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Determination of Water-Soluble Vitamins B_1, B_2, B_3, B_6, B_9, B_{12} and C on ODS Column 5 µm by High Performance Liquid Chromatography with UV-DAD Detection

SAAD ANTAKLI[1,*], NAZEERA SARKEES[2] and TEREZA SARRAF[1]

[1]Department of Chemistry, Faculty of Science, University of Aleppo, Aleppo, Syria
[2]Department of Analytical Chemistry, Faculty of Pharmacy, University of Aleppo, Aleppo, Syria

*Corresponding author: E-mail: antakli@scs-net.org

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HPLC method was developed and applied to the determination of some water-soluble vitamins (B_1, B_2, B_3, B_6, B_9, B_{12} and C) in Syrian pharmaceutical preparations. Reserved phase, ion-pair high performance liquid chromatography with UV-DAD detection was employed. The developed chromatographic method employed an ODS column with dimension (150 × 4.6 mm; 5 µm). The mobile phase was 5.3 mM of hexane-1-sulfonic acid sodium with 0.1 % triethylamine as solvent (A) at pH 3.3 and 5.3 mM of hexane-1-sulfonic acid sodium:acetonitrile (50:50) with 0.1 % triethylamine as solvent (B) at pH 3.5 and paracetamol as internal standard. The method showed acceptable values for precision, recovery and sensitivity.

Keywords: Determination, Vitamins, Reserved phase, Ion-pair.

INTRODUCTION

Vitamins in general play a very important role in our health; even though they only make up a very small part of the food we eat each day[1]. Multivitamin pharmaceutical preparations containing mixtures of these substances are interesting for analysis and most of them include the water-soluble B-group. The simultaneous determination of several water-soluble vitamins is difficult and often many different analyses have been performed. Water-soluble vitamins have been determined by various instrumental methods such as fluorescence[2] which can determine a small concentration of vitamins but it concerns only fluorescent vitamins and it coast lot of time and power, microbiological methods[3] which are typically designed for single vitamin analysis and time consuming, electrochemical assays[4], immunoassays[5,6] and chromatographic methods, including gas chromatography[7], capillary electrophoresis[8-10], thin-layer chromatography[11] and liquid chromatography (LC) with various method for the simultaneous determination of multiple vitamins in food or multivitamin tablets because of its high sensitivity available detection[12-14]. Among these methods, HPLC is a promising and specificity for separation and determination mixture of vitamins with several detections such as electrochemical detection[15], mass spectroscopy[16], fluorescence[17,18] and UV, visible detection[19-22]. HPLC gives ability to determine mixture of vitamins in one step and with high accuracy and good sensitivity for separated vitamins and that help us to save time, solvents and money.

The present paper describes a sensitive and simple ion-pair RP-HPLC validated method using a C_{18} column with UV/VIS-DAD (diode array detector) detection for determination of 7 water-soluble vitamins: thiamine hydrochloride (vitamin B_1), riboflavin (vitamin B_2), nicotinamide (vitamin B_3), pyridoxine hydrochloride (vitamin B_6), folic acid (vitamin B_9), cyanocobalamin (vitamin B_{12}) and ascorbic acid (vitamin C) in multivitamin preparations.

HPLC-DAD detection can give us high absorption of every vitamin so we have the ability to determine each water-soluble vitamin in specific wavelength, so we can determine smaller amount of each in different products correctly and in one step.

EXPERIMENTAL

The chromatograms were obtained by using Hitachi liquid chromatography equipped with a photodiode array detector (PDA) Hitachi L-2455, pump Hitachi L-2130, column oven Hitachi L-2350 and autosampler Hitachi L -2200. The column was from MN Company. Ultrasonic 405 from Hwashin Technology (Korea), Micropipt IsoLap (Germany).

Standard vitamins (B_1, B_2, B_3, B_6, B_9, B_{12} and C) were purchased from Dr Ehrenstorfer (Germany), HPLC grade acetonitrile and methanol. Hexane-1-sulfonic acid sodium, sodium monohydrogen phosphate was purchased from Merck (Germany).

