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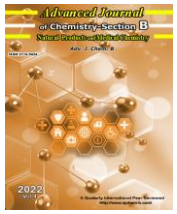

Original Research Article

Development and Validation of a Reverse-Phase HPLC Method for Determination of Some Water-Soluble Vitamins and Preservatives in Pharmaceutical Forms

Seyyed Hossein Hassanpour[1,2*], Aboozar Alidadi[3], Alireza Doroudi[4]

[1]*Department of Nuclear Pharmacy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran*

[2]*Young Researchers and Elite Club, Yassoj Branch, Islamic Azad University, Yasooj, Iran*

[3]*Department of Health Management, Faculty of Health, Baqiyatullah University of Medical Sciences, Tehran, Iran*

[4]*Department of Medicinal Chemistry, Faculty of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

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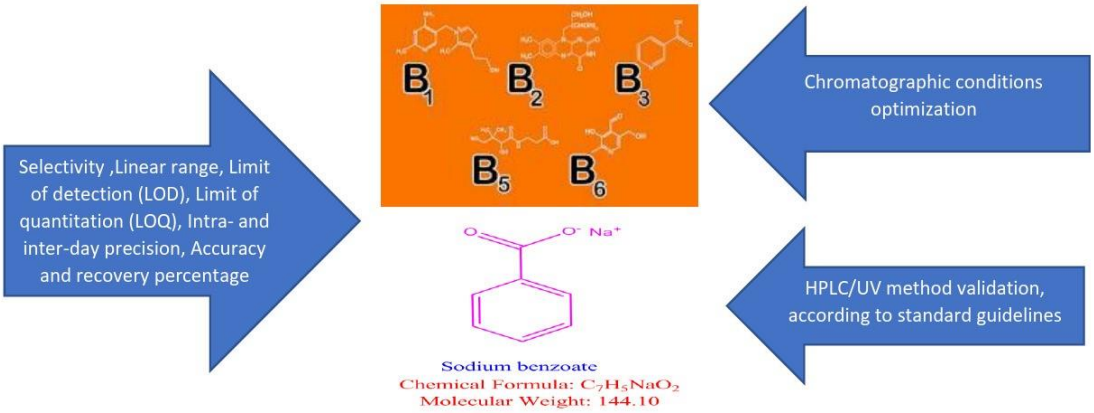
ABSTRACT

Necessary diet components are vitamins as if should be existed in the food. The B vitamins and vitamin C (water-soluble vitamins) are substances required for chemical processes and substances that occur within living organisms. In this research, a simple, rapid, and reliable method was developed to isolate and quantify a primary preservative compound (sodium benzoate), some water-soluble vitamins such as ascorbic acid (C) and five vitamin B compounds. An ODS column was used with a UV detector at different wavelengths at 35 °C. The mobile phase was pH 3.0 phosphate buffer-methanol at a flow rate of 1.0 mL min^{-1} in a gradient elution approach. The mentioned analytes above were separated in 40 min. The linearity, limit of detection (LOD), and limit of quantification (LOQ), accuracy, and instrument precision terms were used in the method validation. The correlation factor near 1 suggests that the developed method has a good linearity range. The results show that recovery percentage was < 99.480% for all compounds. The limit for mean percentage of recovery is 98-102%, and as all the values are within the limit, it can be concluded that the proposed method is accurate. The results showed no HPLC peaks that could disturb the determination of vitamins/preservatives. The data obtained in real sample analysis were consistent with the declared values. The data obtained from subjected multivitamin syrup sample into stress testing (forced degradation) confirmed that there was no interference effects in the quantification of the analytes.

* Corresponding author: Seyyed Hossein Hassanpour
✉ E-mail: Dr.hossein1366@yahoo.com

GRAPHICAL ABSTRACT



Introduction

Essential food ingredients are vitamins that should be supplied in the diet [1]. These foodsrores contain significant quantities of water-soluble and fat-soluble vitamins [2]. The nine members of the first-class (water-soluble) vitamins (including 8 B vitamins and vitamin C) are required in diverse biochemical futures [3]. Vitamin C is an antioxidant agent [4]. The specific and critical metabolic properties of B vitamins are recognized as a complex class of vitamins, and as a result, their deficiency or excess leads to specific weaknesses. [1]. The most relevant vitamin to provide the required energy for the nervous system is vitamin B1 or thiamine, and thus, its deficiency or lack of activity results in neurological manifestations [5,6]. In addition, vitamin B1 deficiency causes beriberi disease accompanied by cardiovascular disorders and digestive impairment [7].

Another member of the vitamin B group is B2 (Riboflavin) with some therapeutic effects including anti-oxidant, anti-inflammatory, anti-aging, anti-nociceptive, and anti-cancer properties. Its protective properties and its ability to reduce the toxic effect of drugs have been confirmed by combining riboflavin with other drugs and compounds [8-10]. Vitamin B3 (niacinamide) and vitamin B6 (pyridoxine) can cause skin inflammation and gastrointestinal tract discomfort [3]. Some symptoms, including fatigue, muscle cramps, headache, paresthesia, weakness, personality changes, numbness, muscle/abdominal cramps, nausea, and muscle incoordination are associated to vitamin B5 deficiency [11].

