Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 6, 2015

Original Article

DETERMINATION OF WATER-SOLUBLE VITAMINS B_1, B_2, B_3, B_6, B_9, B_{12} AND C ON C_{18} COLUMN WITH PARTICLE SIZE 3 µM IN SOME MANUFACTURED FOOD PRODUCTS BY HPLC WITH UV-DAD/FLD DETECTION

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Received: 17 Dec 2014 Revised and Accepted: 15 Jan 2015

ABSTRACT

Objective: Objective of the study was to develop a simple, precise and accurate RP-HPLC ion-pair method, for the determination of water-soluble vitamins (B_1, B_2, B_3, B_6, B_9, B_{12}, and C) in some manufactured food products.

Methods: RP-HPLC with C_{18} BDS (100 x 4.6 mm; 3 µm) column were used. Mobile phase constituents were solvent (A): 5.84 mM of hexane-1-sulfonic acid sodium: acetonitrile (95:5) with 0.1% triethylamine at pH 2.5 and solvent (B): 5.84 mM of hexane-1-sulfonic acid sodium: acetonitrile (50:50) with 0.1% triethylamine at pH 2.5, flow rate 1.6 ml/min, at column temperature 40 °C and suitable detection wavelength.

Results: Two different detectors were used: photo diode array detector (UV-DAD) and the fluorescence detector (FLD). Calibration graphs plotted with five concentrations of each vitamin where linear regression coefficients R^2> 0.9972. LOQ values were 50.0, 81.0, 19.1, 19.0, 30.0, 9.7, 50.0 µg/l with DAD for vitamins B_1, B_2, B_3, B_6, B_9, B_{12} and C respectively, and 5.7, 4.1 µg/l with FLD for vitamins B_2, B_6 respectively. LOD values were 16.5, 26.7, 6.3, 6.3, 9.9, 3.2, 16.5 µg/l with DAD for vitamins B_1, B_2, B_3, B_6, B_9, B_{12} and C respectively, and 1.9, 1.3 µg/l with FLD for vitamins B_2, B_6 respectively.

Conclusion: The proposed method was successfully applied to analysis mixture of seven water-soluble vitamins in pure form and in manufactured food products, with average recovery of 98.14% to 100.96%.

Keywords: Determination, Vitamins, High performance liquid chromatography, Reserved-phase, Ion-pair, Manufactured, Food.

INTRODUCTION

Vitamins are a broad group of organic compounds required to maintain normal cellular and metabolic functions of human and animal body. As it is well known, vitamins are natural constituents of food and well-balanced diet supplies of all the required vitamins [1]. According to their solubility, they are divided into two groups: water-soluble vitamins and fat-soluble vitamins [2].

The importance of vitamins in nutrition was initially understood in the 1920s and 1930s [3], where lack of them can cause serious diseases in humans even though only small concentrations are required to maintain good health [4]. Because of the critical role of vitamins in nutrition, qualitative and quantitative analyses are important issues and a challenging task for food manufacturers [5].

The determination of water-soluble vitamins in various samples is rather difficult due to the chemical instability and complexity of the matrices in which they usually exist. Various analytical methods are available but most of them are time-consuming or not enough an accurate [6]. These techniques were based on the measurement by using electrophoresis [7], thin-layer chromatography [8], spectrophotometric [9-10], spectrofluorimetric [11], enzymatic [12] or microbiological properties [13]. The most widely used method in the determination of vitamins of the B-groups is reversed-phase high-performance liquid chromatography (RP-HPLC) [14-15] with one or more detection methods [16], which used to determine water-soluble vitamins in different types of natural food [17] like cereal [18-19], honey [3], meat [20-21], spinach and wheat bread [21], mushrooms [22], plant food [23], rice [24], wheat flour [25], fruit juices [26], beetroot [27], human milk [28], powder milk [29-30-31-32] and exotic fruits [33].

The present paper describes a sensitive and simple ion-pair RP-HPLC validated method using a C_{18} column with two different detectors, photodiode array detector (UV-DAD) and fluorescence detector (FLD), in one run for the determination of seven water-soluble vitamins: Thiamine hydrochloride (vit. B_1), riboflavin (vit. B_2), nicotinamide (vit. B_3), pyridoxine hydrochloride (vit. B_6), folic acid (vit. B_9), cyanocobalamin (vit. B_{12}) and ascorbic acid (vit. C) in some manufactured food products.

