A Validated Reversed-Phase HPLC Method Toward Quantification of Some Water-Soluble Vitamins and Preservatives in Pharmaceutical Samples

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ABSTRACT: *In this research, a simple, rapid, and reliable method was developed for isolating and quantifying the primary preservative compound (sodium benzoate (NaB)) and some water-soluble vitamins such as ascorbic acid (C) and five vitamin B compounds, including thiamine hydrochloride (B1), riboflavin-5-phosphate sodium (B2), nicotinamide (B3), pantothenic acid (B5), pyridoxine hydrochloride, (B6) in multivitamin syrup. An ODS column (temperature 35 °C) and a UV detector were used. The mobile phase was pH 3.0 phosphate buffer-methanol at a flow rate of 1.0 mL/min in a gradient elution approach. The analytes mentioned above were separated in 40 min. Method validation was reached by evaluating the Limit of Detection (LOD) and Limit of Quantification (LOQ), accuracy, and instrument precision. The data obtained in real sample analysis agreed with the declared values. The data obtained for under stress multivitamin syrup sample (forced degradation) confirmed no interference effects in the quantification of analytes.*

KEYWORDS: *Water-soluble vitamins, Vitamin B, Vitamin C, HPLC–UV, Gradient, Multivitamins.*

INTRODUCTION

Essential food ingredients are vitamins that must be supplied with diet sufficiently [1-5]. These compounds have significant water-soluble and fat-soluble vitamins [6-9]. The nine members of first-class (water-soluble) vitamins (including 8 B vitamins and vitamin C) appear in diverse biochemical futures [10,11]. Vitamin C is an antioxidant agent [12-14]. Various specific and vital metabolic futures of B vitamins as a complex class of vitamins have been confirmed, and thus, their lack or excess results in some particular weaknesses [1]. The most relevant vitamin to provide the required energy for the nervous system is vitamin B1 or thiamine; thus, its deficiency or lack of activity results in neurological manifestations [15,16]. Further, vitamin B1 deficiency causes Beriberi disease accompanied by cardiovascular disorders and digestive impairment [17].

Another member of the vitamin B group is Riboflavin, with sound therapy effects including antioxidant, anti-inflammatory, anti-aging, anti-nociceptive, and anti-cancer properties. Its protective properties and capability to diminish the toxic effect of drugs have been confirmed when riboflavin (B2) is combined with other drugs and compounds [18-20]. Skin inflammation and discomfort from the gastrointestinal tract can be created by a deficiency of vitamin B3 (niacinamide) and vitamin B6 (pyridoxine) [10]. Some

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symptoms' deficiencies, including fatigue, muscle cramps, headache, paresthesia, malaise, personality changes, numbness, muscle/ abdominal cramps, nausea, and impaired muscle coordination, have been related to vitamin B5 deficiency [21].

Usually, to have average growth, metabolism, and physical well-being, a key role is to have a sufficient level of these nutrients [22]. For these goals, various dosages of multivitamin products such as medicines or dietary supplements are emphasized [23]. Determining active compounds in commercial supplements formed by an uncomplicated, rapid, reliable, and cost-effective method is critical due to the widespread utilization of these food and pharmaceutical supplements [24].

Various methods, such as volumetric, spectrophotometric, spectrofluorimetric, electrochemical processes, etc., have been used to determine vitamins from the B-group [25]. 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 [24,26-28].

So far, various HPLC methods, such as Reverse-Phase Liquid Chromatography (RP-LC) without [29-32] and with ion-pair agents [33-39], have been used for simultaneous identification/determination of most of the vitamins. To determine vitamin C [40-46] and pantothenic acid [26,47,48] in different foods quantitatively, specific HPLC methods have been used and developed [40-46]. In contrast, only a few HPLC methods have been reported in the literature [49].

Generally, preservatives, such as natural or synthetic chemicals, must be added to various food, pharmaceuticals, cosmetics, and biological products to prevent microbial growth or undesirable chemical reaction spoilage. Many analytical methods have been reported to determine such as products' vitamins and preservatives [39,50-53]. Thus, introducing/developing novel validating procedures for HPLC is very important in Quality Control (QC) in each pharmaceutical industry for enhancing the quality of the products.

