Chromatographic Analysis of Vitamin B1, B2, B6 and Folic Acid in Multivitamin Pharmaceutical Dosages Available in Local Market

Syeda Kiran Shahzadi, Muhammad Abdul Qadir, and Adil Munir

***Abstract*—In the present study, the RP-HPLC based separation and quantification of four common vitamins: B_1, B_2, B_6, and folic acid in multivitamin pharmaceutical dosages was performed. Simultaneous determination of these vitamins was performed usin Promosil HPLC column C18 at 35 ℃ with methanol – 0.05 M sodium acid phosphate (80 + 20, v/v) as mobile phase at pH 7.0 using UV-Visible detection system at wavelength 254 nm. Sample selection was made on the basis of products that declare the presence and mount of B group vitamins on their labels. All pharmaceutical dosages confirmed the presence of these vitamins. Amount of these vitamins in all formulations were same as declared on label. All the multivitamin pharmaceutical dosages complied with the standard declaration.**

***Index Terms*—RP-HPLC, multivitamins, pharmaceutical formulations, analysis.**

I. Introduction

Vitamins are the organic compounds that are essential for normal health, growth and functioning of human bodies. In foods, they are usually present in minor amounts. Deficiency of vitamins in human body can cause some serious diseases [1]. Vitamins are generally utilized as dietary supplements. They are ordinarily prescribed by doctors as a drug for mild illness to severe chronic illnesses. It is well known fact that vitamins help in boosting immune system, improve health and help in enhanced recovery from an illness [2]

In some recent years, there is an increased interest in humans for food supplements. It has been accounted for that the most widely recognized sort of dietary supplement in the United States is a multivitamin supplements. In US, it was found that the utilization of vitamin supplements expanded from 23.2% in 1987 to 33.9% in 2000 over all races and genders. This supplement utilization was most commonly observed in educated females living a sound lifestyle [3].

One of the significant explanations behind their expanding use is their simple accessibility as over the counter (OTC) agents. OTC medication items are those medications that are accessible to customers without a prescription. OTC specialists for the most part have a low abuse potential; Their advantages exceed their dangers and can be utilized for selfdiagnosed conditions. Expanding wellbeing, training and wage among overall public are vital explanations behind their widespread use [4].

Role of vitamins in human's health and growth had also become well-aware. So consumption of fruits and vegetables as sources of vitamins in daily diet has also increased. But vitamins can easily be leached during storage of fruits and vegetables and by some chemical reactions also, so there was a need to have such available preparations which can replace the possible deficiency of vitamins in daily diet. So the use of multi-vitamin pharmaceutical dosages become widely employed, which lead to the need of a powerful analytical technique for quality control of these pharmaceutical dosages [5]

Vitamins can be chromatographed individually or in combination of two to many vitamins either isocratically or by gradient elution. Simultaneous determination of these vitamins had become most interesting during the last decade. Thus many methods such as micellar liquid chromatography [6], micellar electrokinetic chromatography [7], capillary electrophoresis [8] and High performance liquid chromatography [9] have been developed.

Nowadays, high performance liquid chromatography is the most widely used technique for simultaneous determination of multi-vitamins. These methods involve use of complex buffer system as mobile phase, many types of column packing materials [10] or stationary phases and numerous detectors such as UV-Visible absorbance detector with variable wavelengths, fluorimetric detector [11], photodiode array detector and electrochemical detector [12].

Due to complex composition, multi-vitamin pharmaceutical dosages are interesting for analysis. It has become special provocation for analytical chemists. Due of that, aim of our study was to analyze some common vitamins in multivitamin pharmaceutical dosage forms by using most commonly HPLC technique.

II. Materials and Methods

A. Reagents

All materials, reagents and chemicals used in this study were of analytical reagent grade. Multivitamin tablets and capsules for this analysis were collected locally from Lahore, Pakistan.

B. Standard Preparation

1 mg/ml of working standards i.e. thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride and folic

Manuscript received May 9, 2018; revised August 17, 2018.

Syeda Kiran Shahzadi and Muhammad Abdul Qadir are with Institute of Chemistry, University of the Punjab, Lahore, Pakistan (e-mail: kiran.syyed@gmail.com).

Adil Munir is with Department of Chemistry, Minhaj University, Lahore, Pakistan.

doi: 10.18178/ijcea.2018.9.4.714

acid were dissolved in methanol. Solutions were further serially diluted to the concentration of 5-10 µg/ml for thiamine chloride hydrochloride, 1.5-2 µg/ml for riboflavin, 5-10 µg/ml for pyridoxine hydrochloride and 1-2 µg/ml for folic acid. 50 µl of each solution were used for analysis.