The UV-Vis. photodiode array detector enables to simultaneous determination of investigated compounds at different wavelengths. Thus, in this study, 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 and with FLD detector simultaneously. We can determine the quantity and quality of trace amounts of vitamins in samples with excellent precision, so we can determine the smaller amount of each vitamin in different products correctly and in one step.

Experimental

MATERIALS AND METHODS

The chromatograms were obtained by using Hitachi liquid chromatography equipped with a photodiode array detector (UV-DAD) Hitachi L-2455, fluorescence detector (FLD) L-2485, pump Hitachi L-2130, column oven Hitachi L-2350 and auto sampler Hitachi L-2200. The column Nucleodur BDS C_{18} Gravity from Macherey-Nagel (MN) Company (Germany). Ultrasonic 405 from Hwashin Technology (Korea), Micropipette IsoLap (Germany).

Chemical reagents

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, orthophosphoric acid, triethylamine, hexane-1-sulfonic acid sodium salt, sodium dihydrogen phosphate, sodium hydroxide was purchased from Merck (Germany), methyl paraben was purchased from Clariant (Canada).

Diluted solution (0.05 M of NaH_2PO_4 at pH 6.3)

The diluted solution of sodium dihydrogen phosphate in concentration 0.05 M and pH = 6.3, obtained by dissolving 7.8 g of sodium dihydrogen phosphate dihydrate in 900 mL HPLC water in 1 L volumetric flask. pH adjusted to 6.3 by sodium hydroxide (1 M), then completed to volume with HPLC water.

Stock standard solutions of water-soluble vitamins

Standard stock solutions 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 0.2 g of each vitamin in 40 ml diluted solution in 100 mL volumetric flask, then completed to volume by diluted solution.

Working solutions

The working standard solutions (0.001-1000 mg/l) for all studying vitamins were prepared by diluting a suitable volume of standard stock solution with diluted solution. To construct the calibration curve, five replication (20 µl) of each standard solution was injected immediately after preparation into column. Relative peak areas (peak area of each vitamin divided on the peak area of methyl paraben internal standard) were measured.

Sample preparation

It was performed by two steps, step 1: 6 g of each sample were weighed and divided into three parts, each part was transferred into a 10 ml glass test tube containing 5 ml of methanol, and then the mixture was sonicated for 25 min in ultrasonic bath, and centrifuged for 25 min at 5×10^3 rpm. The supernatant solution of the three glass test tubes was transferred into a 25 ml becker then sonicated for 120 min in ultrasonic bath at 35℃ in the dark for evaporating methanol. 0.1 ml sodium hydroxide (0.1723 M) was added to dissolve the residue. Step 2: In the same time 2 ml of HPLC water were added to the solid precipitate in the three glass test tubes, shaken for 10 min in ultrasonic bath, and 0.1 ml of phosphoric acid (0.05 M) was added to each tube, shaken for 20 min in ultrasonic bath, centrifuged for 25 min at 5×10^3 rpm, the supernatant solutions were transferred to the residue dissolved in the 25 ml becker resulting by step 1, then sonicated for 10 min in ultrasonic bath, and transferred into 10 ml volumetric flask and diluted to the mark with the diluted solution. 2.5 ml from the final sample and 0.25 ml of methyl paraben 1 g/l were transferred to 5 ml volumetric flasks, diluted to the mark with the diluted solution and filtered through a 0.22 µm Millipore filter.

The manufactured food products were subjected to the analytical procedures:

(1) *Vitamilk* milk powder, Régilait, FRANCE, each 100 g contains: 1.65 mg vit. B_1, 2.1 mg vit. B_2, 2.1 mg vit. B_6, 300 µg vit. B_9, 3.75 µg vit. B_{12}, 120 mg vit. C.

(2) *Nido* milk powder, Nestle, Dubai-UAE, each 100 g contains: 0.4 mg vit. B_1, 1.2 mg vit. B_2, 5.3 mg vit. B_3, 0.5 mg vit. B_6, 160 µg vit. B_9, 1.8 µg vit. B_{12}, 50 mg vit. C.

(3) *Dano* milk powder, Arla foods amba, DENMARK, each 100 g contains: 1.1 mg vit. B_2, 3.5 µg vit. B_{12}.

