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New, Rapid and Sensitive Method for Determination of Vitamins B1, B2 and B9 in Mixture by HPLC Method

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Abstract
In this study, three water-soluble vitamins (thiamine, riboflavin, and folate) were separated and determined using HPLC. This method provides a fast, accurate, and reliable method for their determination, with recoveries ranging from 91% to 108%. The RSD% values of B1, B2 and B9 solutions were 2.9, 2.3 and 4.2% respectively. The results presented in this study confirm that the proposed approach is not prone to failure. They appear to be accurate, and the method has been successfully applied to the determination of vitamins B1, B2, and B9 in the presence of certain intermediates.

Keywords
B1, B2, B9, and HPLC

INTRODUCTION

Vitamins are non-energy producing organic compound, essential for normal human metabolism that must be supplied in small quantities in the diet. The importance of vitamins as drugs is primarily in the prevention and treatment of deficiency diseases [9]. Some vitamins do have other empirical uses in pharmacological doses [1]. Vitamin deficiencies occur due to inadequate intake, mal absorption, increased tissue needs, increased excretion, certain genetic abnormalities and drug-vitamin interaction . Myths like ‘they energies the body[2], any physical illness is accompanied by vitamin deficiency’, ‘vitamin intake in normal diet is precariously marginal’, ‘they harmless’ are extensive [23]. Vitamins are traditionally divided into two groups: fat-soluble and water-soluble [14]. Water-soluble vitamins (B-complex and C) are meagerly stored: excess is excreted with little chance of toxicity[17]. They act as cofactors for specific enzymes of intermediary metabolism[4]. B-vitamins are essential for the functioning of the nervous system, which controls our stress response. Our stress response system determines how we feel in the face of everyday stress[26]. Determined the concentrations of (B1, B3, B6, folic and B12 vitamins) in multivitamin tablets by RP-HPLC[30]. The mobile phase for the separation was consist of methanol – 5m M heptanesulphonic acid sodium salt 0.1% triethylamine (25:75 V/V) with pH 2.8 and flow rate (1 ml/min)[3].They concluded that the recovery was ranged from 90.4% to 108.5% .While the precision values of the procedure was between 0.8 and 40[7].The results of the procedure for coefficients and linearity's were more than 0.99. This study was designed to develop a new analytical method on a mixture of soluble vitamins including Thiamine (B1), Riboflavin (B2) and Folic acid (B9) to simultaneous determination the contents of each vitamin with presence the other vitamins[10] .methods were used in this study including. HPLC Method[31], developed and validated two methods,

based on HPLC and UV spectrophotometry for the quantitative determination of some drugs in tablets[13]. The study concluded that the spectrophotometric method is a simple, cheap and less time-consuming method and they stated that the chromatographic method is selective for the determination of the degradation products of drugs [6].The detection limits of the HPLC and spectrophotometric methods were 0.69 and 0.44 µg mL-1, showing that both methods are sufficiently sensitive. Moreover, there is no pharmacopoeial method reported for analysis of this drug[21]. Therefore, both presented methods are useful to the area of pharmaceutical analysis [12].

APPARATUS

Perkin Elmer HPLC Series PE-200 (USA) equipped with a P200 pump, solvent degasser DGU-3A, an automatic sampler AS200, Rheodyne injector with 200 µL loop, UV/VIS detector Series 200 with controlled wavelength at 262 nm and communication Network chromatography Interface Dot Link 600, a Brownlee BIO C18 reversed-phase analytical column, 5µm particle size, 250x4.6 mm dimension.

PHARMACEUTICAL SAMPLES

The contents of vitamins were evaluated in the following pharmaceutical formulations: Vitamins produced (Pregnant plus vital drug) by "Wockhardt", Germany ,containing Folic acid , vitamins and minerals, Tablet. Other product of vitamins tablets produced by Ltd, Coventry, UK. vita life (UK) , Multivitamin capsules under trade name of "B complex" produced by vitamin, Ltd ,London containing thiamin (B_1) ,riboflavin (B_2) ,Folic acid and other vitamins B_{12} and B_3 .