The present study proposed a new HPLC method to determine some water-soluble vitamins and sodium benzoate (as a preservative in more pharmaceutical multivitamin products). Here, a simple and cheap process has reported the simultaneous determination of 6 vitamins in an aqueous solution mixture plus the determination of sodium benzoate as perseverative by the C18 column and phosphate buffer as a carrier phase. As far as we know, no such reports exist in such determination.

EXPERIMENTAL SECTION

Chemical and reagents

Vitamins and preservatives were prepared from different suppliers/ manufacturers as listed below: vitamin B1 and B5, Jiangxi tian xian 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 (sodium benzoate), Fars Chemical Industries Company, Fars, Iran.

The quality of vitamins used (with a commercially pure grade for human nutrition) was controlled by the QC laboratory before use. For this checking, 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 for filtering the prepared aqueous mobile phase. All aqueous solutions were designed in high-purity water from Merck Direct-Q 3 UV (Darmstadt, Germany). Climate Chambers were from Thermolab Scientific Equipment (Maharashtra, India).

Equipment and chromatographic conditions

The 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 for performing the data acquisition. A C18 Nucleodur-HTec reversed-phase column at 35 ºC was used (250mm×4.6mm×5µm, MACHEREY-NAGEL GmbH & Co.

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

Time	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

KG, Dueren, Germany). A gradient elution approach consisting of methanol (solvent A) and a phosphate buffer (Solvent B: 0.05 M concerning each NaH_2PO_4 and Na_2HPO_4 salt; pH 3.0) at a flow rate of 1 mL/min was used as adjusted 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.

Preparation of solutions

Standard solutions

Stock solutions were prepared daily in a 50 mL volumetric flask by dissolving an aliquot 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 reached the mark by distilled water. The prepared stock solutions were kept in dark conditions.

For preparing 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 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 2000–3500 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). In tightly closed bottles, these solutions were kept in the dark and were stable for 36 h at room temperature.

Quality control samples

A multivitamin syrup and its blank 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. Blank did not contain water-soluble vitamins and preservatives.

An aliquot of 5 mL of the sample was added to a 100 mL volumetric flask to analyze the multivitamin syrup solution. An aliquot of 5 mL of the blank was added to a 100 mL volumetric flask to analyze the blank solution. After sonication for 10 minutes, it reached the mark by 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). No supernatant for the tested products was observed.

Method validation

For validating the HPLC method, some characteristics, including specificity, linear range, the Limit of Detection (LOD), The Limit of Quantitation (LOQ), and intra- and inter-day precision, accuracy, and recovery percent were evaluated as illustrated in the following sections [54-56].

System suitability

For evaluating the system suitability, some HPLC factors such as retention time (t_R), resolution factor (R_s), tailing factor (T_F), theoretical plates (T.P), and RSD% were estimated by replicating measurements (n=3) of the standard solution. Based on the literature [57], peak separation between adjacent peaks must have $R_s \geq 2$ and $T_F \leq 2$ as acceptable values.

Selectivity or specificity

The specificity or selectivity for an analytical method can be stated through the absence of interference and by comparing the results obtained with those obtained with an orthogonal procedure. Further, these can be obtained by the analytical method's scientific rules [54-56].

The method's selectivity was tested by comparing the HPLC chromatograms of the individual vitamin and preservative standard solutions with vitamins/preservatives' usual mixture, blank, 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 each analyte's corresponding standard solution and appropriately diluted with water, as mentioned earlier. 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 $R^2 > 0.995$ [58]. Further, 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.

The following formulas were used to calculate LOD and AOQ Figs. of merit:

$$\mathrm{LOD} = \frac{3.3\,\sigma}{\mathrm{m}}$$

$$\mathrm{LOQ} = \frac{10\,\sigma}{\mathrm{m}}$$

Where,

σ = the residual deviation of the regression line (in the calibration curve)

m = the slope of the calibration curve

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. 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 added to 100 mL volumetric flasks and diluted the mark with 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 obtained relative standard deviations (RSDs) measure the process precision, which should be ≤2%. [59].

Accuracy and recovery

The recovery approach was used to test the accuracy of the method suggested, and it was considered 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 to 50 mL volumetric flasks and diluted to the mark by water, respectively. Triplicate measurements were done on each spiked sample.