Usually, to have an average growth, metabolism and physical well-being, the key role is to have sufficient levels of these nutrients [12]. Therefore, it is recommended to use various dosages of multivitamin products as medicines or dietary supplements [13]. The determination of active compounds in commercial supplements by an uncomplicated, rapid, reliable, and cost-effective method is a healthcare key factor due to the widespread utilization of these food and pharmaceutical supplements [14].

Various methods such as volumetric, spectrophotometric, spectrofluorimetric, electrochemical processes, etc. have been used to determine B-group vitamins [15]. Especially from the 1980s, various instrumental methods, including electrochemical method, spectrophotometry/spectrofluorimetry, derivative UV spectrophotometry, normal phase, and reversed-phase TLC and HPLC have been

used/developed for the quantitative determination of some water-soluble vitamins, especially the B-group vitamins [14, 16-18].

For simultaneous identification and determination of most vitamins, various HPLC methods are used, such as reversed-phase liquid chromatography (RP-LC) without ion-pair reagents [19-22], and also RP-LC with ion-pair chromatography [23-29]. For quantitative determination of vitamin C [30-36] and pantothenic acid [16,37,38] in various foods, specific HPLC methods have been used and developed [30-36]. In contrast, only a few HPLC methods for vitamins have been reported in the literature [39].

In general, to prevent spoilage from microbial growth or undesirable chemical reactions, we should add preservatives, as natural or synthetic chemicals, to various products such as food, pharmaceuticals, cosmetics, biological samples, etc. Many analytical methods have been reported to determine vitamins and preservatives in such products [29, 40-43]. Thus, introducing/developing novel techniques and validating HPLC techniques is very important in quality control (QC) in each pharmaceutical industry because it plays an essential role in the products quality.

This study described a novel and simple a reverse-phase High-performance liquid chromatography (RP-HPLC) method to determine some water-soluble vitamins and sodium benzoate used as a preservative in pharmaceutical multivitamin products. This research evaluated the linearity and range of HPLC method in sodium benzoate and water-soluble vitamins such as ascorbic acid (C) and five vitamin B compounds. This method is precisely able to estimate LOQ and LOD of sodium benzoate and water-soluble vitamins. (LOQ=12.00 µg/mL, LOD= 4.00 µg/mL for sodium benzoate, LOQ= 361.80 µg/mL and LOD=119.40 µg/mL for vitamin C, LOQ=2.40 µg/mL and LOD= 0.80 µg/mL for vitamin B1, LOQ= 2.30 µg/mL and LOD=0.80 µg/mL for vitamin B2, LOQ=9.80 µg/mL and LOD=3.20 µg/mL for vitamin B3, LOQ=1.26 µg/mL and LOD=4.20 µg/mL for vitamin B5, LOQ=5.80 µg/mL and LOD=1.90 µg/mL for vitamin B6)

Experimental

Chemical and reagents

Vitamins and preservatives were obtained from different suppliers/manufacturers as listed below: vitamin B1 and B5, Jiangxi Tianxin Pharmaceutical Co., Ltd, Shanghai, China; vitamin B2, Supriya Life Science Ltd., Mumbai, India; vitamin B3, Tianjin TEDA Co., Ltd, Tianjin, China; vitamins B6, Xinfa Pharmaceutical Co., Ltd, Kenli County, China; vitamins C, Shandong Pharmaceutical Co. Ltd, Shandong, China; NaB, Fars Chemical Industries Company, Fars, Iran.

The used vitamins are a pure grade for human nutrition, and their quality was checked by a QC laboratory section before use. In this regard, the preservatives that met USP requirements were considered. NaH_2PO_4 and Na_2HPO_4 salts (Merck) were used to prepare the chromatography buffer. Methanol (HPLC gradient grade) and orthophosphoric acid (analytical grade purity) were purchased from Merck (Darmstadt, Germany). The solution pH was adjusted by a Denver UB-10 pH Meter, Denver Instrument, Inc. A 0.45 µm PTFE membrane filter (Sartorius Stedim Biotech GmbH, Gottingen, Germany) was used to filter the aqueous mobile phase. All aqueous solutions were prepared in high purity water obtained from Merck Direct-Q 3 UV (Darmstadt, Germany). Climate Chambers were purchased from Thermolab Scientific Equipment (Maharashtra, India).

Equipment and Chromatographic conditions

The used HPLC system was an Agilent 1260 Infinity I (Waldron, Germany). The instrument is equipped with a solvent delivery module in a quaternary gradient mode (Agilent G1311C), an Agilent G1314F VWD detector, an auto-sampler

(Agilent G7129A), and an Agilent G7116A thermostated column compartment (G1316A). An Open Lab **64®-bit** software was used to perform data acquisition. A C18 Nucleodur-HTec reversed-phase column at 35 ºC was used (250 mm×4.6 mm×5 µm, MACHEREY-NAGEL GmbH & Co. KG, Dueren, Germany). A gradient elution approach consisting of methanol (solvent A) and a phosphate buffer (Solvent B: 0.05 M each concerning NaH_2PO_4 and Na_2HPO_4 salts; pH 3.0) at a flow rate of 1 mL/min was used as presented in Table 1. The sample injection volume was 20 µL. A UV detector was used at detection wavelengths of 291 nm (vitamins B_6 and C), 261 nm (vitamins B1 and B3), and 220 nm (vitamins B5 and B2 and NaB). Sodium benzoate and vitamins were identified by comparing their retention times to corresponding standards.