(4) *Milky gold* milk powder, AGL, EGYPT, each 100 g contains: 8 mg vit. C.

(5) *Windmill* cacao & milk powder, Al-Saada, Aleppo-SYRIA, each 100 g contains: 0.03 mg vit. B_1, 0.15 mg vit. B_2, 0.07 mg vit. B_3, 0.04 mg vit. B_6, 2.28 µg vit. B_9, 1.34 mg vit. C.

(6) *Bebelac* infant food, Mutlu Bebek, TURKY, each 100 g contains: 20 mg vit. C.

(7) *Squeeze* orange juice powder, Katakit, Damascus-SYRIA, each 100 g contains: 60 mg vit. C.

Chromatographic conditions

A C_{18} BDS column (10 cm x 4.6 mm; 3 µm) was used. Mobile phase was 5.84 mM of hexane-1-sulfonic acid sodium: acetonitrile (95:5) with 0.1% triethylamine as solvent (A) at pH 2.5 (adjusted by orthophosphoric acid 1 M) and 5.84 mM of hexane-1-sulfonic acid sodium: acetonitrile (50:50) with 0.1% triethylamine as solvent (B) at pH 2.5 (adjusted by orthophosphoric acid 1 M).

The column was operated at 40 °C. The flow rate was 1.6 ml/min and the injected volume 20 µl. starting with solvent A 100%. A gradient elution was performed till the mobile composition 50% of A and 50% of B for 5 min. Detection was performed for UV-DAD detector: 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}. For FLD Detector was programmed at λ_{ex} = 296 nm, λ_{em} = 390 nm for vitamin B_6 during the first three minutes, then at λ_{ex} = 450 nm, λ_{em} = 530 nm for vitamin B_2 from 3 minute to 4.6 minute.

RESULTS AND DISCUSSION

Under new chromatographic conditions, the typical chromatograms of standard solution of water-soluble vitamins (B_1, B_2, B_3, B_6, B_9, B_{12} and C) at 270 nm for UV-DAD detector as a general view and for FLD detector at the same time, with methyl paraben as internal standard are presented in fig. (1). The values of retention times were: 0.887 min for C, 1.340 min for B_3, 2.207 min for B_6, 3.560 min for B_1, 3.860 min for B_9, 4.267 min for B_2 4.493 min for B_{12}, and 3.253 min for methyl paraben, for DAD detector and 2.413 min for B_6, 4.513 min for B_2 for FLD detector. Fig. (2) represent the typical chromatograms of vitamins (B_1, B_2, B_3, B_6, B_9, B_{12} and C) standard solutions with DAD detector 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 methyl paraben as internal standard.

Fig. 1: (a): general view chromatogram of (B_1, B_2, B_3, B_6, B_9, B_{12} and C) and methyl paraben as an internal standard at 270 nm with UV-DAD detection. (b): chromatogram of (B_6, B_2) with FLD detection

The Linearity of the method was determined by injecting five replicated solutions of each concentration between 0.001-1000 mg/l. Good Linearities were obtained with correlation coefficients R^2>0.9972. The important parameters of calibration curves in addition to limit of detection (LOD) and limit of quantification (LOQ) were presented in table 1 for vitamins (B_1, B_2, B_3, B_6, B_9, B_{12} and C) with UV-DAD detector and in table 2 for vitamins (B_2, B_6) with FLD detector. The limits of detection for vit. B_2 and vit. B_6 with UV-DAD were estimated to be 26.7 µg/l, 6.3 µg/l respectively, where it was estimated for FLD to be 1.9 µg/l, 1.3 µg/l respectively. By consequence the vitamins pyridoxine hydrochloride and riboflavin, were determined by fluorescence detection with greater sensitivity than UV-DAD.

Analytical application

The most common methods for the extraction of water-soluble vitamins from food products involve pre-treatment through complex chemical reactions or solid-phase extraction SPE procedure, followed by individual methods for the determination of each

vitamin [23]. In the present study the determination of the water-soluble vitamins based firstly on folic acid extraction with methanol and secondly on acid digestion and water-soluble vitamins extraction, followed by HPLC analysis. The new chromatographic conditions were applied to separate and determinate seven water-soluble vitamins (B_1, B_2, B_3, B_6, B_9, B_{12} and C) in seven different manufactured food products: milk powder (Vitamilk, Nido, Dano and Milky Gold), cacao & milk powder (Windmill), infant food (Bebelac) and orange juice powder (Squeeze). We present in fig. (3) typical separation chromatograms of vitamins (B_1, B_2, B_3, B_6, B_9, B_{12} and C) with methyl paraben as internal standard, for one of these food samples (Nido), with UV-DAD detector and for FLD detector at the same time.

