential spectrophotometric method are in agreement with those of the colorimetric method. Although spectrophotometric method is simple and less time-consuming as compared to the colorimetric method. The multicomponent spectrophotometric method has been applied only to B-complex syrups and the results obtained are higher than those of the other methods. This may be attributed to slightly lower values of the extinction coefficient used in the calculations

The results indicate that formulations containing vitamin C when assayed colorimetrically give slightly higher values in certain cases since the greater dilution of the assay solution minimized the effect of any reducing material that might tend to destory pyridoxine chlorimide colour [13]. Formulation containing iron (Fe^{++}ions) in addition to vitamin C may still give higher values.

The values of standard deviation and coefficient of variation for these methods are reported in Table 3. A statistical comparision of the assay procedures indicates that colorimetric method is more precise than the spectro-

Table 3. Statistical data on the assay methods.

Assay method	Mean	Standard deviation	Coefficient of variation
Colorimetric	10.06	0.232	2.188
Spectrophotometric	9.09	0.279	2.828

The results are based on 10 determinations (10 μg/ml).

photometric method.

In the presence of photodecomposition products of vitamin B_6 erraneous results may be obtained as the colorimetric and spectrophotometric methods do not involve any separation of vitamin B_6 from other components prior to assay. Such an interference may be eliminated provided the assays are performed in conjunction with TLC separation. In conclusion a consideration of the above factors in these techniques will be helpful for a better control in pharmaceutical manufacturing.

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