Table 1. Mobile phase gradient, methanol (solvent A) and a phosphate buffer (Solvent B: 0.05 M with respect to each NaH_2PO_4 and Na_2HPO_4 salts; pH 3.0) at flow rate 1 mL/min.

Time Minutes	Buffer (Solvent B)	Methanol (Solvent A)
0	98	2
4	98	2
12	81	19
20	71	29
30	50	50
35	50	50
35.1	98	2
40	98	2

Preparation of solutions

Standard solutions

Stock solutions were daily prepared in a 50 mL volumetric flask by dissolving an aliquot amount of each analyte listed below. 2500 mg (C), 15 mg (B1), 19 mg (B2), 80 mg (B3), 100 mg (B5), 50 mg (B6), and 140 mg (NaB). Each analyte was added into a 50 mL volumetric flask, and after complete dissolution at 10 min stirring, it was made up to the mark by distilled water. The prepared stock solutions were kept in the dark conditions.

To prepare the standard working solution, 5 mL of the prepared stock solution was added into a 100 mL volumetric flask and reached the mark by water. This standard is used for the assay tests.

Calibration standards

To prepare calibration standards, 4, 4.5, 5, 5.5,6, and 7 mL of standard stock solution were separately added into 50 mL volumetric flasks and diluted to the mark by water. The final concentration of these working standards was in the range of 2000.00–3500.00 µg/mL for (C), 12–21 µg/mL for (B1), 12–21 µg/mL for (B2), 64–112 µg/mL for (B3), 80–140 µg/mL for (B5), 40–70 µg/mL for (B6), and 112–196 µg/mL for (NaB). These solutions were kept in the dark in tightly closed bottles and were stable for 36 h at room temperature.

Quality control samples

A multivitamin syrup and its placebo were from the Raha pharmaceutical Co. (Isfahan, Iran) that was analyzed for its content. The experimental multivitamin syrup was declared to contain: C, 250.00 mg/5 mL; B1, 1.50 mg/5 mL; B2, 1.50 mg/5 mL; B6, 5.00 mg/5 mL; B5, 10.00 mg/5 mL; vitamin B3, 8.00 mg/5 mL, and NaB preservative, 14.00 mg/5 mL, which should be in the range of 90% to 120% of the labeled amounts in the sample preparation based on USP 43

requirement. The placebo did not contain water-soluble vitamins and preservatives.

To analyze the multivitamin syrup solution, an aliquot of 5 mL of the sample was added to a 100 mL volumetric flask. To analyze the Placebo solution, an aliquot of 5 mL of the Placebo was added to a 100 mL volumetric flask. After sonication for 10 minutes, it reached the mark with water and was covered with aluminum foil for light protection. It was injected into the HPLC instrument after centrifuging this sample for 5 min at 4000 rpm (hettich centrifuge rotofix 32A).

Method validation

To validate the HPLC method, some characteristics including selectivity, linear range, limit of detection (LOD), limit of quantitation (LOQ), and intra- and inter-day precision, accuracy and recovery percent were evaluated, as illustrated in the following sections.

System suitability

To evaluate the system suitability, some HPLC factors such as retention time (t_R), resolution factor (R_s), and tailing factor (T_F) were estimated by replicate measurements (n=3) of a standard solution (section 2.3.1). Based on the literature [44], peak separation between two adjacent peaks must have $R_s \geq 2$ and $T_F \leq 2$ as acceptable values.

Selectivity

The method's selectivity was tested by comparing the HPLC chromatograms of the individual vitamin and preservative standard solutions with vitamins/preservatives standard mixture, placebo, and syrup samples.

Linearity (calibration curve), detection, and quantitation limits

To evaluate the linearity, LOD, and LOQ for the proposed method, some analytical solutions were prepared from the corresponding standard solution of each analyte and diluted with water appropriately, as mentioned earlier (Section 2.3.2). The plots of peak areas against six standard analyte concentrations were constructed as calibration curves that showed a proportional signal-concentration behavior in the concentration ranges of 2000.00–3500.00 µg/mL for (C), 12–21 µg/mL for (B1), 12–21 µg/mL for (B2), 64–112 µg/mL for (B3), 80–140 µg/mL for (B5), 40–70 µg/mL for (B6), and 112–196 µg/mL for (NaB). The correlation coefficient (R^2) of the plots was determined that need a requirement of $R^2 > 0.995$ [45]. Furthermore, LOD and LOQ were estimated. LOD was calculated as an analyte concentration. The corresponding HPLC peak is at least 3 times greater than the baseline noise, and the signal could be detected from the baseline noise disturbances. In contrast, LOQ was calculated based on the analyte concentration that creates an analyte response 10 times greater than the baseline noise.

