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SIMULTANEOUS DETERMINATION OF VITAMINS B1, B2 AND B6 IN MULTIVITAMIN TABLET AND BIOLOGICAL FLUID BY RP-HPLC

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Thiamine (Vit. B_1),
Riboflavin (Vit. B_2),
Pyridoxine (Vit.B_3),
HPLC

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ABSTRACT

Multivitamin tablets containing various substances of varying characteristics may have a problem in quantitative analysis. This research has developed HPLC method for simultaneous determination of three vitamin components that is thiamine (Vit. B_1), riboflavin (Vit. B_2) and pyridoxine (Vit. B_3) in tablet formulation. The chromatographic separation was achieved by using a C-18 column with dimension of 4.6 mm I.D.X 250 mm and particle size of 5µm. A mixture of methanol: water (22:78) was used as mobile phase. The aqueous mobile phase contained O- phosphoric acid adjusted to pH 2.5, with flow rate of 1mL/min. The effluent was monitored at 290 nm at ambient temperature. Effective separation and quantification was achieved in less than 10 min. The method was simple, accurate, precise, and could be successfully applied for the analysis of thiamine, riboflavin, and pyridoxine multivitamin tablets. The HPLC results revealed that % vitamins were found to be for B_1, B_2 and B_6 respectively in marketed multivitamin formulation. HPLC analysis of biological fluid (urine) showed that % Vit.B_1 in 2, 4, 6 hrs were found to be 0.69, 0.12 and 0.015.

INTRODUCTION: The vitamins are a disparate group of compounds; they have little in common either chemically or in their metabolic functions. Nutritionally, they form a cohesive group of organic compounds that are required in the diet in small amounts (micrograms or milligrams per day) for the maintenance of normal health and metabolic integrity. They are thus, differentiated from the essential minerals and trace elements (which are inorganic) and from essential amino and fatty acids, which are required in larger amounts.

The discovery of the vitamins began with experiments performed by Hopkins at the beginning of the twentieth century; he fed rats on a defined diet providing the then known nutrients: fats, proteins, carbohydrates, and mineral salts.

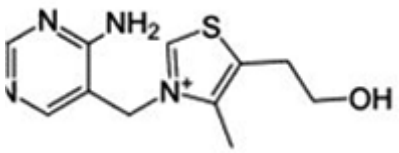
The animals failed to grow, but the addition of a small amount of milk to the diet both permitted the animals to maintain normal growth and restored growth to the animals that had previously been fed the defined diet. He suggested that milk contained one or more "accessory growth factors" – essential nutrients present in small amounts, because the addition of only a small amount of milk to the diet was sufficient to maintain normal growth and development.

The first of the accessory food factors to be isolated and identified was found to be chemically an amine; therefore, in 1912, Funk coined the term *vitamine*, from the Latin vita for "life" and amine, for the prominent chemical reactive group. Although subsequent accessory growth factors were not found to be amines, the name has been retained–with the loss of the final"-e" to avoid chemical confusion.

The decision as to whether the word should correctly be pronounced "vitamin" or "veitamin" [1]. The use of therapeutic multivitamins is indicated in cases of deficiency in pathological conditions in which the nutritional requirements are greatly increased or in conditions in which absorption, utilization, or excretion of vitamins is abnormal.

Multivitamin pharmaceutical preparations containing mixtures of these substances are very interesting for analysis, and most of them include the water-soluble B-group. The term B-group vitamins usually refers to thiamine, riboflavin, pyridoxine, nicotinic acid, pantothenic acid, biotin, cyanocobalamine and folic acid [2].

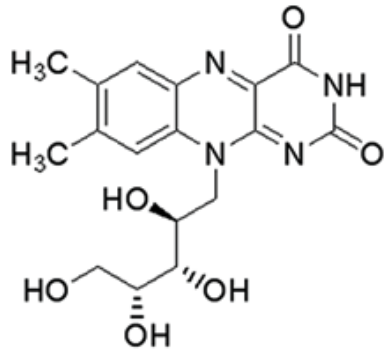
Vitamins are reported to reduce the damage by free radicals and check degenerative disease [3]. The vitamin B_1 family consists of the pyrimidyl-substituted thiazole, thiamine, and its phosphate esters. The principal biologically active form of thiamine, TDP, is a coenzyme in several enzyme complexes that play vital roles in the metabolism of carbohydrates, fats, and alcohol [4].



Thiamine B_1: Riboflavin, or vitamin B_2 having molecular formula $C_{17}H_{20}N_4O_6$, serves as a coenzyme--one that must combine with a portion of another enzyme to be effective--in the metabolism of carbohydrates, fats, and, especially, respiratory proteins. It also serves in the maintenance of mucous membranes.