Sample Preparations Tablets

Place one tablet in a 50 ml beaker, add 1 ml sodium hydroxide solution (0.01 M) with little amount of distilled water, grind the tablet with a glass rod to form powder, shake vigorously, then filter through Whatman filter paper (#41) and complete to 50 ml with distilled water washings. Further dilution is accomplished by transferring 1 ml of the previous solution to 25 ml measuring flask and completed to the mark with distilled water.

Procedure

HPLC method

Thiamine (B_1)

An aliquot of standard Thiamin solutions (2.5 - 25 µg/m1) were placed in a 10 ml measuring flask and completed to the mark with distilled water. The solution is then transferred to quartz cell with 10 mm path length and the absorbance is recorded against water blank at wavelength 263 nm. The unknown samples were measured directly without further dilution and the concentrations were calculated according to the calibration curve [8].

Riboflavin (B_2)

An aliquot of standard Riboflavin (B_2) solutions (2.5 - 25 µg/m1) were placed in a 10 ml measuring flask and completed to the mark with distilled water. The solution is then transferred to quartz cell with 10 mm path length and the absorbance is recorded against water blank at wavelength 264 nm. The unknown samples were measured directly without further dilution and the concentrations were calculated according to the calibration curve [15].

Folic acid (B_9)

An aliquot of standard Folic acid (B_9) solutions (20 - 150 µg/m1) were placed in a 10 ml measuring flask and completed to the mark with distilled water. The solution then transferred to quartz cell with 10 mm path length and the absorbance is recorded against water blank at wavelength 278 nm. The unknown samples were measured directly without further dilution and the concentrations were calculated according to the calibration curve [20].

Preparation of synthetic binary mixtures

Accurately measured aliquots of the suitable working standard solutions of vitamins are transferred into a series of 10 ml volumetric flasks to prepare different synthetic binary mixtures of Thiamin with either riboflavin or Folic acid in different concentrations. The solutions are then diluted with distilled water to the volume and scanned [18].

Preparation of pharmaceutical samples

One tablet is dissolved in water using glass rod and completed to 50 ml with distilled water. Aliquot of 1 ml is transferred to (25) ml volumetric flasks and diluted with water. The final solutions are scanned

Reverse Phase High Performance Liquid Chromatographic Analysis

After series trials the mobile phase which selected in study was 0.04 mol l^{-1} KH_2PO_4 (pH =7) : acetonitrile, 75:25 . The flow-rate was (1) ml min^{-1}. The column was operated at room temperature (20°C). The mobile was first degassed and (1 µl) Thiamin solution (20 -200 µg/ml) was injected into 200 µL loop and the column elute was monitored with a UV detector at 263 nm. The same conditions were applied on the other vitamins but by used concentrations of (5- 25 µg/ml and 100 – 500 µg/ml) for riboflavin and folic acid , respectively. Identification of the studied vitamins in a sample was ascertained by comparing its retention time with that of standard solutions and their concentrations were calculated from the calibration curve of integrated peak areas versus the corresponding concentrations of standard solutions.

RESULTS AND DISCUSSION

After trials of investigations of different parameters mobile phase composition (various ratios of solvents) ,and flow rate , the best retention time was chosen for separation of the studied vitamins . From the chromatogram shown in Figure (1) , it is evident that the mobile phase consisting of (Buffer solution : acetonitrile) of ratio (75 : 25) gave fast and good separation comparing with the other applied mobile phase ratios of (70 : 30) , (77 : 23) and (80 :20) , Figures (2 - 4) . Also different **P**H values were used during the separation including **P**H values of 4 , 6 and 7 (Figures 5 , 6 and 7) . The obtained results showed that the **P**H value of (7) gave the good separation comparing with the other **P**H values of 4 and 6 , Figures (6) and (7).