Precision

The assay precision was evaluated by repeatability (intra-day) and intermediate precision (inter-day). For these tests, three analytical concentrations of each analyte were obtained from the QC samples' concentrations that their ranges are 2000, 2500, and 3500 µg/mL for (C), 12, 15, and 21 µg/mL for (B1), 12, 15, and 21 µg/mL for (B2), 64, 80, and 112 µg/mL for (B3), 80, 100, and 140 µg/mL for (B5), 40, 50, and 70 µg/mL for (B6) and 112, 140, and 196 µg/mL for (NaB). To prepare these solutions 4, 5, and 7 mL of multivitamin syrup were separately added into 100 mL volumetric flasks and diluted to the mark by water, respectively.

To test the intra-day precision, five replicate measurements were carried out in a day, while the inter-day precision was tested for 3 days. The relative standard deviations (RSDs) are considered a measure of the process precision which should be ≤2%. [46].

Accuracy and Recovery

To test the accuracy of the suggested method, the recovery approach was used, and it was considered that the recovery of a known amount

of analyte spiked into the placebo. The spiked samples were prepared in three levels over a range that covered the expected content of the analyte (2000, 2500, and 3500 µg/mL for (C), 12, 15, and 21 µg/mL for (B1), 12, 15, and 21 µg/mL for (B2), 64, 80, and 112 µg/mL for (B3), 80, 100, and 140 µg/mL for (B5), 40, 50, and 70 µg/mL for (B6) and 112, 140, and 196 µg/mL for (NaB)). To prepare these solutions 4, 5, and 7 mL of standard stock solution (section 2.3.1) and 5 mL of placebo were separately added into 50 mL volumetric flasks and diluted to the mark by water, respectively. Triplicate measurements were done on each spiked sample.

Stability studies-stress testing

To elucidate selectivity and the stability-indicating nature of the proposed analytical method, stress testing was done on the experimental multivitamin preparation to generate degradation products that were used [45]. Both suitable multivitamin syrup formulation and placebo were subjected to temperature stress (in a climate chamber at 40 ˚C/75 %RH (relative humidity)), until all combinations were reached. Control samples were stored at ambient conditions. The test periods up to 1 and 3 months were applied.

The light stress was also applied, at which a quality control solution in tightly closed quartz cells were irradiated by direct sunlight for 10 h at the outside temperature of 24–26 ˚C. Control samples of the exact solutions were stored protected from light in similar quartz cells at room temperature.

Results and Discussion

Method development and optimization

The HPLC conditions for separating and determining mixed vitamin compounds and sodium benzoate were optimized, and the resulting chromatogram is shown in Figure 1.

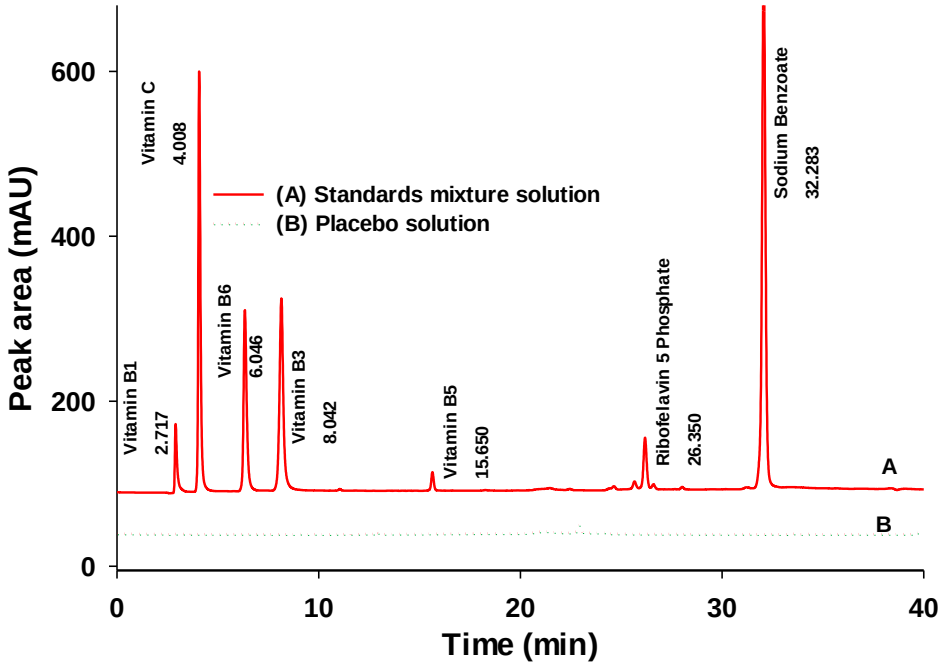


Figure 1. (A) HPLC chromatogram of standards mixture solution (section 2.3.1), **(B)** HPLC chromatogram of placebo solution (section 2.3.3). Peaks: vitamin C, t_R ~2.7 min; C, t_R ~4.0 min; B6, t_R ~6.0 min; B3, t_R ~8.0 min; B5, t_R ~15.6 min; B2, t_R ~26.3 min; NaB, t_R ~32.2 min. By C18 (250mm×4.6mm×5µm) column at 35 ºC, with gradient elution (Table 1) at flow rate 1 mL/min and injection volume 20 µL. A UV detector was used at detection wavelengths of 291 nm (B6 and C), 261 nm (vitamins B1 and B3), and 220 nm (vitamins B5, B2, and NaB)

