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RESEARCH ARTICLE

Simultaneous and Rapid Separation and Determination of Vitamins B1, B2, PP, and B6 Using Ion-pair Rp-HPLC and Ultra Violet Detection in Multivitamin Tablets

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ABSTRACT

Introduction: Multivitamin tablet containing various substances of varying characteristics may have a problem in quantitative analysis. This research has developed and validated of ion-pair RP-HPLC method for simultaneous determination of four B vitamins, thiamine hydrochloride (vit. B1), riboflavin (vit. B2), niacinamide (vit. PP) and pyridoxine hydrochloride (vit. B6).

Chromatographic Conditions: The method was developed based on mixture (water: methanol: glacial acetic acid) ratio(72:27:1 v/v) as the mobile phase that contain in every 100 ml 140mg, 5 mM heptanesulphonic acid sodium salt as the ion pairing reagent were investigated and established.

The best results for the simultaneous determination of B1, B2, B3, B6 vitamins were obtained with above mobile phase where the pH was adjusted to 5 ± 0.2 by using 2N sodium hydroxide solution to prevent peaks interfering during separation the mixture of studied compounds, mobile phase was filtered through 0. 45µm Millipore filter, flow rate 1ml/min and octadecyle column ODS C18(250x4. 6, 5µm), measurements were made at λ=280 nm.

Results: A simple accurate, rapid, and sensitive method ion-pair RP-HPLC was developed and validated for the simultaneous determination some B-group vitamins, niacinamide (vit. PP), pyridoxine hydrochloride (vit. B6), riboflavin (vit. B2), and thiamine hydrochloride (vit. B1) multivitamin tablets.

Keywords: HPLC, Multivitamin preparations, Niacinamide, Pyridoxine hydrochloride, Riboflavin, Thiamine hydrochloride

INTRODUCTION

Vitamins of Group B belong to the most important biologically active substances, as they ensure the normal performance of human body through participation in the biosynthesis of proteins and functioning of the central nervous, cardiovascular, and gastrointestinal system.

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They income into the body with plant items or food of animal origin, some of them are synthesized by intestinal microflora.

Most of the Group B vitamins are present in food of plant origin: Cereals, bran, rough flour, yeast and lesser extent in meat, kidney, liver, fish, and milk. And dietary product.[1]

Group B vitamins belong to organic substances of different chemical structure, each of them has important individual feature also in the action of human body.[2,3,4]

Taking into account the great amount of vitamins,

multivitamins, and multidrug formulations based on Group B vitamins produced by various manufacturers, the production of vitamins, quality control, validity periods, and use in medical practice is not possible without the careful control over their manufacture, storage, and use.

Therefore, it seems natural in the last decades, areal boom in the search for novel methods of analysis of Group B vitamins in various objects was observed. The most frequently used methods are chromatography[5-10] chemiluminescence[11,12] spectrophotometry,[13,14] capillary zone electrophoresis,[15,16] and flow-injection analysis with various types of signal registration,[17-19] some of vitamins can be determined simultaneously,[20-22] rather than electrochemical methods are used.

Various potentiometry and voltametametry methods have been used to determine vitamins in pharmaceutical formulations,[23,24] foods,[25] biological items,[26] shampoos and creams, and multivitamin formulas.[27,28]

Effective separation and quantification of the four water-soluble vitamins were achieved in <15 min, Thomas *et al.* reported that the running time was about 60 min using a C18 column (250 × 4.6 mm) with 5 μm of particle size and with gradient elution of mobile phase methanol–15 mM 1-hexane sulfonic acid sodium salt solution pH 3.00 for determination of thiamine hydrochloride, riboflavin, nicotinamide, and pyridoxine hydrochloride.

The aim of present paper is to develop sensitive, rapid, and simple ion-pair RP-HPLC method with UV/VIS detection for determination of B-group vitamins:

Thiamine hydrochloride, riboflavin, niacinamide, and pyridoxine hydrochloride in pharmaceutical formulation (tablet).