Stability studies

To elucidate the selectivity and stability-indicating nature of the proposed analytical method, stress testing was done on the experimental multivitamin preparation

Table 2: Results from the evaluation of the specificity of the HPLC method (section 2.2)

Compound	t_R (min)	R_S	T_F	T.P	RSD%
Vitamin B1	2.717	–	1.648	6024.736	0.325
Vitamin C	4.008	7.730	1.206	11169.748	0.567
Vitamin B6	6.046	10336	1.196	13529.427	0.251
Vitamin B3	8.042	8.099	1.035	13892.709	0.098
Vitamin B5	15.650	34.758	1.087	106765.946	0.124
Vitamin B2	26.35	48.429	1.066	175326.088	0.203
NaB	32.283	23.216	1.078	249552.359	0.024

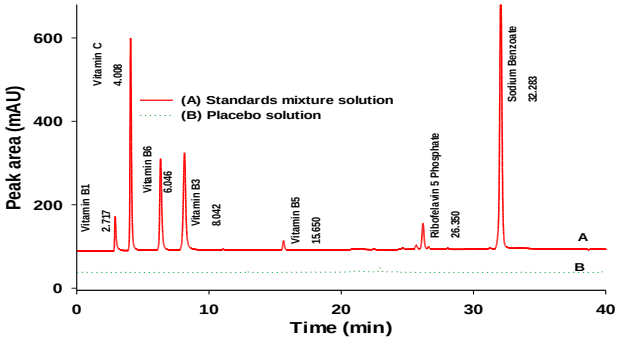

Fig. 1: (A) HPLC chromatogram of standards mixture solution (section 2.3.1), (B) HPLC chromatogram of placebo solution (section 2.3.3). Peaks: vitamin B1, t_R ~2.7 min; C, t_R ~4.0 min; B6, t_R ~6.0 min; B3, t_R ~8.0 min; B5, t_R ~15.6 min; B2, t_R ~26.3 min; NaB, t_R ~32.2 min. By C18 (250mm×4.6mm×5µm) 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 and B2, and NaB)

to generate closely degraded products [58]. 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 quality control solutions 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 and 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 Fig. 1.

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

Method validation

Standard methods/ procedures used to validate the method were adopted from the literature [54-56].

Selectivity

HPLC chromatograms in Figs 1 belong to the standard mixture solutions (section 2.3.1) and blank solution (placebo) (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 ($R \geq 2$).

Linearity

Fig. 2 shows the calibration plots obtained for the analytes investigated the correlation coefficients evaluated their logic. The characteristics of the 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 evaluating the linearity and range of the HPLC method (section 2.2)

Compound	Regression plot	R^2	LOQ (µg/mL)	LOD (µg/mL)
Vitamin C	$y = 1441.8\ x + 340.28$	0.9993	361.80	119.40
Vitamin B1	$y = 48474.0\ x - 42.32$	0.9992	2.40	0.80
Vitamin B2	$y = 41385.0\ x + 48.63$	0.9992	2.30	0.80
Vitamin B3	$y = 42258.0\ x - 126.00$	0.9995	9.80	3.20
Vitamin B5	$y = 2032.6\ x - 2.67$	0.9995	1.26	4.20
Vitamin 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