To reach the optimal separation by UV-HPLC analysis, the influencing factors such as the optimal column, pH of eluent, and isocratic or gradient elution program were examined and optimized. A gradient elution program (using the conditions illustrated in Section (2.2) equipment and chromatographic conditions) and a Macherey-Nagel C18 Nucleodur-HTec reversed-phase column were based on the Rs factor selected. The mobile phase pH was varied using orthophosphoric acid, and the change in Rs was followed. The best condition was achieved at pH 3.0. The t_R, R_S, and T_F factors were evaluated for proving the method specificity, and the results are summarized in Table 2. Rs values > 7.0 and T_F values < 2.0 were obtained. The separation results obtained for each compound confirmed the good specificity of the proposed method.

Table 2. Results from the specificity evaluation of the HPLC method (Section 2.2).

Compound	t_R (min)	R_S	T_F
B1	2.717	–	1.648
C	4.008	7.730	1.206
B6	6.046	10336	1.196
B3	8.042	8.099	1.035
B5	15.650	34.758	1.087
B2 (Ribofelavin)	26.35	48.429	1.066
NaB	32.283	23.216	1.078

Method Validation

Selectivity

HPLC chromatograms in Figure 1 belong to the standards mixture solutions (Section 2.3.1) and placebo solution (Section 2.3.3), respectively. In the chromatogram of the placebo syrup, some small peaks appeared, which were significantly different from the HPLC peaks of vitamin/preservative in the syrup formulation, and thus well separated from them (R≥2).

Linearity

Figure 2 depicts the calibration plots obtained for the analytes investigated the correlation coefficients evaluated their logics. The characteristics of calibration curves were assessed by the linearity range, LOD, and LOQ, as summarized in Table 3. The correlation factor near 1 suggests that the developed method has a good linearity range. The variation in the linear range depends on the nature of the individual compounds.

Table 3. Results from the linearity evaluation and range of the HPLC method (Section 2.2).

Compound	Regression plot	R^2	LOQ (µg/mL)	LOD (µg/mL)
C	y = 1441.8 x + 340.28	0.9993	361.80	119.40
B1	y = 48474.0 x – 42.32	0.9992	2.40	0.80
B2	y = 41385.0 x + 48.63	0.9992	2.30	0.80
B3	y = 42258.0 x – 126.00	0.9995	9.80	3.20
B5	y = 2032.6 x - 2.67	0.9995	1.26	4.20
B6	y = 51135.0 x -119.16	0.9996	5.80	1.90
NaB	y = 56331.0 x -30.899	0.9998	12.00	4.00