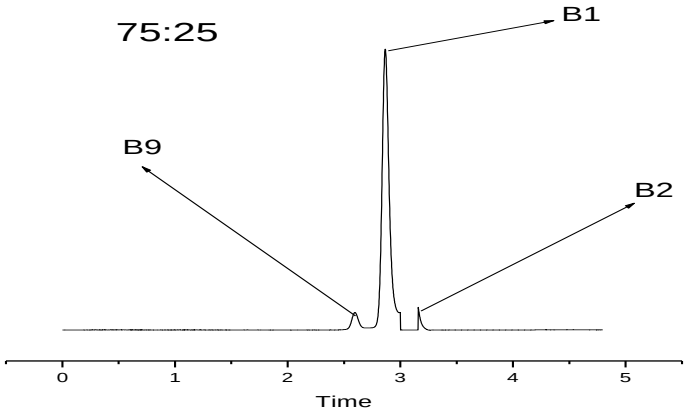

Fig. 1 The effect of the mobile phase (75:25) on the separation

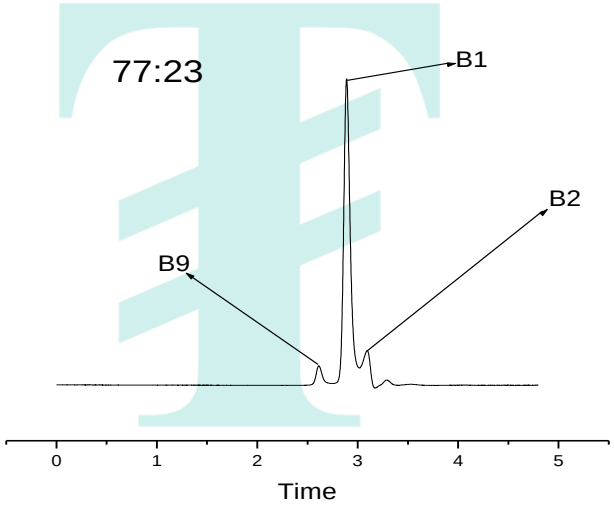

Fig. 2 The effect of the mobile phase (77:23) on the separation

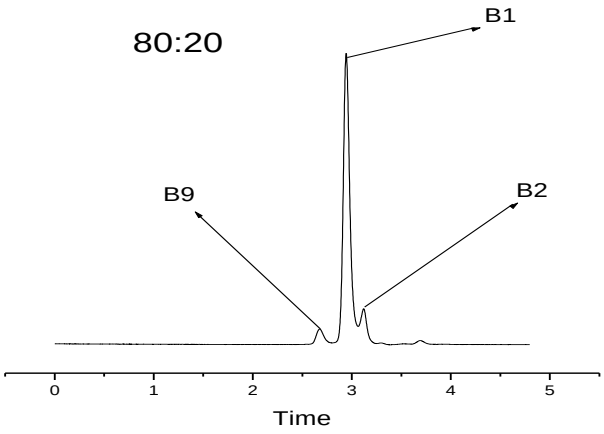

Fig. 3 The effect of the mobile phase (80:20) on the separation

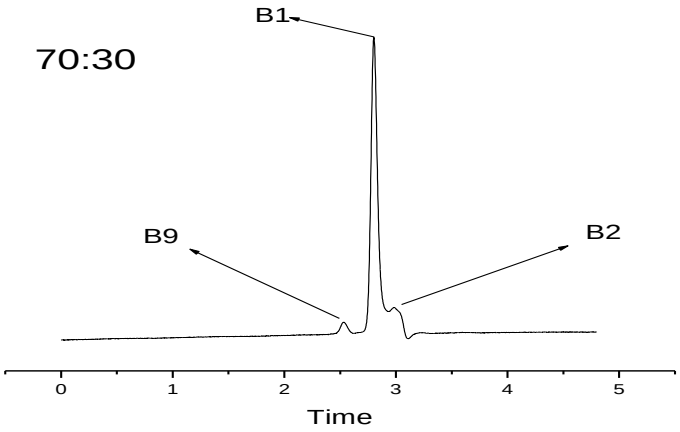

Fig. 4 The effect of the mobile phase (70:30) on the separation

Also the effect of PH values on the separation was studied by modified the applied PH values the results were shown in Figures (5 -7).