Standard preparation: Standard stock solution of vitamins (B_1, B_2, B_3, B_6, B_9, B_{12} and C) in concentration of (2000 mg/L) for all, obtained by dissolving required amount of them in diluted solution (0.05M of Na_2HPO_4 at pH 6.3). The working standard solutions (0.1-1500 mg/L) for all vitamins were prepared by diluting the standard stock solution with diluted solution.

Calibration curve: To construct the calibration curve five replication (20 µL) of each standard solution were injected immediately after preparation into column and peak area of chromatograms were measured.

Sample preparation: 20 tablets or capsules containing water soluble vitamins were weighed and finely powdered. The average mass of one tablet or capsule was transferred into a 50 mL volumetric flask and 20 mL of methanol was added, then the volume reached by diluted solution to the obtained volume. After putting the flask to ultrasonic for 20 min, (5 mL of this solution for Minavite, 10 mL for Baricomplex, Cenvite, B-complex, Obarvite, 1 mL for Damvita, Barkaneurin) was transferred into a 25 mL volumetric flask and 5 mL of paracetamol (1 mM) was added, diluted to the mark with the diluted solution and filtered through a 0.45 µm Millipore filter. For determining water soluble vitamins in ampules, the content of 5 ampules was transferred into a 50 mL volumetric flask diluted to the mark with the diluted solution, then 2 mL of this solution was transferred into a 25 mL volumetric flask and 5 mL of paracetamol was added, diluted to the mark with the diluted solution and filtered through a 0.45 µm Millipore filter. For determination of vitamin B_{12} 20 tablets or capsules containing water soluble vitamins were weighed and finely powdered. The average mass of four tablets or capsules was transferred into a 10 mL volumetric flask and 10 mL of diluted solution was added. After putting the flask to ultrasonic for 20 min, 0.5 mL of this solution was transferred into a 10 mL volumetric flask and 0.2 mL of paracetamol was added. For ampules, the content of 3 ampules was transferred into a 10 mL volumetric flask and 2 mL of paracetamol was added. All solutions diluted to the mark with the diluted solution and filtered through a 0.45 µm Millipore filter.

Chromatographic condition: A C_{18} column (15 cm × 4.6 mm; 5 µm) was used. Mobile phase was 5.3 mM of hexansulfonic acid with 0.1 % triethylamine at pH 3.3 (adjusted by orthophosphoric acid 1 M) as solvent (A) and 5.3 mM of hexansulfonic acid and acetonitrile (50:50) with 0.1 % triethylamine at pH 3.5 (adjusted by orthophosphoric acid 1 M) as solvent B. The column was operated at (25 ºC). The flow rate was 1 mL/min and the injected volume 20 µL. Starting with solvent A 95 % and solvent B 5 %. A gradient elution was performed till the mobile phase composition 35 % of A and 65 % of B for 9 min, then it was constant for next 4 min. Detection was performed at 246 nm for vitamins C and B_1, 267 nm for vitamin B_2, 260 for vitamin B_3, 290 for vitamin B_6, 282 nm for vitamin B_9, 361 nm for vitamin B_{12}.

RESULTS AND DISCUSSION

Under new chromatographic conditions, the topical chromatogram of standard solution of water soluble vitamins (WSV) at 270 nm as a general view with paracetamol as internal standard is presented in Fig. 1. The values of retention times were: 2.07 min for C, 5.20 min for B_3, 9.31 min for B_6, 11.19 min for B_9, 11.61 min for B_1, 12.15 min for B_{12}, 12.73 min for B_2 and 8.45 min for paracetamol. Fig. 2 represent the typical chromatograms of water soluble vitamins standard solutions at 246 nm for C and B_1, 267 nm for B_2, 260 for B_3, 290 for B_6, 282 nm for B_9, 361 nm for B_{12} with paracetamol as internal standard.