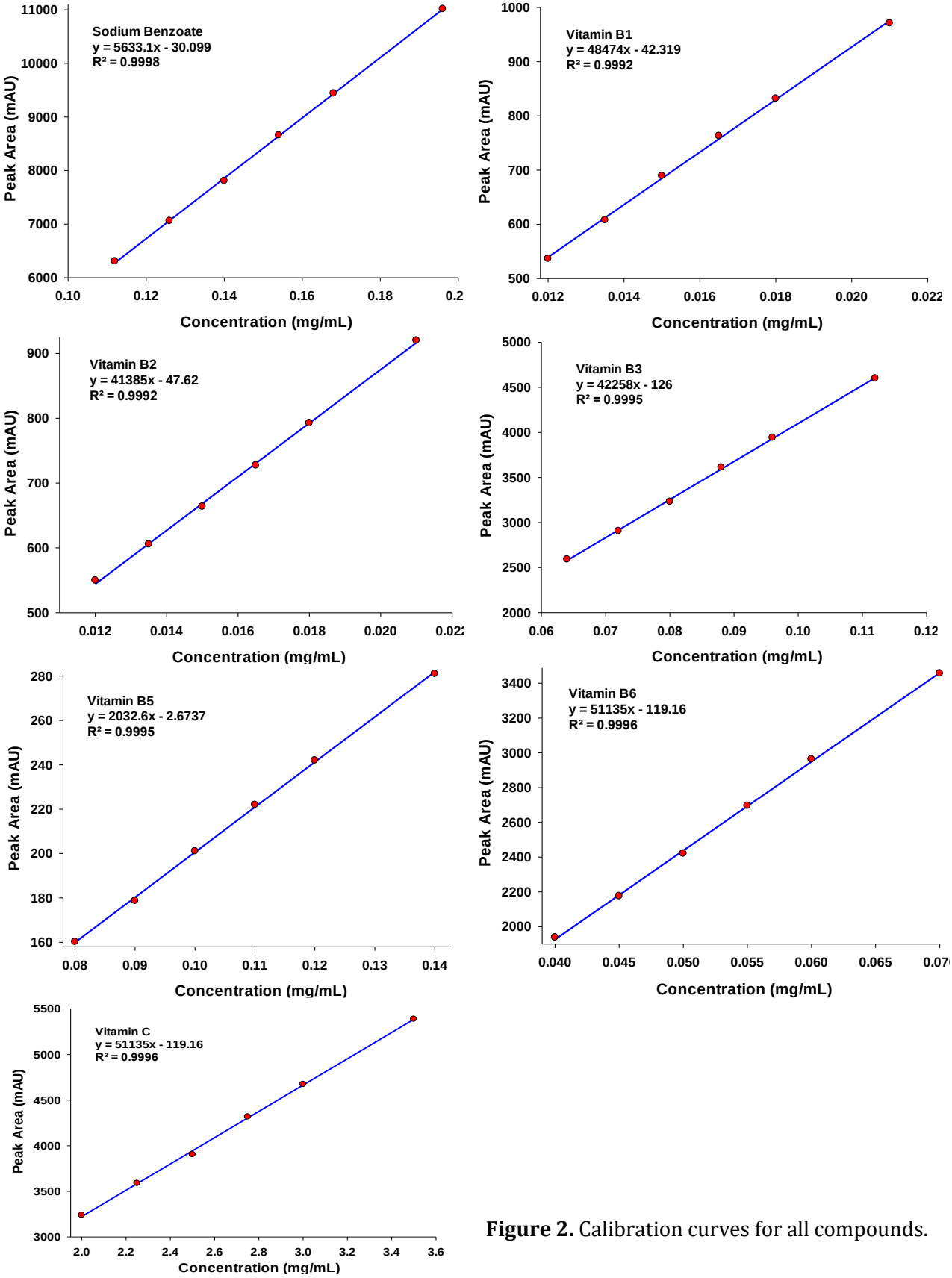


Figure 2. Calibration curves for all compounds.

3.2.3. Precision

The intra- and inter-day coefficient of variation (RSD %) was considered to measure the developed assay method precision. The t_R and peak area criteria were used to confirm the intra- and inter-day precision, and the results are collected in Table 4. The RSDs of the intra- and inter-day on all compounds for t_{Rs} were <0.15% and <0.53%, respectively, while for the intra-day peak areas for all compounds, the RSDs were in the range of 0.067–1.649%. All compounds showed the inter-day peak areas RSDs in the range of 0.28–0.891%. Based on the obtained results and relatively RSD values, a relatively high reproducibility was confirmed for the proposed developed method.

Table 4. Results from the evaluation of intra- and inter-day precision of the HPLC method (Section 2.2).

Compound	1				2				3			
	t_R-RSD (%)		AREA-RSD (%)		t_R-RSD (%)		AREA-RSD (%)		t_R-RSD (%)		AREA-RSD (%)	
	Intra-day[a]	Inter-day[b]	Intra-day[a]	Inter-day[b]	Intra-day[a]	Inter-day[b]	Intra-day[a]	Inter-day[b]	Intra-day[a]	Inter-day[b]	Intra-day[a]	Inter-day[b]
C	0.060	0.308	0.164	0.215	0.106	0.081	0.428	0.366	0.040	0.046	0.152	0.330
B1	0.062	0.176	0.712	0.803	0.093	0.045	0.559	0.496	0.071	0.049	0.181	0.511
B2	0.012	0.078	0.769	0.444	0.011	0.015	0.658	0.682	0.013	0.009	1.649	0.891
B3	0.078	0.056	0.074	0.471	0.100	0.059	0.067	0.160	0.082	0.086	0.049	0.100
B5	0.010	0.017	0.244	0.376	0.030	0.019	0.139	0.463	0.015	0.012	0.828	0.496
B6	0.128	0.532	0.179	0.104	0.155	0.118	0.409	0.224	0.098	0.059	0.382	0.028
NaB	0.011	0.008	0.230	0.476	0.014	0.010	0.319	0.736	0.005	0.004	0.381	0.520

t_R retention time, *AREA* peak area.

[a] Intra-day at five times in 1 day (n = 5).

[b] Inter-day on 3 different days (n = 3).

Accuracy and recovery

The method accuracy was obtained by testing the spiked placebo samples for each vitamin and preservative was evaluated. The methods used and solution preparation are illustrated in section 2.4.5. The results are presented in Table 5. The results show recovery% was < 99.48 % for all compounds. The limit for mean % recovery is 98-102%, and as all the values are within the limit, it can be concluded that the proposed method is accurate.

Table 5. Results from evaluation of the recovery of HPLC method (Section 2.2).