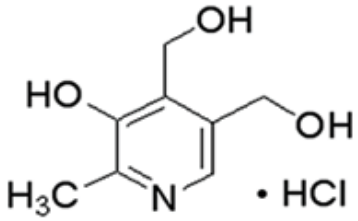
Other functions include the elimination of soreness of mouth and lips, also important for good muscle tone, for antibody production, for cell respiration and growth, for good vision, skin, hair and nails and for red blood cell formation.

The best sources of riboflavin are liver, milk, eggs, meat, soy products, fish, cheese, dark green vegetables, whole grain and enriched cereals, pasta, bread, legumes, and mushrooms. A lack of riboflavin in the body reduces energy levels. Riboflavin is also needed for the formation of hair, skin, and nails [5].



RIBOFLAVIN

Pyridoxine, or vitamin B_6 chemically 4,5-Bis(hydroxymethyl)-2-methylpyridin-3-ol having molecular formula $C_8H_{11}NO_3$, is necessary for the absorption and metabolism of amino acids. It also plays roles in the use of fats in the body and in the formation of red blood cells. Pyridoxine deficiency is characterized by skin disorders, cracks at the mouth corners, smooth tongue, convulsions, dizziness, nausea, anemia, and kidney stones. The best sources of pyridoxine are whole grains, cereals, bread, liver, avocadoes, spinach, green beans, and bananas [6].



PYRIDOXINE HYDROCHLORIDE

PHYSIOLOGIC ROLES AND DEFICIENCY SIGNS OF VITAMIN B_1, B_2 & B_6

Vitamin	Physiologic roles	Clinical signs of deficiency
Thiamin (B1)	Coenzyme functions in metabolism of carbohydrates and branched chain amino acids	Beriberi, polyneuritis, and Wernicke-Korsakoff syndrome
Riboflavin (B2)	Coenzyme functions in numerous oxidation and reduction reactions	Growth, cheilosis, angular stomatitis, and dermatitis
Vitamin B6 (pyridoxine)	Coenzyme functions in metabolism of amino acids glycogen and sphingoid, bases	Nasolateral seborrhea, glossitis and peripheral neuropathy (epileptiform convulsions in infants)[7]

The main objective of the present study to develop a simple, efficient, reliable and cost-effective high performance liquid chromatographic (HPLC) method for the quantization of water soluble vitamins in marketed multivitamin tablets and biological fluid(urine).

MATERIAL AND METHODS:

Reagents and Solvents: The following chemicals were used: Thiamine HCl, Riboflavin, Pyridoxine HCl Hexane sulphonic acid sodium salt, Potassium dihydrogen phosphate, Triethylamine, O-phosphoric acid Methanol and Water (HPLC grade). All chemicals were obtained from Shyam Brothers, 27- Sindhi Market, Bhopal (M.P.).

Dosage form: Multivitamin tablets, manufactured by Sapco laboratories (P) Ltd. (Each film coated tablet contains: thiamine chloride hydrochloride 10 mg Riboflavin 10 mg and pyridoxine hydrochloride 5 mg).

Standard Stock Solution: 01 mg of vitamin B_1 (thiamine HCl), vitamin B_2 (riboflavin) and vitamin B_6 (pyridoxine HCL) were separately dissolved in 10 ml of mobile phase respectively.

For buffer preparation, 1.08 g of hexane sulphonic acid sodium salt and 1.36 g of potassium dihydrogen phosphate were dissolved in 940 ml of HPLC water and 5 ml of triethylamine was added to it and the pH was adjusted to 3.0 with orthophosphoric acid. To prepare the mobile phase, water and methanol were mixed with a ratio of 78:22 and filtered through 0.45 µ membrane filter and degassed by using bath sonicator.

Sample Preparation Water soluble vitamins can be extracted from simple matrices such as multivitamin tablets (after homogenization) with water in an ultrasonic bath. Only 250 mg from the total sample are transferred into a 50 ml volume flask. Approximately 40 ml of 0.5% oxalic acid solution was added and the sample stirred.

After 20 min treatment in an ultrasonic bath, the sample solution must be cooled down and the volume adjusted to 50 ml with 0.5% oxalic acid. Before injection the sample was filtered through a 0.45 µm syringe filter.

Biological Fluid Sample: We observed the level of each vitamin in the urine of a volunteer after his swallowing an overdose of vitamins (three pills). The vitamin pill was composed of 70 mg vitamin C, 10 mg B_1, 10 mg B_2, and 5 mg B_6. We collected urine samples 1, 2, 3, 5, and 8 hrs after eating the vitamin pills. Water soluble vitamins are known to be almost excreted in 2 to 8 hr. after dosage. Each urine sample was stored in a refrigerator under 4°C and was centrifuged at 10 000 rpm for 20 min. Then the supernatant was taken and 0.1% (v:v) 1M HCl were added.