C. Sample Preparation

Each tablet were weighed and triturated into fine powder. Powdered sample was suspended in 50 ml of methanol, sonicated for 15 min and dilute to 100 ml with methanol. 50 µl of each was for HPLC analysis.

D. Chromatographic Conditions

Shimadzu LC system (LC 20A pump) equipped with UV/Visible detector was used for analysis. A single wavelength of 254 nm was used for simultaneous detection. A Promosil HPLC column C18 was used at 35 ℃. Methanol – 0.05 M sodium acid phosphate (80 + 20, v/v) at pH 7.0 was used as mobile phase. Flow rate was adjusted to 0.8 ml/min. 50 µl of sample and standard solutions were injected in a single injection.

III. Results and Discussion

The present research work describes an analysis based study of multivitamin pharmaceutical dosages using a simple and a sensitive Reverse-phased-HPLC with UV/Visible detector. This method offers simultaneous determination of some common B-group vitamins: Vitamin B_1 (Thiamine chloride hydrochloride), Vitamin B_2 (Riboflavin), Vitamin B_6 (Pyridoxine hydrochloride) and folic acid in multivitamin pharmaceutical dosages. Six random multivitamin tablets and capsules were selected and collected from local market of Lahore, Pakistan.

For the separation and quantification of vitamins in multivitamin formulations, best results were obtained with methanol – 0.05 M sodium acid phosphate (80 + 20, v/v) as mobile phase. Effect of minor parameters such as eluent composition, pH value, flow rate and temperature was investigated. There was observed a minor or no effect of these parameters on analytical results. Mobile phase composition was changed to ±5%, the column temperature from 25 to 40 ℃ and pH from 5 to 8. There was no remarkable effect on retention time. These minor changes didn't interfere in quantification results too. So the analysis was performed with methanol – 0.05 M sodium acid phosphate (80 + 20, v/v) as mobile phase, pH 7, 35 ℃ temperature and 0.8 ml/min flow rate.

TABLE I: Determination of Vitamin B_1, B_2, B_6 and Folic Acid in Multivitamin Pharmaceutical Dosages

Multivitamin pharmaceutical Dosage	Vitamins	Amount Found	Recovery %	Range %	RSD %	t_n
	B_1	2.79 mg	99.7	95-105	0.17	0.21
Multivitamin pharmaceutical Dosage-1	B_2	3.25 mg	101.58	95-105	0.77	0.74
	B_6	3.9mg	99.7	95-105	1.26	1.72
	FA	403.6 mg	100.98	95-105	0.44	2.12
Multivitamin pharmaceutical Dosage-2	B_2	2.06 mg	103	95-105	1.47	2.76
	B_6	1.014 mg	101.4	95-105	0.69	1.57
	FA	0.49 mg	98.3	95-105	1.01	1.42
Multivitamin pharmaceutical Dosage-3	B_1	14.95 mg	99.67	95-105	0.16	0.63
	B_2	10.03 mg	100.33	95-105	0.14	0.93
	B_6	12.01 mg	100.08	95-105	0.05	1.10
	FA	149.5 mcg	99.4	95-105	0.17	0.91
Multivitamin pharmaceutical Dosage-4	B_1	50.09 mg	100.18	95-105	0.09	1.23
	B_2	14.73 mg	98.2	95-105	0.91	1.42
	B_6	5.025 mg	100.5	95-105	0.25	0.94
	FA	498.58mcg	98.3	95-105	0.14	1.42
Multivitamin pharmaceutical Dosage-5	B_1	1.996 mg	99.8	95-105	0.1	0.62
	B_2	2.502 mg	100.09	95-105	0.04	1.12
	B_6	2.592	100.9	95-10	1.8	1.25

		mg		5		
Multivitamin pharmaceutical Dosage-6	B_1	14.94 mg	99.6	95-105	0.20	0.72
	B_2	15.12 mg	100.8	95-105	0.39	1.43
	B_6	9.96 mg	99.6	95-105	0.20	1.71

The representative HPLC chromatograms of the standard solution of Vitamin B_1, Vitamin B_2, Vitamin B_6 and folic acid are presented in Fig. 1 (a,b,c,d) respectively. Values of retention times as seen from the Fig.1 are: 3.205 min for Vitamin B_1, 2.9511 min for Vitamin B_2, 2.801 min for Vitamin B_6 and 4.189 min for folic acid. Good linear curves were obtained with working standard concentration range: 5-10 µg/ml for Vitamins B_1 and B_6, 1.5-2 µg/ml for Vitamins B_2 and 1-2 µg/ml for folic acid. Value of correlation coefficients were above 0.99.