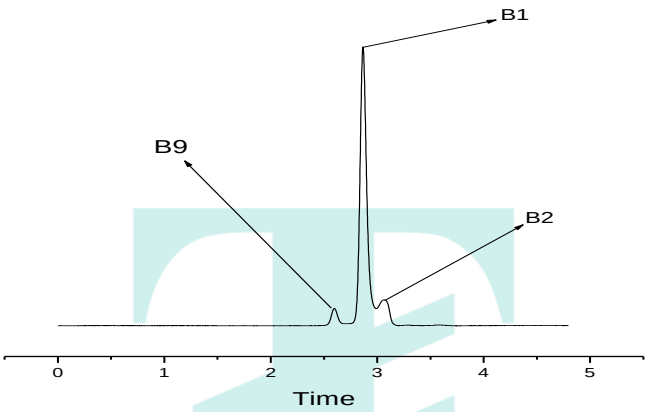

Fig. 5 The effect of (PH = 4) on the separation of B_1, B_2 and B_9

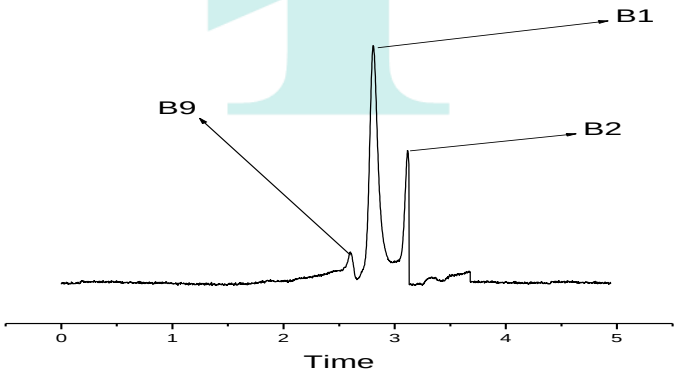

Fig. 6 The effect of(PH= 6) on the separation of B_1, B_2 and B_9

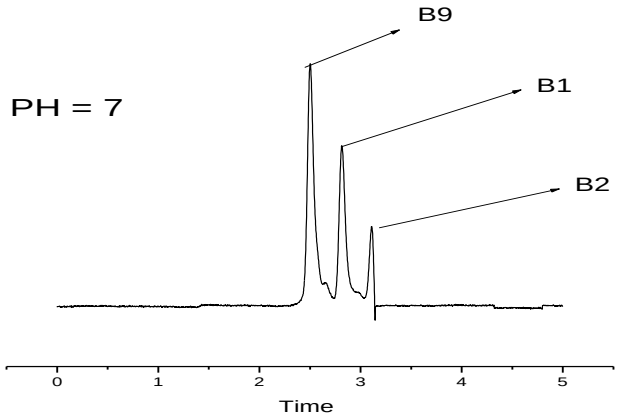

Fig. 7 The effect of (PH= 7) on the separation of B_1, B_2 and B_9

Also after series of attempts the total of chromatographic run time is 3.3 min, the flow rate is 1 min/ml which required to move the mobile phase from the injection loop to the column to the detector . On the other side the value of U.V which applied in the HPLC was chosen according to the λ_{max} value which obtained from the spectrophotometric measurements during this study , where the results showed that λ_{max} of the studied vitamins were : 263 , 264 and 278 nm for B_1 , B_2 and B_9 , respectively [25]. The best wave length which gave good separation was 262 nm (Also after series of investigations). The best optimizing the conditions of separation which chosen in this study were shown in Table 1.