HPLC analysis of water soluble vitamins in marketed multivitamin tablets and biological fluid (urine) by HPLC: The HPLC analysis was performed using a LC-100, Cyberlab^{TM}, Salo Torrace, Millburry, MAO 1527, USA with LC-UV-100 UV detector. A CAPCELL (C-18) HPLC-packed column (4.6 mm I.D.X 250 mm), type MG 5 µm, number AKAD/05245 was used for the chromatographic separations. The mobile phase consisted of Water and o-phosphoric acid: methanol (78:22). The flow rate was 1.0 mL/min, and a column temperature of 25°C. The injection volume was 20µl, and UV detection was effected at 290 nm. The sample and standard solution were subjected to HPLC column and the obtained records were superimposed on the retention time values of the standard samples.

$$\text{Vitamin content \%} = \frac{A1 \times W_2 \times \text{dilution factor}}{A_2 \times W_1}$$

Where, A_1 = Peak area of sample solution; A_2 = Peak area of standard solution; W_1 = Weight in g of sample; W_2 = weight in g of standard

RESULTS AND DISCUSSION: The best result of RP-HPLC method for the simultaneous determination of thiamine hydrochloride, riboflavin and pyridoxine hydrochloride was obtained by using a C-18 column with dimension of 4.6 mm I.D.X 250 mm and particle size of 5µm. A mixture of methanol: H_2O(22:78) with O-phosphoric acid 1% to adjusted a pH 2.8 using as mobile phase with flow rate of 1mL/min. The effluent was monitored at 290 nm. Under the described experimental conditions, the three water-soluble vitamins were selectively separated (**Figure 1**), no significant interfering peaks were observed at the retention times of the vitamins.

Effective separation and quantification of the three water-soluble vitamins was achieved in less than 10 min (**Figure 1 - 5**). The HPLC analysis data shown in table 1 and the corresponding RT value of vit.B_1, vit. B_2 ,Vit. B_6 (Standard) and sample were tabulated in **table 1**. The % vitamin content of the vit. B_1, vit. B_2 & vit. B_6 in the marketed multivitamin tablets were found to be 0.2, 0.25 & 0.76 respectively. HPLC analysis of biological fluid (urine) showed that % Vit.B_1 in 2, 4, 6 hrs were found to be 0.69, 0.12 and 0.015.

TABLE: 1 HPLC ANALYSIS OF WATER SOLUBLE VITAMINS

S. No	Vitamin	RT(min)	Height	Area	Conc.	Half width	Res	Theo.Plate	Tail.Factor
1	Vit.B1(Std)	2.602	141150	2623167.1	40.012	18.58	0.77	390.73	1.53
2	Vit.B2(Std)	2.759	233	2616.8	69.6570	11.23	2.21	1282.43	1.61
3	Vit.B6(Std)	2.926	2828	137982.7	96.7556	48.79	0.98	71.68	1.16
4	Vit.B1(Sample)	3.890	5572	70158.6	1.9125	12.22	0.71	1569.90	1.28
5	Vit.B2(Sample)	2.96	923	10293.2	3.6687	11.15	3.69	1297.44	1.60
6	Vit.B6(Sample)	4.394	12150	179567.0	64.0015	14.78	1.30	1761.55	3.29