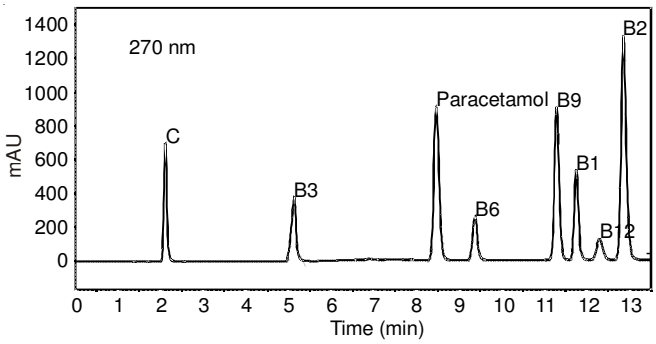

Fig. 1. Chromatogram of water soluble vitamins at 270 nm with paracetamol as internal standard

The linearity of the method was determined by injection five replicated solutions of concentration between 0.01-1500 mg/L. Good linearities were obtained with correlation coefficients > 0.99. The important parameters of calibration curves in addition to limit of detection (LOD) and limit of quantification (LOQ) are presented in Table-1.

The new chromatographic conditions were applied on seven different Syrian pharmaceutical preparations. Typical separation chromatograms (Fig. 3) of water soluble vitamins for one of these pharmaceutical preparations (Damvita) at 270 nm as a general view, at 246 nm for C and B_1, 267 nm for B_2, 260 for B_3, 290 for B_6, 282 nm for B_9, 361 nm for B_{12} with paracetamol as internal standard. Recoveries were tested by the standard addition procedure. Three addition levels were used for each water-soluble vitamin in multivitamin samples. Five replicated injections were performed for each addition

TABLE-1
LINEARITY OF STANDARD CURVES, LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ) FOR SEVEN WATER-SOLUBLE VITAMINS

Vitamin	y=ax+b	r	Concentration range (mg/L)	LOQ (mg/L)	LOD (mg/L)
Ascorbic acid (C)	y = 0.0027x - 0.0028	0.9991	0.17 - 300	0.17	0.05
Niacin (B_3)	y = 0.0018x + 0.0035	0.9993	1.49 - 700	1.49	0.45
Pyridoxine (B_6)	y = 0.0024x + 0.0005	0.9988	1.76 - 250	1.76	0.53
Folic acid (B_9)	y = 0.0032x + 0.0035	0.9990	1.35 - 250	1.35	0.41
Thiamin (B_1)	y = 0.0023 + 0.0118	0.9906	0.51 - 250	0.51	0.15
Cyanocobalamin (B_{12})	y = 0.0010x + 0.0010	0.9985	1.80 - 1000	1.80	0.54
Riboflavin (B_2)	y = 0.0050x - 0.0082	0.9976	0.71 - 250	0.71	0.21