Table 1 The best Optimizing the conditions of separation

Parameter	Value
PH	7
Mobile Phase	75 : 25
Wave length	262
Retention time (t_R)	3.3 min

The applied method was subjected to validation for various parameters including linearity, accuracy, limit of diction (LOD) , limit of quantification (LOQ) ,Suitability of method, precision and accuracy [29]. The method is unaffected by slight variations in experimental conditions such as pH and reagent concentration. Moreover, the methods are free from interference by common additives and excipients [10]. The wide applicability of the new procedures for routine quality control is well established by the assay of vitamins (vitamin B_1, vitamin B_2, and vitamin B9) [11].

The HPLC method is a versatile method and may offer advantages over the such derivative method for the selective determination [19]. On the other side the HPLC also gave high recovery values and the separation was appeared within short time comparing with some studies which used the HPLC method in same manner [31].

Linearity

The linearity of the concentration against peak height was studied according to values of slope , intercept of the regression line and correlation coefficient (r^2). The fruitful linearity was obtained for the compounds between the concentrations and peak area , where the concentration which gave good linearity were ranged as following 25 – 200 µg/ml, 5 – 25 µg/ml and 100 – 400 µg/ml for B1, B2 and B9, respectively. Table 2 shows the linearity results of the studied vitamins.

Table 2 The linearity results of the HPLC method

Parameter / Vitamin	Range	slope	intercept	R^2	Sy/x	LOD mg/ml	LOQ mg /ml
B1	25- 200	13539.16	18457.68	0.93	418375	0.092	0.309
B2	5 - 25	49200	-268000	0.96	147241	0.0089	0.029
B9	100 - 400	593.76	-85015	0.94	33423	0.168	0.562

Applications

The proposed HPLC method was applied to determine vitamins B1, B2 and B9 in some pharmaceuticals products[3] . The pharmaceuticals containing the studied vitamins alone or along with other vitamin s. The applied method showed fruitful results , where , complete separation was obtained [16]. Three different types of drug samples containing B_1 , B_2 and B_9 , beside to different intergradient's (vitamins and minerals) were selected as application of the proposed methods, the drugs including (vita life, B complex and Pregnant plus drug) samples [5] . The chromatograms of the studied samples were shown in Figures (14 – 16) , the values of retention times of the drugs samples were compared with the retention time of each vitamin of standard solutions [22] . The results showed that the retention times of the drugs were appeared at 2.67 , 3.0 and 3.2 min for B_9 , B_1 and B_2 , respectively and harmony with the retention times of the standard solutions [28]. The contents of the drugs which obtained from of HPLC method were illustrated in Table 6.

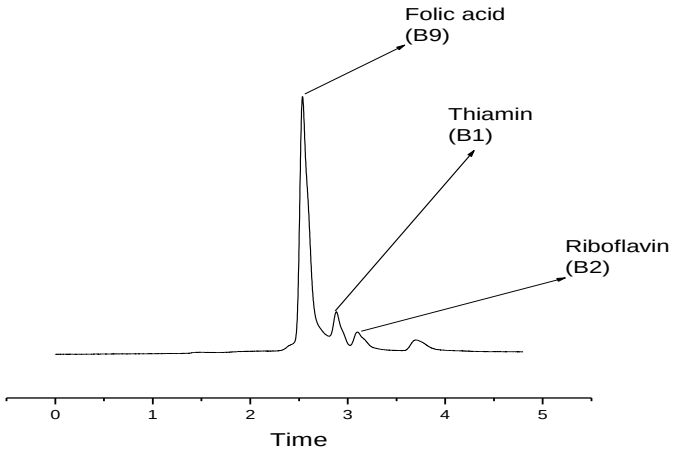

Fig. 8 The chromatogram of (Vita life drug)
Times (2.67 for folic, 3.01 for B_1 and 3.23 for B_2)