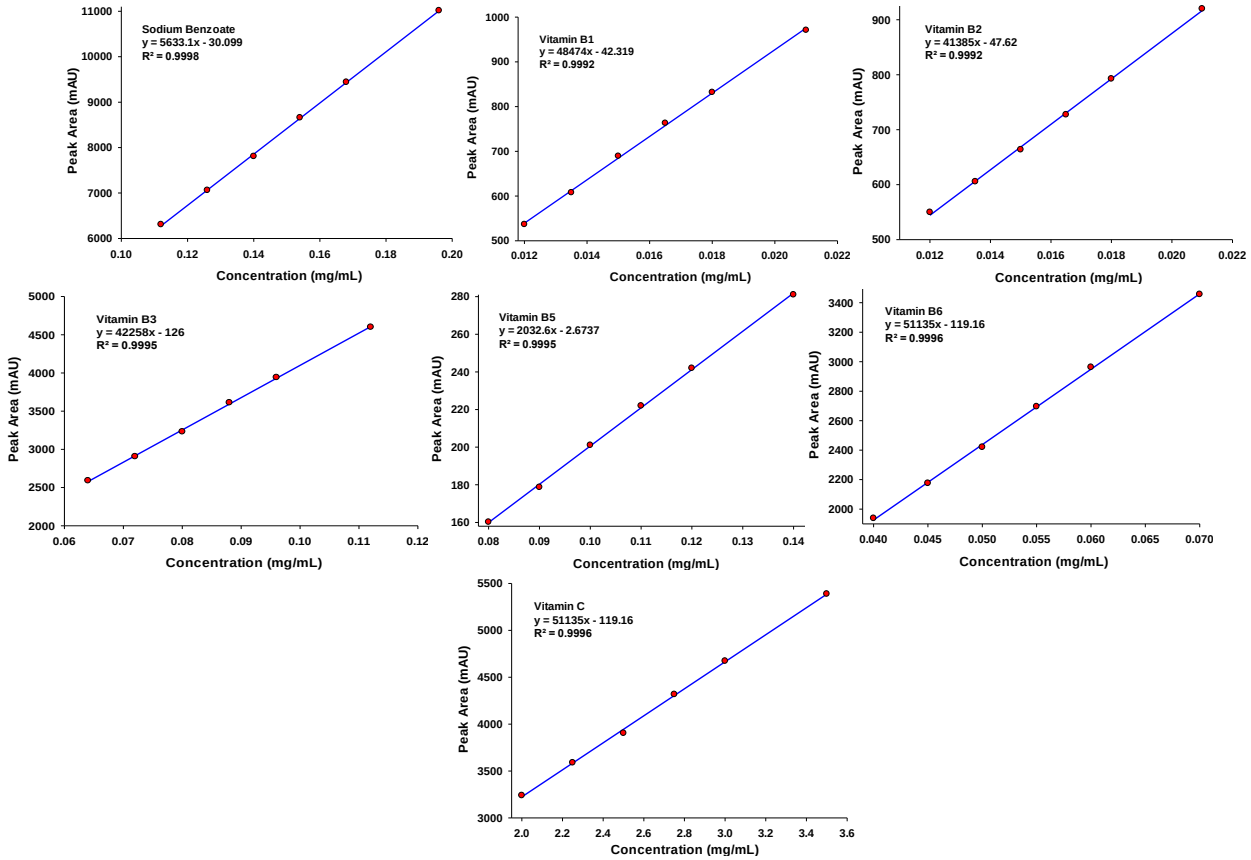

Fig 2: Calibration curves for all compounds

Precision

The intra- and inter-day relative standard deviation (RSD %) 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 0.028–0.891% range. Based on the obtained results and relatively RSD values, a relatively high reproducibility was confirmed for the proposed developed method.

Accuracy and recovery

The method accuracy was evaluated by testing the spiked placebo samples for each vitamins and preservative. The methods used and solution preparation are illustrated in section 2.4.5. The results are displayed in Table 5. The results show that recovery% was > 99.26 % 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 4: Results from evaluating the 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)

Table 5: Results from evaluating the recovery of the 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

Prepared bright yellow experimental syrup solution had a pleasant taste, and its non-stressed (initial analysis) samples' chromatogram is depicted in Fig. 3. Applying 3 months of stress (at 40 C/75% RH conditions), the syrup color was significantly yellow to dark brown, accompanied by some sediments. Further, vitamins C, B2, and B5 were relatively degraded. Some peaks belonging to the degradation products were detected but rather well separated with high-resolution efficiency. The degradation extent of the vitamins after 3 months at 40 C/75%RH was as drastic as the first month.

In contrast, 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 had a yellow color at 40 °C/75% RH. In addition, 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.

After sunlight stress, the degradation extents of the 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 not susceptible degradation was achieved for NaB. High resolution was achieved to separate the examined vitamins/preservative from the sunlight stress degradation products. Table 6 shows the obtained results for sunlight stress.