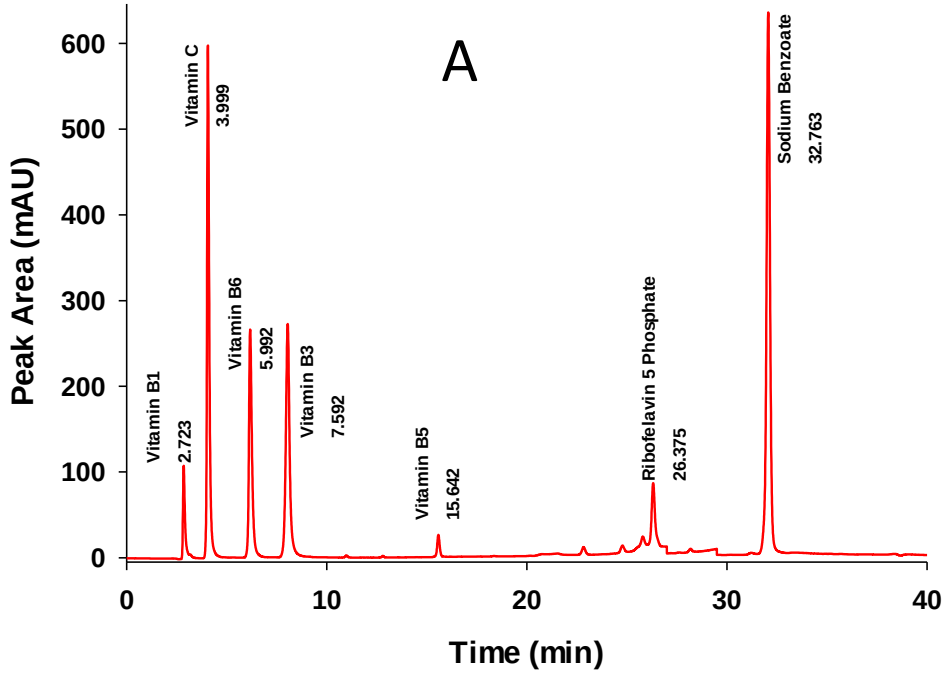
Compound	Spiked standard Conc. (mg/mL)	Recovery %	Spiked standard Conc. (mg/mL)	Recovery %	Spiked standard Conc. (mg/mL)	Recovery %	Average Recovery %
C	2.000	101.875	2.500	100.930	3.000	100.425	101.077
B1	0.015	99.650	0.019	100.500	0.023	100.592	100.247
B2	0.015	100.625	0.019	101.870	0.023	101.350	101.282
B3	0.064	99.263	0.080	99.910	0.096	99.292	99.488
B5	0.087	100.000	0.109	99.710	0.130	100.242	99.984
B6	0.040	99.875	0.050	100.100	0.060	99.883	99.953
NaB	0.112	100.425	0.140	100.040	0.168	99.367	99.944

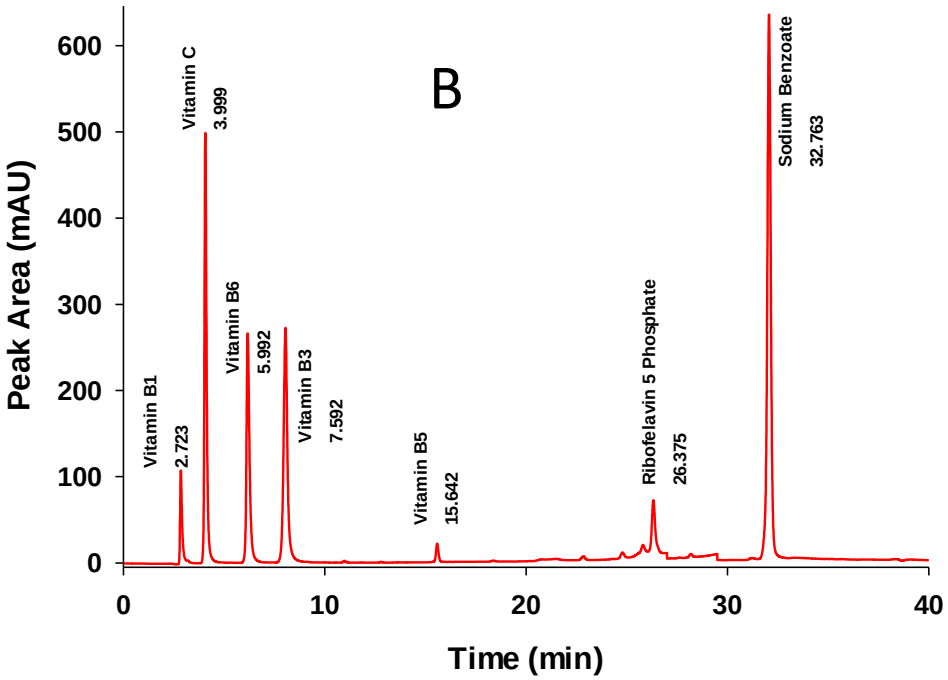
Stability studies-stress testing

To prepare bright yellow experimental syrup solution had a pleasant taste, and its non-stressed (initial analysis) samples' chromatogram is depicted in Figure 3. Applying 3 months of stress (at 40 C/75%RH condition), the syrup color was significantly yellow to dark brown, accompanied by some sediments. Furthermore, vitamin C, B2,

and B5 were relatively degraded. Some peaks belonging to the degradation products were detected but rather good separated with high-resolution efficiency. The degradation extent of vitamins after 3 months at 40 C/75%RH was as drastic as the first month.