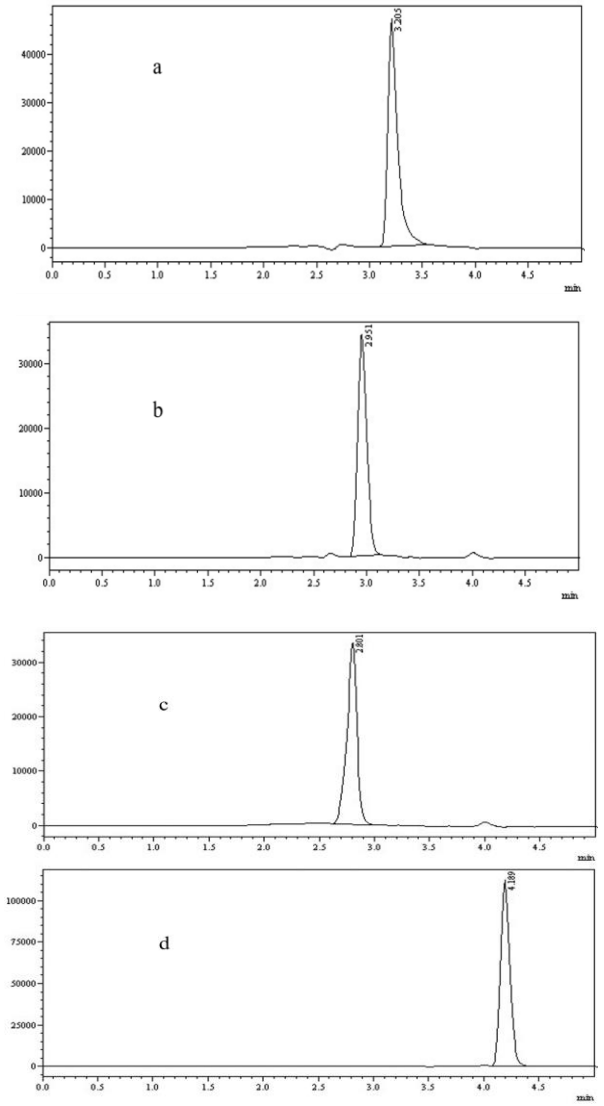

Fig. 1. Representative chromatograms of the standard solution of Vitamin B_1 (a) B_2 (b), B_6 (c) and folic acid (d).

Individual vitamin peak in sample chromatograms was identified by comparing retention time with that of standard peaks. The best fit standard curves were prepared by linear regression of peak areas and concentrations.

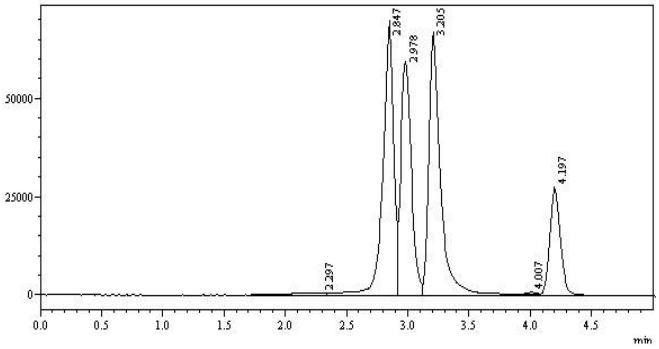

Fig. 2. Representative chromatogram of the multivitamin pharmaceutical dosage-1.

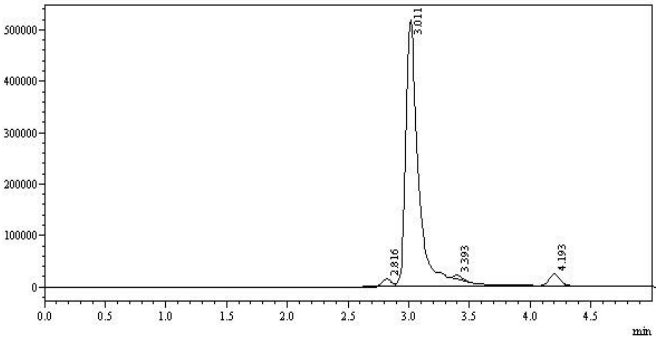

Fig. 3. Representative chromatogram of the multivitamin pharmaceutical dosage-2.

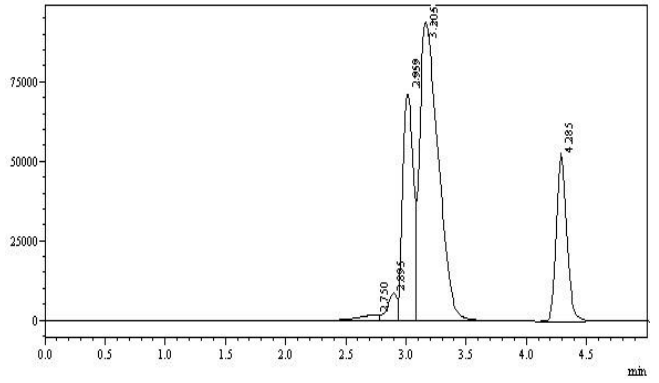

Fig. 4. Representative chromatogram of the multivitamin pharmaceutical dosage-3.