It would be advantageous in the routine analyzed the four water-soluble vitamins in tablet preparations, if they could be determined simultaneously in a single chromatographic run.

MATERIALS AND METHODS

Instrument

The HPLC system (shimadzu-japan) consists of control unit (scl-10-A vp), oven(CTO-10A vp), and two types of detectors: Ultra violet Detector (SPD-10AV vp), fluorescent Detector (RF-10AXL), degassing unit (DGU-14A) isocratic pump, with valve 20-μL loop, and LC-solution software were used for collected data, chromatographic analysis was performed on RP-C18 (250 mm × 4.6 mm, 5 μm particle size) column from Knauar (Germany).

Reagents and materials

The present paper describes a sensitive and simple Ion-pair RP-HPLC method with UV/VIS detection for simultaneous determination of B-group vitamins, Table 1 below summarize studied compound [Figures 1-4].

All chemicals and reagents were of analytical grade and the water was distilled and filtered through a membrane filter 0.45 μm.

Thiamine hydrochloride, riboflavin, niacinamide, pyridoxine hydrochloride, and BASF – Germany were used as working standards. Methanol for HPLC, glacial acetic acid, (Merck), heptanesulphonic acid sodium salt, and acetonitrilee (Sigma) were used to prepare the mobile phase and sodium hydroxide 2N (Merck) for adjusting mobile phase pH value.

Dosage form

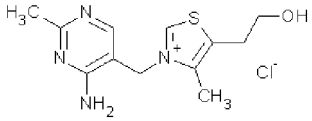
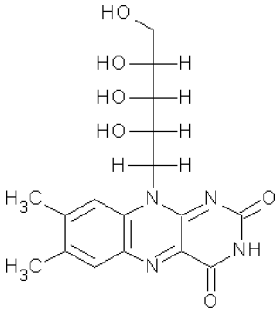
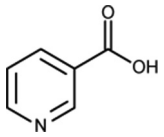
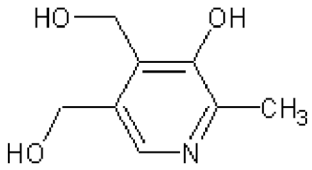
Vitamin B complex® coated tablet: thiamine hydrochloride (vit. B1) 5 mg, riboflavin (vit. B2) 2 mg, pyridoxine hydrochloride (vit. B6) 2 mg, and niacinamide (vit. PP) 20 mg.

Mobile phase preparation

Several mobile phases were examined in our search, mixture (water: methanol: glacial acetic acid) ratio (72:27:1 v/v) that contains in every 100 mL 140 mg. 5 Mm heptane sulfonic acid sodium salt as the ion pairing reagent were investigated and established.

The best results for the simultaneous determination of B1, B2, B3, and B6 vitamins were obtained after adjusting pH to 5 ± 0. 2 using 2N sodium hydroxide solution, the mobile phase was degassed by filtering through a Milli-Q 0. 45 μm pore membrane filter.

Table 1: Chemical structure, IUPAC name, and generic name of studied compound

Chemical structure and IUPAC name	Vitamin and generic name
 (3-[(4-amino-2-methyl-5-pyrimidinyl) methyl-5-(2-hydroxyethyl)-4-methyl thiazolium chloride hydrochloride) **Figure 1:** Chemical structure and IUPAC name of thiamine hydrochloride	Vitamin B1 Thiamine hydrochloride
 7,8-dimethyl-10-(2,3,4,5-tetrahydroxypentyl) benzo[g] pteridine-2,4-dione **Figure 2:** Chemical structure and IUPAC name of riboflavin	Vitamin B2 riboflavin
 (3-pyridine carboxamide) **Figure 3:** Chemical structure and IUPAC name of niacinamide	Vitamin B3 (pp) niacinamide
 (5-hydroxy-6-methyl-3,4-pyridine dimethanol hydrochloride) **Figure 4:** Chemical structure and IUPAC name of Pyridoxinehydrochloride	Vitamin B6 Pyridoxinehydrochloride