We present in fig. (4) the typical chromatograms of vitamins (B_1, B_2, B_3, B_6, B_9, B_{12} and C) for the same sample (Nido) with DAD detector 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 methyl paraben as internal standard.

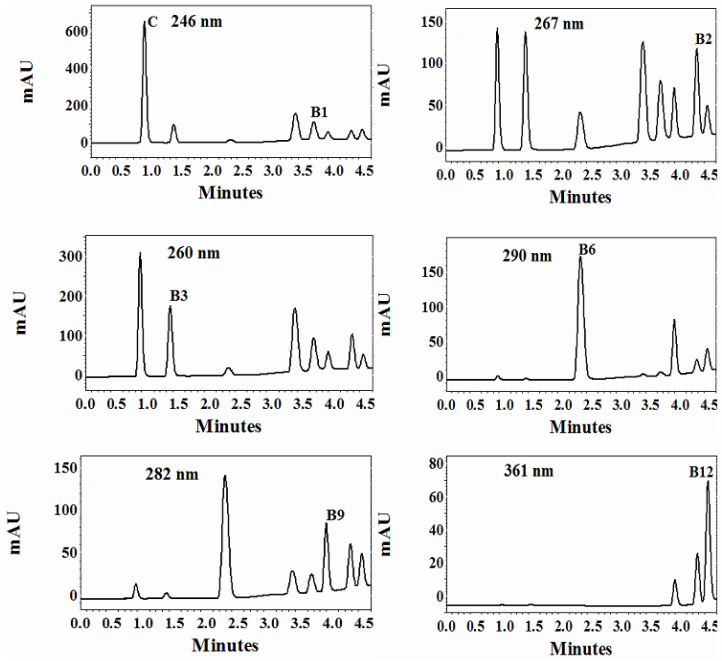

Fig. 2: Typical chromatograms of water soluble vitamins standard solution at 246 nm for C and B_1, 267 nm for B_2, 260 for B_3, 290 for B_6, 282 nm for B_9, 361 nm for B_{12} with methyl paraben as internal standard

Table 1: Linearity of calibration curves, limit of detection (LOD) and limit of quantification (LOQ) for seven water-soluble vitamins with UV-DAD detection

Vitamin	Y = ax+b	R^2	Concentration Linearity mg/l	LOQ mg/l	LOD mg/l
Thiamin. HCl (B_1)	y = 0.0304x-0.0024	0.9994	0.085-100	0.0500	0.0165
Riboflavin (B_2)	y = 0.0692x+0.0084	0.9999	0.100-200	0.0810	0.0267
Niacin (B_3)	y = 0.0308x-0.0004	0.9999	0.025-300	0.0191	0.0063
Pyridoxine. HCl (B_6)	y = 0.0319x-0.0006	0.9994	0.020-300	0.0190	0.0063
Folic acid (B_9)	y = 0.0384x+0.0017	0.9998	0.050-250	0.0300	0.0099
Cyanocobalamin (B_{12})	y = 0.0116x+0.0025	0.9988	0.010-600	0.0097	0.0032
Ascorbic acid (C)	y = 0.0243x-0.0045	0.9972	0.190-400	0.0500	0.0165

Table 2: Linearity of calibration curves, limit of detection (LOD) and limit of quantification (LOQ) for two water-soluble vitamins with FLD detection

Vitamin	Y = ax+b	R^2	Concentration range mg/l	LOQ mg/l	LOD mg/l
Riboflavin (B_2)	y = 2.5604x+0.3475	0.9999	0.010-08	0.0057	0.0019
Pyridoxine. HCl (B_6)	y = 2.7078x+1.0731	0.9994	0.005-10	0.0041	0.0013