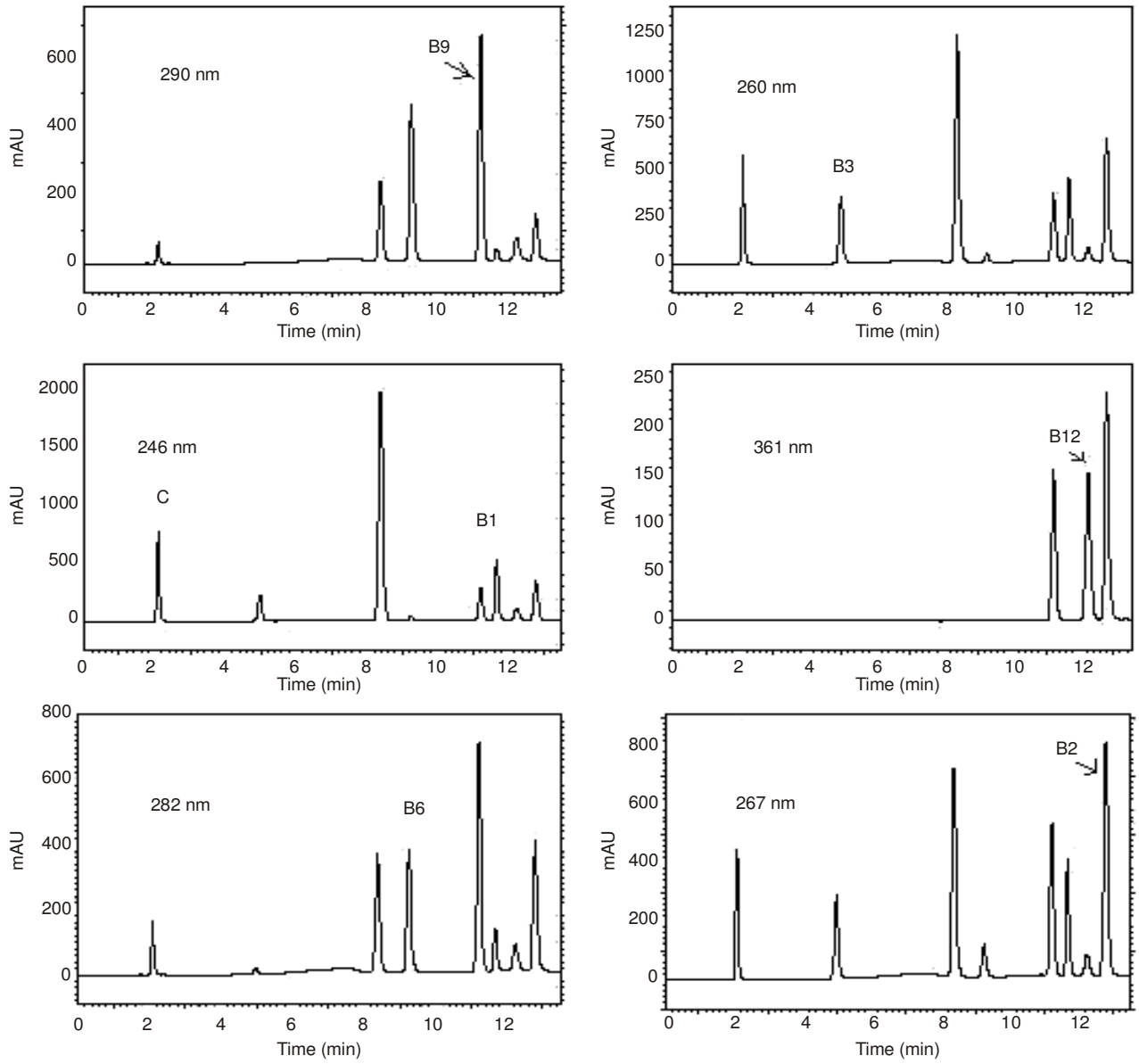

Fig. 2. Typical chromatograms of water soluble vitamins standard solutions at 246 nm for vitamin C and B_1, 267 nm for B_2, 260 for B_3, 290 for B_6, 282 nm for B_9, 361 nm for B_{12} with paracetamol as internal standard

level. Mean recoveries was calculated of three different concentrations. The recoveries and the vitamins results obtained on C_{18} column for seven Syrian pharmaceutical products are presented in Table-2.

Conclusion

In this work we developed and put new chromatographic conditions for determining water-soluble vitamins (B_1, B_2, B_3, B_6, B_9, B_{12} and C) in seven Syrian pharmaceutical products. Under the suggested chromatographic conditions the water soluble vitamins have been separated and determined on C_{18} column. The presence of very little amount of triethylamine in the mobile phase lead to obtain better symmetric and fine peaks.

The new method has been validated in terms of sensitivity, linearity, precision and accuracy. Low limit of detection and quantification due to UV-DAD detection, very good linearity in large concentration intervals and acceptable precision.

The results confirm that the proposed method is simple, accurate and precise and can be successfully applied for the routine analysis of the above vitamins in B-complex tablets. The investigated vitamins were completely separated with good resolution within an analysis time not exceed 13 min. Under the recent study, we could have a good determination, separation a fine peaks of soluble vitamins (B_1, B_2, B_3, B_6, B_9, B_{12} and C) in a short time.