Chromatographic conditions

HPLC analysis was carried out using HPLC equipped with fluorescence (HPLC-FL) (Shimadzo Technologies, Japan). 20 µL of sample solution was injected into Rp-C18 (250 mm × 4. 6 mm, 5 µm particle size). A mixture (water: methanol: glacial acetic acid) ratio(72:27:1 v/v) as the mobile phase that contain in every 100 ml 140mg of 5 mM heptane sulphonic acid sodium salt as the ion pairing reagent ,the flow rate was 1. 0 mL/min,and the measurements were made at λ=280 nm.

Procedure

Solvent preparation

Mixture of (water: acetonitrile:glacial acetic acid) with ratio (94:5:1 v/v) was selected as suitable solvent for studied materials, due this mixture of solvent dose not absorb light at selected wave length which was 280 nm, in addition to all studied vitamins which are very soluble in this solvent mixture.

Preparation of standard solution

Accurately weighed amounts, 200 mg niacinamide, 20 mg pyridoxine hydrochloride, 20 mg riboflavin, and 50 mg thiamine hydrochloride, were taken to that 25. 0 flask, suitable amount of selected solvent was added, the flask was immersed in a hot water bath maintained at 65–70°C for 10 min and mixed on a vortex-mixer until complete solubility, cooled to room temperature, the flask was made up to the mark with same solvent, 5 mL of this solution was transferred into a 50 mL volumetric flask, diluted to the mark with the same solvent, and filtered

through 0. 45 μm membrane filter, clear filtrate was used, and the filtrate can be used within 3 h of filtration.

Preparation of sample solution

This was sample preparation of B1, B2, B6, and pp. Twenty tablets were weighed and triturated to a fine powder. The average mass of one tablet was transferred into a 25 mL volumetric flask and solution was added. The flask was immersed in a hot water bath maintained at 65–70°C for 10 min and mixed on a vortex-mixer until complete solubility, cooled to room temperature, and the flask were made up to the mark with same solvent and filtered through 0.45 μ membrane filter. The obtained concentrations for standard solution thiamine hydrochloride (vit. B1) riboflavin (vit. B2), pyridoxine hydrochloride (vit. B6), and niacinamide (vit. PP) compare to the labeled amount per tablet show in the Table 2.

HPLC procedure

Before injection into the chromatographic system, all analytical solutions were degassed by sonication. All the prepared sample solutions were first chromatographed to ensure that interfering peaks were not present. 10 μL and 100 μL aliquots of the standard solutions and sample solutions were injected. Triplicate injections were made for each solution.

RESULTS AND DISCUSSION

The aim of this study was to develop a simple, accurate, and precise HPLC method for simultaneous determination and separation of four vitamin niacinamide (vit. PP), pyridoxine hydrochloride (vit. B6), riboflavin (vit. B2), and thiamine hydrochloride (vit. B1) in pharmaceutical formulation (tablets). The method was developed based on mixture (water: methanol:glacial acetic acid) ratio (72:27:1 v/v) as the mobile phase that contains in every 100 mL 140 mg 5 mM heptane sulfonic acid sodium salt as the ion pairing and octadecyle column ODS c18 (250 × 4.6 mm, 5 μm) with UV detector 280 nm. Vitamins separation has various characteristics; there were acids, bases, or neutral compounds under certain circumstances.

At the RP-HPLC, the neutral compounds would be retained on column depend of their polarity, but the ionic compounds would be eluted spontaneously. A mixture of ionic and neutral compounds could be separated by RP–ion-pair chromatography. The ionic compounds were pairing with counter ion and distributed between the mobile and stationary phase as a non-ionic molecule, using an alkyl sulfonate as a counter ion, the cation such as thiamine could be a non-ionic molecule and retained on column due to the lipophylicity of the alkyl chain.