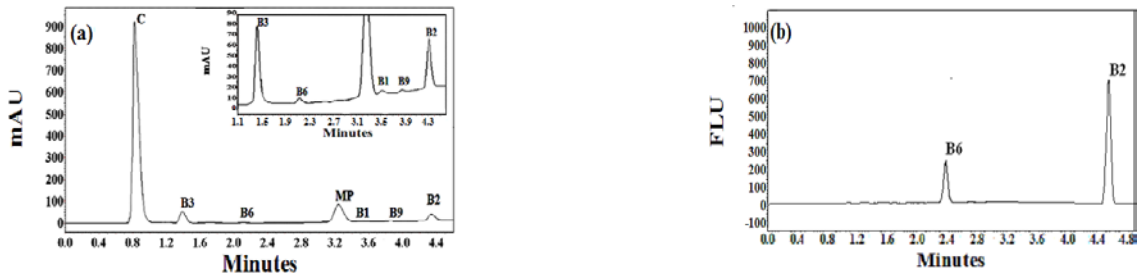

Fig. 3: (a): general view chromatogram of (B_1, B_2, B_3, B_6, B_9, B_{12} and C) and methyl paraben as internal standard for (Nido) powder milk, at 270 nm with UV-DAD. (b): Chromatograms of (B_2, B_6) for (Nido) powder milk, with FLD

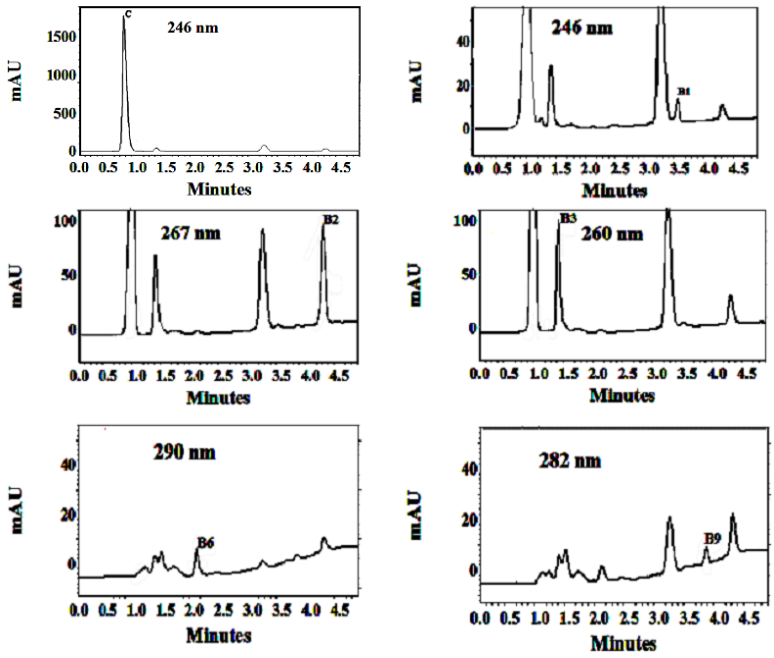

Fig. 4: Chromatograms of water soluble vitamins for (Nido) powder milk, with UV-DAD at 270 nm as a general view, 246 nm for C and B_1, 267 nm for B_2, 260 for B_3, 290 for B_6, 282 nm for B_9, 361 nm for B_{12} with methyl paraben as internal standard

Recoveries were tested, by the standard addition procedure. Three addition levels were used for each water-soluble vitamin in each sample. Five replicated injections were performed for each addition level of all studied samples. Mean recoveries were calculated of three different concentrations. Tables (3, 4) present the results of the recoveries for water-soluble vitamins obtained with UV-DAD detector and FLD detector respectively, on C_{18} column for seven different manufactured food products. The recoveries of the food samples for UV-DAD and FLD were in the ranges 98.14-100.96% and 98.73-99.44% respectively.

The obtained results agree with the labeled values for all investigated vitamins in the studied manufactured food products. No interference was found during analysis of the food products.