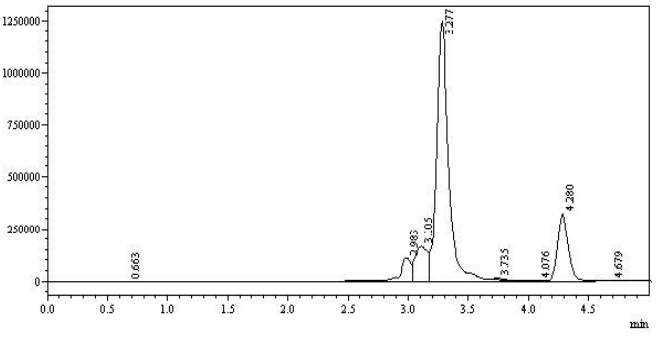

Fig. 5. Representative chromatogram of the multivitamin pharmaceutical dosage-4.

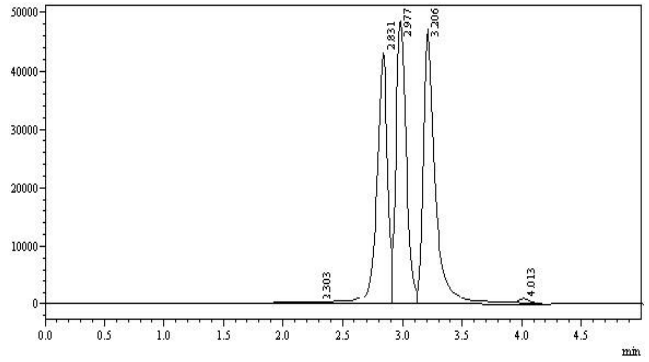

Fig. 6. Representative chromatogram of the multivitamin pharmaceutical dosage-5.

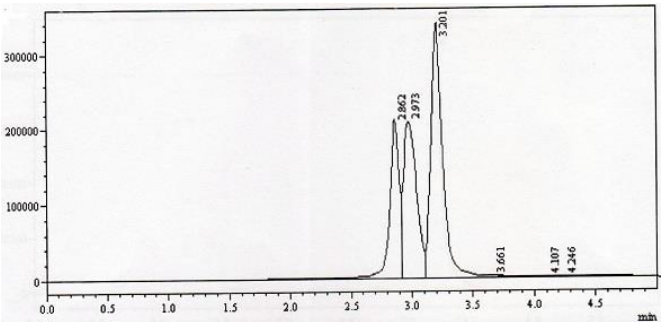

Fig. 7. Representative chromatogram of the multivitamin pharmaceutical dosage-6.

The results of quantification of four common B-group vitamins in multivitamin formulations are shown in Table I Sample selection was made on the basis of products that declare presence of B group vitamins on their labels. All of the tested pharmaceutical formulations contained most of the B group vitamins with an amount slightly deviated from that of declared on the label. The RSD values for vitamin B_1, vitamin B_2, vitamin B_6 and folic acid in sample solutions were in the range of 0.04 to 1.8 (below 2 %) which indicates satisfactory repeatability of the analytical system. The percentage of recovery of vitamin B_1, vitamin B_2, vitamin B_6 and folic acid were within the limit (95-105%) range show the validity and accuracy of the method.

IV. Conclusion

In the present analysis, six random multivitamin pharmaceutical dosages were qualitatively and quantatively analyzed for the presence of Vitamin B_1, B_2, B_6 and folic acid by a well know RP-HPLC method. All pharmaceutical dosages confirmed the presence of these vitamins. Amount of these vitamins in all formulations were same as declared on label.

Acknowledgement

Authors are acknowledged to Director Institute of Chemistry, Minhaj University, Lahore, Pakistan and Director Institute of Chemistry, University of the Punjab, Lahore, Pakistan to support this research work by providing facilities.

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Syeda Kiran Shahzadi was born in Pakista in September 1st, 1989. She got PhD scholar at Institute of Chemistry, University of the Punjab, Lahore, Pakistan. She majors in analytical/medicinal chemistry. She has been working as visiting lecturer at Department of Science Education, University of the Punjab, Lahore, Pakistan. Previously she been working as visiting research scholar at William G. Lawrie Department of Chemical and Biomolecular Engineering, Columbus, Ohio, USA. She has published 7 research papers in reputed journals and 3 conference papers.