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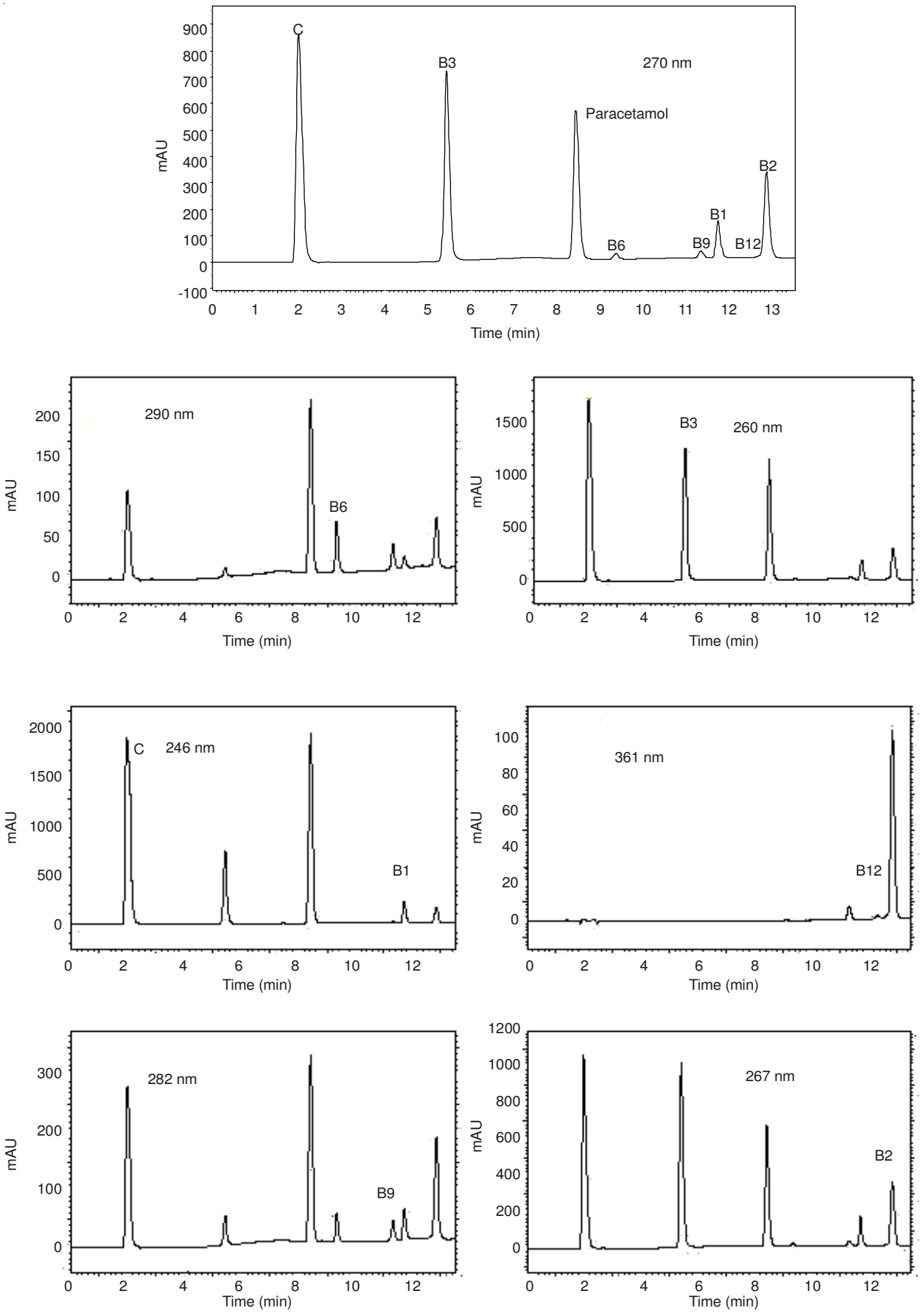