Table 3: Vitamins amounts mg/100 g and recoveries% of water-soluble vitamins (B_1, B_2, B_3, B_6, B_9, B_{12} and C) in studied manufactured food products on C_{18} column with UV-DAD detection

Commercial name	Labeled mg/100 g	*$\bar{x}$, mg/100 g	RSD%	Recovery%
Vitamin B_1				
Vitamilk milk powder, Régilait, FRANCE	1.65000	1.6434	1.23	99.17
Nido milk powder, Nestle, Dubai-UAE	0.40000	0.3964	1.45	98.30
Windmill cacao & milk powder, Al-Saada, Aleppo-SYRIA	0.03000	0.0295	3.07	98.30
Vitamin B_2				
Vitamilk milk powder, Régilait, FRANCE	2.10000	2.0832	1.14	99.43
Nido milk powder, Nestle, Dubai-UAE	1.20000	1.1866	1.26	99.05
Dano milk powder, Arla foods amba, DENMARK	1.10000	1.0671	1.35	98.73
Windmill cacao & milk powder, Al-Saada, Aleppo-SYRIA	0.15000	0.1473	2.46	99.07
Vitamin B_3				
Nido milk powder, Nestle, Dubai-UAE	5.30000	5.2682	1.33	99.10
Windmill cacao & milk powder, Al-Saada, Aleppo-SYRIA	0.07000	0.0687	2.11	99.45
Vitamin B_6				
Vitamilk milk powder, Régilait, FRANCE	2.10000	2.0815	1.18	99.28
Nido milk powder, Nestle, Dubai-UAE	0.50000	0.4930	1.44	99.17
Windmill cacao & milk powder, Al-Saada, Aleppo-SYRIA	0.04000	0.0392	1.99	98.95
Vitamin B_9				
Vitamilk milk powder, Régilait, FRANCE	0.30000	0.2958	1.49	100.01
Nido milk powder, Nestle, Dubai-UAE	0.16000	0.1570	1.72	99.58
Windmill cacao & milk powder, Al-Saada, Aleppo-SYRIA	0.00228	N/D	-	-
Vitamin B_{12}				
Vitamilk milk powder, Régilait, FRANCE	0.00375	0.00368	2.13	98.74
Nido milk powder, Nestle, Dubai-UAE	0.00180	N/D	-	-
Dano milk powder, Arla foods amba, DENMARK	0.00350	0.00337	2.31	98.14
Vitamin C				
Vitamilk milk powder, Régilait, FRANCE	120.00000	119.8333	0.32	100.96
Nido milk powder, Nestle, Dubai-UAE	50.00000	50.0200	0.60	100.52
Milky gold milk powder, AGL, EGYPT	8.00000	7.9920	1.66	100.53
Windmill cacao & milk powder, Al-Saada, Aleppo-SYRIA	1.34000	1.3300	2.80	100.94
Bebelac infant food, Mutlu Bebek, TURKY	20.00000	19.9700	1.15	100.24
Squeeze powdered orange juice, Katakit, Damascus-SYRIA	60.00000	59.8800	1.51	100.29

Table 4: Vitamins amounts mg/100g and recoveries% of water-soluble vitamins (B_2, B_6) in some manufactured food products on C_{18} column with FLD detection

Commercial name	Labeled mg/100 g	$\bar{x}^*$, mg/100 g	RSD%	Recovery%
Vitamin B_2				
Vitamilk milk powder, Régilait, FRANCE	2.10	2.0839	1.11	99.44
Nido milk powder, Nestle, Dubai-UAE	1.20	1.1870	1.24	99.06
Dano milk powder, Arla foods amba, DENMARK	1.10	1.1071	1.31	98.73
Windmill cacao & milk powder, Al-Saada, Aleppo-SYRIA	0.15	0.1486	1.97	99.08
Vitamin B_6				
Vitamilk milk powder, Régilait, FRANCE	2.10	2.0832	1.08	99.34
Nido milk powder, Nestle, Dubai-UAE	0.50	0.4940	1.42	99.26
Windmill cacao & milk powder, Al-Saada, Aleppo-SYRIA	0.04	0.0394	1.95	98.99

CONCLUSION

Simultaneous determination of water-soluble vitamins is complicated by many factors. First of all, due to their diverse chemical properties, it is difficult to separate all of them in one chromatographic run. On the other hand, their difference in solubility and stability presents another challenge with the optimization of sample preparation procedures. Furthermore, these vitamins are added into pharmaceuticals and fortified food products at different amounts.

In the present study, a simple and rapid method was developed for the simultaneous determination of seven water-soluble vitamins (B_1, B_2, B_3, B_6, B_9, B_{12}, and C), by High-performance liquid chromatography with two different detectors photo diode array detector (UV-DAD) and fluorescence detector (FLD), requiring no expensive solid phase extraction SPE in the HPLC procedure. The validation of the method confirmed that it is very sensitive, selective, linear, accurate and reproducible.