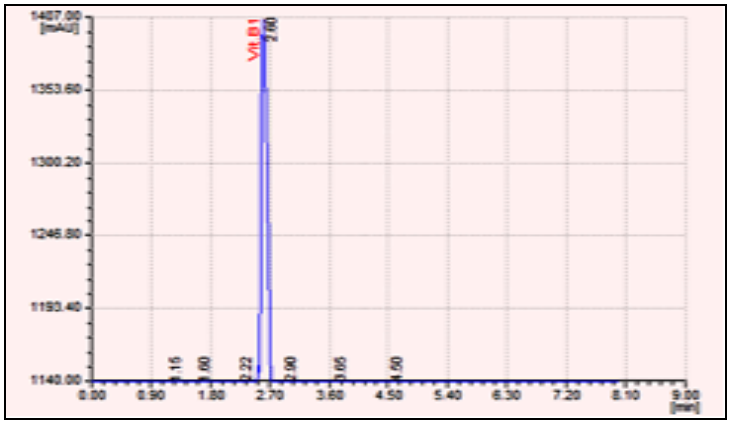


FIG. 1: STANDARD OF VIT. B1

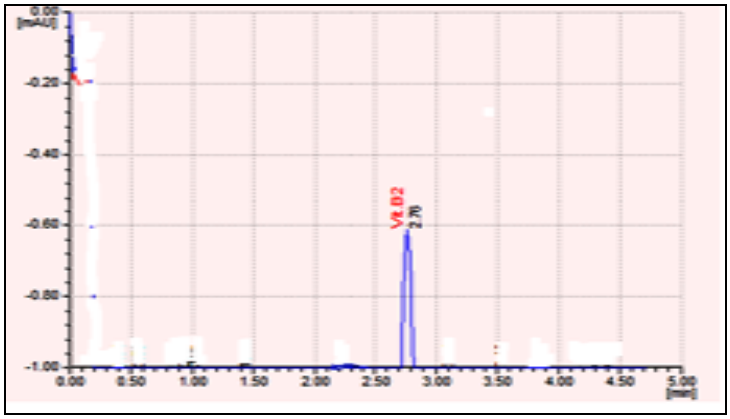


FIG. 2: STANDARD OF VIT. B2

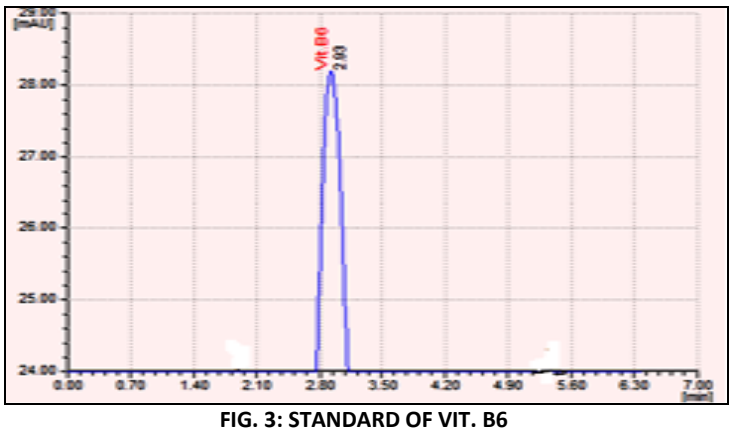


FIG. 3: STANDARD OF VIT. B6

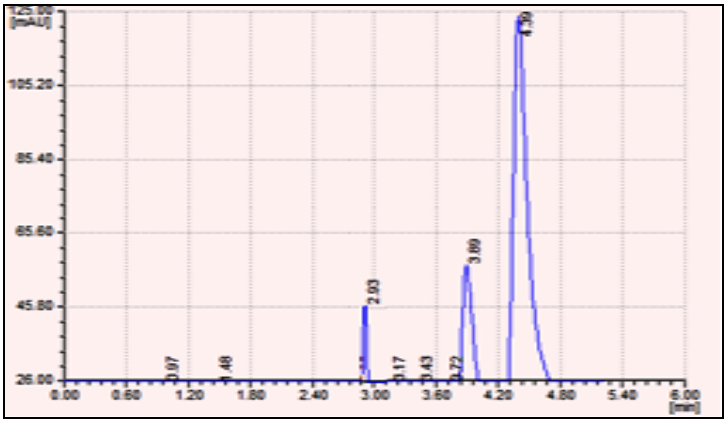


FIG. 4: HPLC CHROMATOGRAM OF MULTIVITAMIN TABLET

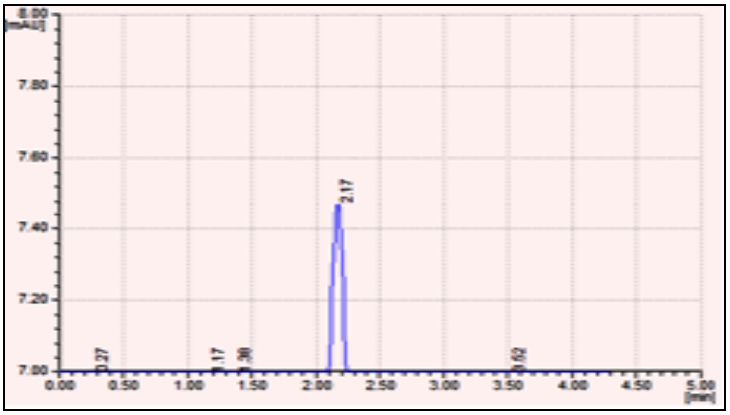


FIG. 5: HPLC CHROMATOGRAM OF BIOLOGICAL SAMPLE

CONCLUSIONS: The simultaneous determination of the three water-soluble vitamins was performed by RP-HPLC using C-18 column of (4.6 mm I.D. X 250 mm) dimension and 5µm of particle size and mobile phase methanol: H_2O (22:78) with O-phosphoric acid 1% to adjusted a pH 2.8. The effluent was monitored at 290 nm. The method was simple, accurate, precise, and could be successfully applied for the analysis of thiamine hydrochloride, riboflavin and Pyridoxine Hydrochloride in multivitamin tablets and also biological fluid.

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