In contrast, the relatively high stability was achieved for sodium benzoate as a preservative agent at the tested temperature conditions after 3 months, and about 7% of its initial amount was degraded. Placebo syrup was also subjected to the same temperature conditions, and it has a yellow color at 40 °C/75% RH. It showed no HPLC peaks that could disturb the determination of vitamins/preservatives. The vitamins/preservatives' assay results for the temperature conditions are listed in Table 6.





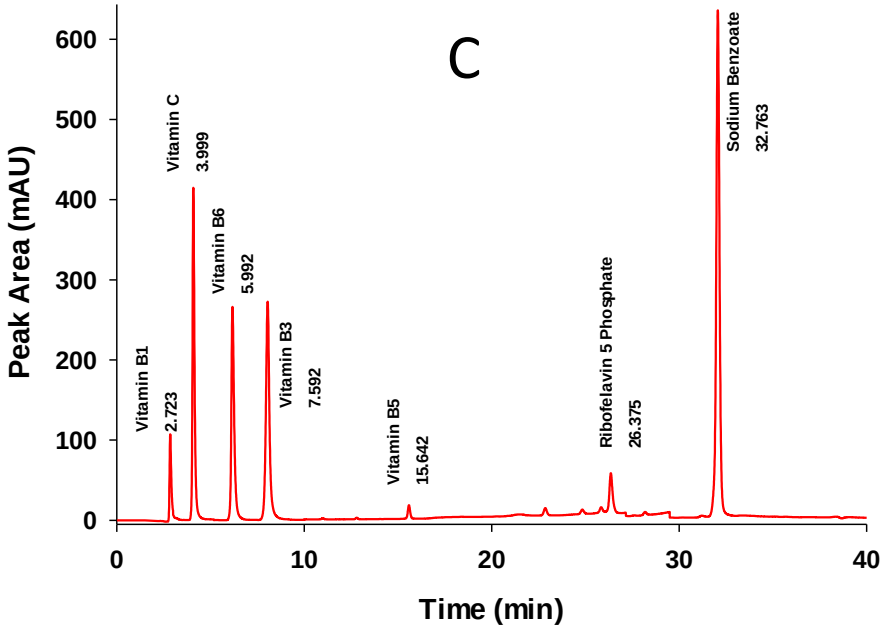


Figure 3. HPLC chromatogram of experimental syrup preparation initial analysis (A), and for experimental syrup preparation at stress conditions (section 2.4.6) after Month 1 (B), and Month 3 (C)

After sunlight stress, the degradation extents of components in the quality control samples were about 70% (vitamin B2), 50% (vitamins B6 and B1), 40% (vitamin C), 20% (B3), and 8% (B5), while no susceptible degradation was achieved for NaB. High resolution was achieved to separate the examined vitamins/preservatives from the sunlight stress degradation products. Table 6 represents the obtained results for sunlight stress.

Table 6. Vitamins/preservatives assay% for the experimental syrup preparation at Stress conditions (Section 2.4.6).

	Assay%					
		Month 1		**Month 3**		
Compound	***Initial analyze***	**Control sample (room cond.)**	**40 ºC/75% RH**	***Control sample (room cond.)***	***40 ºC/75% RH***	**Sunlight 10 h (24–26 ºC)**
C	98.08	96.23	88.12	94.44	71.25	62.21
B1	99.65	98.57	97.11	99.57	96.54	45.87
B2	97.37	94.25	90.14	88.69	80.11	23.11
B3	98.00	96.99	97.55	98.50	98.12	83.14
B5	96.41	95.00	60.77	90.21	50.87	93.25
B6	100.07	99.08	99.99	97.54	96.12	59.69
NaB	99.98	98.99	98.01	99.14	96.99	98.74

Conclusion

A simple HPLC–UV method was successfully validated in accuracy, precision, sensitivity, linearity, and stability to simultaneously analyze six water-soluble vitamins (C, B1, B2, B3, B5, and B6) and preservatives (sodium benzoate). A simple phosphate buffer mobile phase was used, with easy preparation. The proposed method with little or no variation was confirmed that is suitable for assaying the vitamins/preservatives in the actual samples. An analyzing time of about 40 min with good reproducibility and quantitative ability was reached for exploring such a relatively complex sample. Good results were obtained for the stressed samples (under temperature and sunlight stress of the multivitamin syrup), and the assay method's stability-indicating nature was confirmed. Furthermore, the stress sample's degradation products of vitamins/preservatives were separated well. Therefore, according to all the results obtained in this research, it can be concluded that this method can be approved and developed to determinate a number of water-soluble vitamins, including B1, B2, B3, B5, and B6, as well as the preservative compound (sodium benzoate).

Compliance with Ethical Standards

The authors declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants.

Informed consent

Informed consent was obtained from all participants included in the study.

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