Among the two detectors used in this study, FLD detection is selective and highly sensitive for the determination of vitamins fluorescent B_2, B_6. DAD detection, on the other hand, provides sufficient specificity and sensitivity to the analysis of the seven vitamins in food matrices.

The successful application of the simple low-cost and time-efficient method to the determination of water-soluble vitamins in manufactured food products make it highly desirable for the quality control of foodstuffs in the food industry.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Vidovic C, Stojanovic B, Veljkovic J, Prazic-Arsic L, Roglic G, Manojlovic D. Simultaneous determination of some water-soluble vitamins and preservatives in multivitamin syrup by validated stability-indicating high-performance liquid chromatography method. J Chromatogr A 2008;1202(2):155-62.
2. Chen Z, Chen B, Yao S. High-performance liquid chromatography/electrospray ionization-mass spectrometry for simultaneous determination of taurine and 10 water-soluble vitamins in multivitamin tablets. Anal Chim Acta 2006;569(1-2):169-75.
3. Ciulu M, Solinas S, Floris I, Panzanelli A, Pilo MI, Piu PC, *et al*. RP-HPLC determination of water-soluble vitamins in honey. Talanta 2011;83(3):924-9.
4. Heudi O, Kilinc T, Fontannaz P. Separation of water-soluble vitamins by reversed-phase high performance liquid chromatography with ultra-violet detection: Application to polyvitaminated premixes. J Chromatogr A 2005;1070(1-2):49-56.
5. Ekinci R, Kadakal C. Determination of seven water-soluble vitamins in tarhana, a traditional Turkish cereal food, by high performance liquid chromatography. Acta Chromatogr 2005;15:289-97.
6. Marszall ML, Lebiedzinska A, Czarnowski W, Szefer P. High performance liquid chromatography method for the simultaneous determination of thiamine hydrochloride, pyridoxine hydrochloride and cyanocobalamin in pharmaceutical formulations using coulometric electrochemical and ultraviolet detection. J Chromatogr A 2005;1094(1-2):91-8.
7. Fotsing L, Fillet M, Bechet I, Hubert Ph, Crommen J. Determination of six water-soluble vitamins in a pharmaceutical formulation by capillary electrophoresis. J Pharm Biomed Anal 1997;15(8):1113-23.
8. Kartsova LA, Koroleva OA. Simultaneous determination of water-and fat-soluble vitamins by high-performance thin-layer chromatography using an aqueous micellar mobile phase. J Anal Chem 2007;62(3):255-9.
9. Rajput GK, Kumar A, Kumar A, Srivastav G. To develop a simple (UV-Vis spectrometric) method for the estimation of water soluble vitamins. Int J Drug Res 2011;2(3):232-9.
10. Saradhi DRSV, Prryanka A, Baby Sirisha P, Meherjaha SK, Jyothsna N. Enzymatic and spectroscopic determination of riboflavin using amylase phosphate. IJPPS 2012;4(3):170-2.
11. Mohamed AM, Mohamed HA, Abdel-Latif NM, Mohamed MR. Spectrofluorimetric determination of some water-soluble vitamins. J AOAC Int 2011;94(6):1758-69.
12. Okamoto H, Nakajima T, Ito Y. Simultaneous determination of water-soluble vitamins in a vitamin-enriched drink by an in-capillary enzyme reaction method. J Chromatogr A 2003;986(1):153-61.
13. Ball GFM. Water-soluble vitamin assays in human nutrition. Chapman & Hall; 1994;317-64.
14. Monferrer-Pons L, Capella-Peiro ME, Gil-Agusti M, Esteve-Romero J. Micellar liquid chromatography determination of B vitamins with direct injection and ultraviolet absorbance detection. J Chromatogr A 2003;984(2):223-31.
15. Poongothai S, Ilavarasan R, Karrunakaran CM. Simultaneous and accurate determination of vitamins B_1, B_6, B_{12} and alpha-lipoic acid in multivitamin capsule by reverse-phase high performance liquid chromatographic method. IJPPS 2010;2(4):133-9.
16. Chen P, Atkinson R, Wolf WR. Single laboratory validation of a high-performance liquid chromatographic-diode array detector-fluorescence detector/mass spectrometric method for simultaneous determination of water-soluble vitamins in multivitamin dietary tablets. J AOAC Int 2009;92(2):680-7.
17. Kirilov B, Obreshkova D, Tsvetkova D. Validation of HPLC method for determination of antioxidant vitamin C and vitamin B_6 in food supplements and drugs. IJPPS 2012;4(1):300-4.
18. Suh JH, Yang DH, Lee BK, Eom HY, Kim U, Kim J, *et al*. Simultaneous determination of B groupe vitamins in supplemented food products by high performance liquid chromatography-diode array detection. J Bull Korean Chem Soc 2011;32(8):2648-56.
19. Saint Jose Rodriguez R, Fernandez-Ruiz V, Camara M, Sanchez-Mata MC. Simultaneous determination of vitamin B1 and B_2 in complex cereal foods, by reverse phase isocratic HPLC-UV. J Cereal Sci 2012;55(3):293-9.
20. Esteve MJ, Farré R, Frigola A, Pilamunga C. Contents of vitamins B_1, B_2, B_6, and B_{12} in pork and meat products. J Meat Sci 2002;62(1):73-8.