Fig. 3. Chromatograms of water soluble vitamins for (Damvita) preparation at 270 nm as a general view, 246 nm for C and B_1, 267 nm for B_2, 260 for B_3, 290 for B_6, 282 nm for B_9, 361 nm for B_{12} with paracetamol as internal standard

TABLE-2
RECOVERIES AND AMOUNT OF WATER-SOLUBLE VITAMINS (C, B_1, B_2, B_3, B_6, B_9, B_{12}) IN SEVEN SYRIAN PHARMACEUTICAL PRODUCTS ON C_{18} COLUMN

Product	Company	Vit. C mg/dose		RSD (%)	Rec. (%)	Vit. B_1 (mg/dose)		RSD (%)	Rec. (%)
Minavite (capsules)	Shiffa	75	74.43	1.064	99.2	20	19.837	1.298	99.18
Baricomplex (tablets)	Barakat	–	–	–	–	15	15.617	4.03	104.1
Barkaneurine (tablets)	Barakat	–	–	–	–	100	99.445	0.585	99.445
Cenvite (tablets)	Pharmasyr	60	59.8	1.4	99.65	1.5	1.52	0.93	101.3
Damvita (capsules)	Ultra Medica	150	149.42	0.09	99.61	10	10.117	1.001	101.17
B-complex (tablets)	Asia	–	–	–	–	5	5.1103	1.123	102.21
Obarvite (ampules)	Obari	–	–	–	–	10	10.177	3.747	101.77
Product	Company	Vit. B_2 (mg/dose)		RSD (%)	Rec. (%)	Vit. B_3 (mg/dose)		RSD (%)	Rec. (%)
Minavite (capsules)	Shiffa	10	10.339	1.869	103.39	40	40.11	0.621	100.27
Baricomplex (tablets)	Barakat	15	14.81	0.767	98.73	50	49.33	1.32	98.66
Barkaneurine (tablets)	Barakat	–	–	–	–	–	–	–	–
Cenvite (tablets)	Pharmasyr	1.7	1.701	1.6	100.06	20	20.05	1.117	100.25
Damvita (capsules)	Ultra Medica	10	10.03	0.894	100.3	100	101.61	1.304	101.61
B-complex (tablets)	Asia	2	2.0568	2.460	102.84	20	21.2153	1.5152	106.08
Obarvite (ampules)	Obari	4	4.0378	0.572	100.94	40	40.9893	2.3974	102.47
Product	Company	Vit. B_6 (mg/dose)		RSD (%)	Rec. (%)	Vit. B_9 (mg/dose)		RSD (%)	Rec. (%)
Minavite (capsules)	Shiffa	5	5.039	1.363	100. 79	–	–	–	–
Baricomplex (tablets)	Barakat	10	10.08	4.75	100.8	–	–	–	–
Barkaneurine (tablets)	Barakat	200	200.65	0.799	100.32	–	–	–	–
Cenvite (tablets)	Pharmasyr	2	2.03	0.591	101.5	0.4	0.395	1.772	98.75
Damvita (capsules)	Ultra Medica	3	3.0588	1.665	101.96	1.5	1.5103	2.166	100.68
B-complex (tablets)	Asia	2	2.1404	3.838	107.02	–	–	–	–
Obarvite (ampules)	Obari	4	4.0345	0.652	100.86	–	–	–	–
Product	Company	Vit. B_{12} (mg/dose)		RSD (%)	Rec. (%)				
Minavite (capsules)	Shiffa	–	–	–	–				
Baricomplex (tablets)	Barakat	0.01	0.00982	2.449	98.2				
Barkaneurine (tablets)	Barakat	0.5	0.4998	1.69	99.96				
Cenvite (tablets)	Pharmasyr	0.006	0.0062	1.012	103.33				
Damvita (capsules)	Ultra Medica	0.005	0.005012	0.463	100.24				
B-complex (tablets)	Asia	–	–	–	–				
Obarvite (ampules)	Obari	0.008	0.00806	2.977	100.87				

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