CONCLUSIONS

A simple HPLC–UV method was successfully validated in accuracy, precision, sensitivity, linearity, and stability to analyze six water-soluble vitamins (C, B1, B2, B3, B5, and B6) and preservative (sodium benzoate). A simple phosphate buffer mobile phase was used, with easy prepare preparation. The proposed method with little or no variation was confirmed suitable for assaying the vitamins/preservative in actual samples. An analyzing

Table 6: Vitamins/preservative assay% for the experimental syrup preparation under stress conditions (section 2.4.6)

Compound	Assay%					
	Initial analyze	Month 1		Month 3		Sunlight 10 h (24–26 ºC)
		The control sample (room cond.)	40 ºC/75% RH	The control sample (room cond.)	40 ºC/75% RH	
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

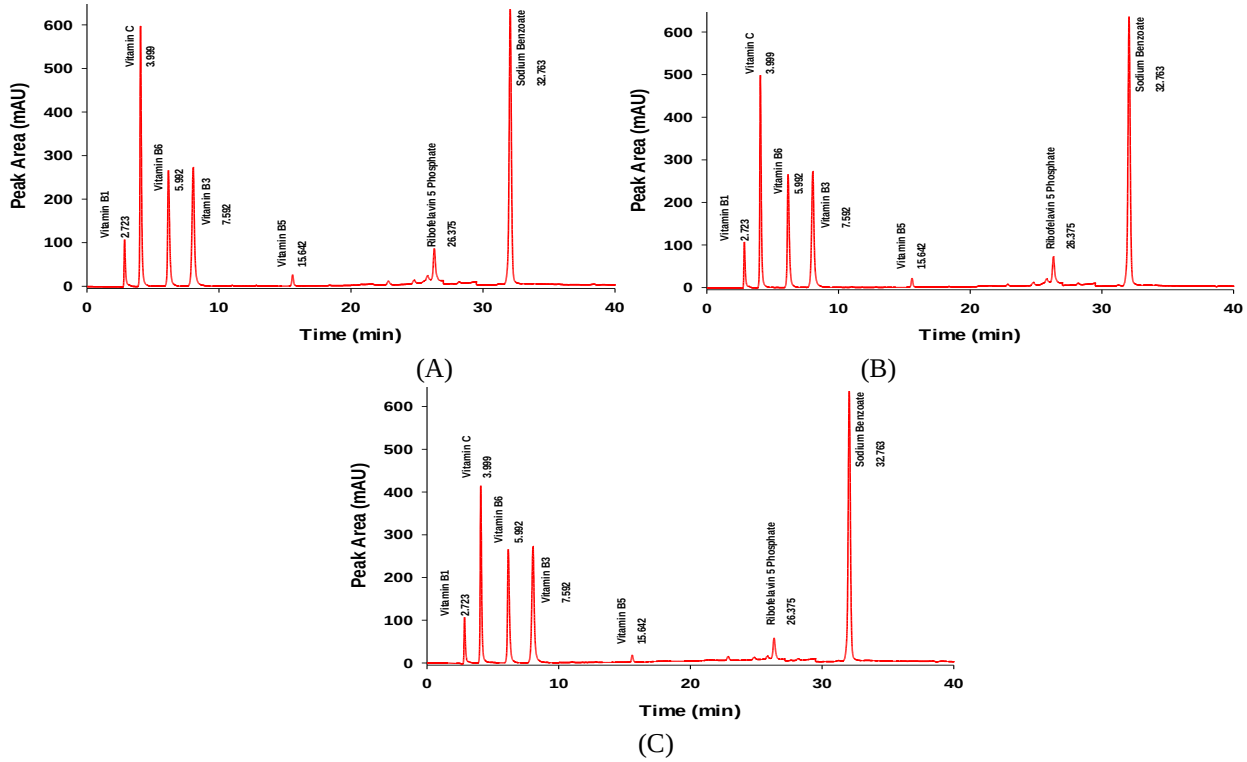

Fig. 3: HPLC chromatograms of syrup prepared for initial analysis (A), the syrup under stress conditions for 1 month 1 (B), and 3 months (C) (see section 2.4.6)

time of about 40 min with good reproducibility and quantitative ability was reached for exploring such a relatively complex real sample. The results obtained for the stressed samples (under temperature and sunlight stress of the multivitamin syrup) were used to confirm the assay method's stability. Further, the stress sample's degradation products of vitamins/preservative were separated well. Thus, this proposed validated HPLC-UV method can be used for routine drug analysis.

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