21. Freisleben A, Schieberle P, Rychlik M. Comparison of folate quantification in foods by high-performance liquid chromatography-fluorescence detection to that by stable isotope dilution assays using high-performance liquid chromatography-tandem mass spectrometry. J Anal Biochem 2003;315(2):247-55.
22. Furlani RPZ, Godoy HT. Vitamins B_1 and B_2 contents in cultivated mushrooms. J Food Chem 2008;106(2):816-9.
23. Lebiedzinska A, Marszall ML, Kuta J, Szefer P. Reversed-phase high-performance liquid chromatography method with coulometric electrochemical and ultraviolet detection for the quantification of vitamins B_1 (thiamine), B_6 (pyridoxamine, pyridoxal and pyridoxine) and B_{12} in animal and plant food. J Chromatogr A 2007;1173(1-2):71-80.
24. De Brouwer V, Storozhenko S, Stove CP, Van Daele J, Van Der Straeten D, *et al*. Ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) for the sensitive determination of folate in rice. J Chromatogr B 2010;878(3-4):509-13.
25. Alaburda J, De Almeida AP, Shundo L, Ruvieri V, Sabino M. Determination of folic acid in fortified wheat flours. J Food Compos Anal 2008;21(4):336-42.
26. Rodriguez-Bernaldo de Quiros A, Fernandez-Arias M, Lopez-Hernandez J. A screening method for the determination of ascorbic acid in fruit juices and soft drinks. J Food Chem 2009;116(2):509-12.
27. Jastrebova J, Witthoft C, Grahn A, Svensson U, Jagerstad M. HPLC determination of folates in raw and processed beetroots. J Food Chem 2003;80(4):579-88.
28. Romeu-Nadal M, Morera-Pons S, Castellote AI, Lopez-Sabater MC. Rapid high-performance liquid chromatographic method for vitamin C determination in human milk versus an enzymatic method. J Chromatogr B 2006;830(1):41-6.
29. Mittermayr R, Kalman A, Trisconi MJ, Heudi O. Determination of vitamin B_5 in a range of fortified food products by reversed-phase liquid chromatography-mass spectrometry with electrospray ionization. J Chromatogr A 2004;1032(1-2):1-6.
30. Chavez-Servin JL, Castellote AI, Rivero M, Lopez-Sabater MC. Analysis of vitamins A, E and C, iron and selenium contents in infant milk-based powdered formula during full shelf-life. J Food Chem 2008;107(3):1187-97.
31. Heudi O, Kilinc T, Fontannaz P, Marley E. Determination of vitamin B_{12} in food products and in premixes by reversed-phase high performance liquid chromatography and immunoaffinity extraction. J Chromatogr A 2006;1101(1-2):63-8.
32. Lu B, Ren Y, Huang B, Liao W, Cai Z, Tie X. Simultaneous determination of four water-soluble vitamins in fortified infant foods by ultra-performance liquid chromatography coupled with triple quadrupole mass spectrometry. J Chromatogr Sci 2008;46:225-32.
33. Valente A, Albuquerque TG, Sanches-Silva A, Costa HS. Ascorbic acid content in exotic fruits: a contribution to produce quality data for food composition databases. J Food Res Int 2011;44(7):2237-42.