Solution of 1-hexane sulfonic acid sodium salt was used to decrease the retention time. The typical chromatogram of standard studied compounds is shown in Figure 5.

The optimization procedure included studies concerning the composition of the mobile phase,

Table 2: Prepared standard solution concentrations comparing to the labeled dosage form concentration

Prepared standard solution concentration mg/mL	Labeled concentration per tablet mg/ tab	Vitamin
0. 20	5 mg	B1 (Thiamine hydrochloride chloride)
0. 08	2 mg	B2 (riboflavin)
0. 08	2 mg	B6 (Pyridoxine hydrochloride)
0. 80	20 mg	PP (niacinamide)

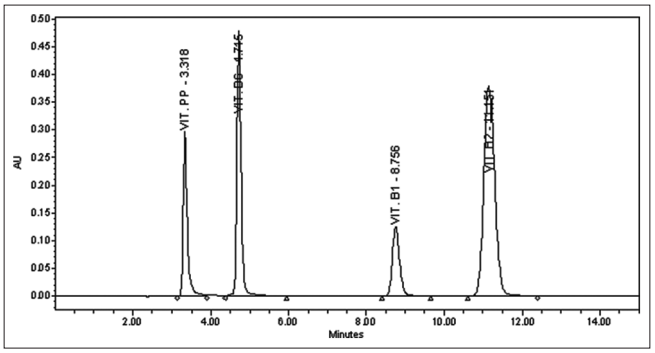


Figure 5: Representative chromatogram of the standard solution of vitamin B1, B2, PP, and B6

flow-rate, and temperature. After establishing the optimal conditions for the separation, the selectivity, linearity, precision, limit of detection, and limit of quantification were determined, the chromatographic parameters, that is, capacity factor, selectivity factor, resolution factor, and factor symmetry were calculated on the basis of the experimentally obtained, values of retention times, and width peaks for all the investigated B-complex vitamins. Under the described experimental conditions, there is no scientific difference between the values of retention times for samples and standards of studied compounds, Table 3 shows peaks summary statics (mean, StdDev, RSD%) which indicates suitability of our selected chromatographic conditions for separation and determination B group vitamins.

The values of selectivity factor were 1.5 for nicotinamide/pyridoxine hydrochloride/,1. 4 thiamine hydrochloride/pyridoxine hydrochloride and 1.6 for ribofalavin/thiamine hydrochloride. The resolution factors Rs between the chromatographic peaks were calculated from the equation Rs=2 $(t_2–t_1)/(W_1+W_2)$, where t_2 and t_1 are the retention times of the two components and W_1 and W_2 are the peak widths at the base of the two respective peaks: 4.2 for pyridoxine hydrochloride/nicotinamide, 4.6 for riboflavin/pyridoxine hydrochloride, and 8. 4 for thiamine hydrochloride/riboflavin. The representative chromatogram of the working standard solution of B1, B2, PP, and B6 is presented in Figure 5.

The assay was selective, no significant interfering peaks were observed at the retention times of the vitamins. All excipients eluted at different times and did not interfere with the analyzed compounds. The representative chromatogram of the sample solutions of vitamin B1, B2, PP, and B6 presented in Figure 6.

The linearity of the method was determined by injecting five solutions of concentration between 50% and 150% of the expected concentration, analysis was performed in triplicate to determinate the linearity of the assay. Good linearitys were obtained with correlation coefficients above 0.99. The important parameters of calibration curves, that is, slope(a), intercept (b), and correlation coefficient (r) are presented in Table 4.