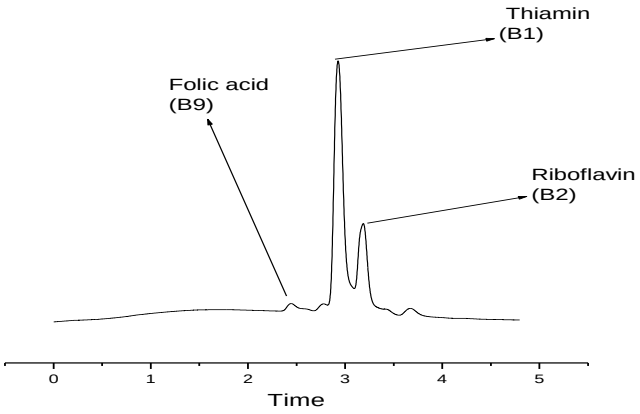

Fig. 9 The chromatogram of (B-Complex drug)
Times (2.67 for folic, 3.09 for B1 and 3.22 for B2)

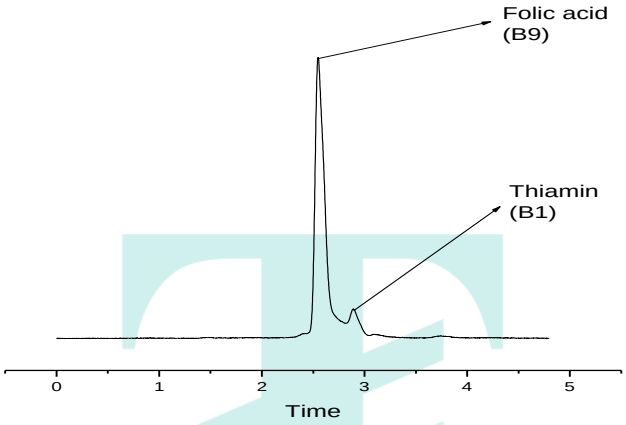

Fig. 10 The chromatogram of (Pregnant plus drug)
Times (2.66 for folic B_9& 3.01 for B_1)

Table 3 The recovery values (%) of the contents of B_1, B_2 and B_9 in the studied drug samples by using HPLC method

Sample / Parameter		Label Claim		Found value		Recovery %
Pregnancy Plus vital	B1	1.2	B1	1	83
	B2	1.5	B2	1.40	93
	B9	600	B9	680	113
Vitamin B complex	B1	1.1	B1	0.80	72
	B2	1.40	B2	1.30	92.8
	B9	200	B9	210	105
Vita Life	B1	8	B1	6	75
	B2	4	B2	3.36	84
	B9	400	B9	500	125

Also the recovery values of the samples compared with those of the prepared mixtures of the studied vitamins [27] .The obtained results showed that the high values recovery were recorded in the range of (90 – 110%) according to the precision values of statistical analysis [24].

CONCLUSION

In conclusion three water-soluble vitamins (Thiamine, riboflavin and folic acid) were separated and analyzed by RP - HPLC. The method provided a rapid , accurate and reliable method for their determination with recoveries ranging from 91 to 108 %. , the RSD% values were 2.9 , 2.3 and 4.2 % for the solutions of B_1 , B_2 and B_9 , respectively . The developed method was further applied to pharmaceutical or other samples. The binary eluent system used for water-soluble and the isocratic eluent system used for soluble vitamins provide good separation high selectivity and resolution within a minimum analysis time of 3.3 min .The simplicity of the procedure should make it highly desirable for quality control of multi-vitamin products in food industries , plant extractions and drug intergrading's . The results given in Table (6) confirm that the proposed method is not liable to interferences. They seem to be accurate, and the method was successfully applied to the determination of vitamins B_1, B_2 and B_9 in the presence of some intergradient. It's new method for the mobile phase ratio. Rapid, where the separation time is completed within 3 min comparing with many of published methods. Sensitive to determine different types of soluble water vitamins, especially which selected in this study (Some of B vitamins) Unlike the HPLC procedures, the instrument is simple and affordable.

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