Table 3: Peaks summary statics for standards and samples of studied compound

Vitamin	RT (min)	AUC
B1 (standard)	8.765	1611889
B1 (sample)	8.729	1619110
Mean	8.743	1617999.207
SD	0.019	1570.739
RSD%	0.22	0.10
B2 (standard)	11.151	6810166
B2 (sample)	11.101	6828197
Mean	11.126	681918.430
SD	0.035	12749.695
RSD%	0.31	0.19
PP (standard)	3.318	2164957
PP (sample)	3.314	2165263
Mean	3.316	2165109. 937
SD	0.003	216.869
RSD%	0.08	0.01
B6 (standard)	4.715	3587391
B6 (sample)	4.705	3593818
Mean	4.710	3590604.207
SD	0.007	4544.235
RSD%	0.15	0.13

Table 4: The important parameters for the calibration curves

Vitamin	y=ax+b	R
B1	y=33472.3x+659. 06	0. 9997
B2	y=4163.216x–1118	0. 9994
B pp	y=1570.58x+2484.68	0. 9995
B6	y=30344.56x–2616.38	0. 9980

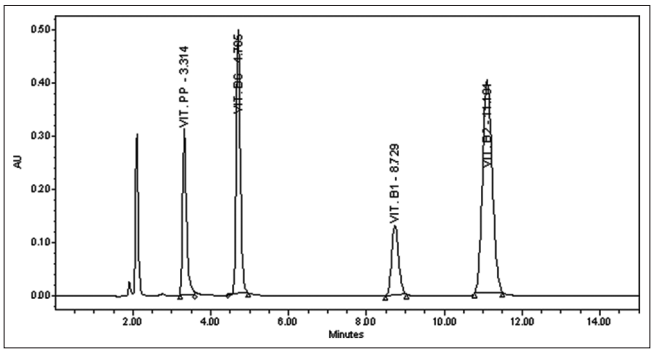


Figure 6: Representative chromatogram of the sample solution of vitamin B1, B2, PP, and B6

The precision of the procedure was checked by analysis of ten working standard solutions (B1 = 10 μg/mL, PP = 40 μg/mL, B6 = 4 μg/mL, and B2 = 4 μg/mL). The RSD values 1. 3%; 0.6%; 0.1%; and 0.4% for B1, PP, B6, and B2, respectively, were indicative of the satisfactory repeatability and

Table 5: LOD and LOQ

Vitamin	LOD (μg/mL)	LOQ (μg/mL)
B1	0. 3125	0. 6250
B2	0. 1250	0. 2499
B pp	0. 3125	0. 6250
B6	0. 0780	0. 1538

LOD: Limits of detection, LOQ: Limits of quantification

Table 6: Results of the determination of B-group vitamins in B complex® coated tablets

Vitamin	Amount in B complex® tablet	Found	Recovery %	RSD %
B1	5.0 mg (3.5–5.5 mg)	4.520 mg	90.4	1.2
B2	2.0 mg (1.25–2.75 mg)	2.104 mg	105.2	0.4
B pp	20.0 mg (17.0–23.0 mg)	21.38 mg	106.9	1.7
B6	2.0 mg (1.25–2.75 mg)	2.104 mg	105.2	2.0

thereby the precision of the system. The limit of detection (LOD) and limit of quantification (LOQ) for the investigated vitamins were experimentally determined and they are presented in Table 5.
The accuracy was carried out at 80%, 100%, and 120% of specification limit, Table 6 shows recoveries mean, concentrations found mean and relative standard deviation mean for each vitamin, good accuracy and reproducibility of used method were obtained.

CONCLUSION

The simultaneous determination of the four water-soluble vitamins thiamine hydrochloride, riboflavin, nicotinamide, and pyridoxine hydrochloride was performed on column of c18 (250 × 4. 6,5 μm), a mixture of (water: methanol:glacial acetic acid) ratio (72:27:1 v/v) as the mobile phase with flow rate of 1mL/men, the effluent was monitored at 280 nm. The simplicity, rabidity, specify, and accuracy of the procedure should make it highly desirable for quality control of multivitamin products in the pharmaceutical and health food industries.

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