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Original Article

Analytical Method Development and Validation for the Simultaneous Quantification of Water-Soluble Vitamins (B Complex) at Single Wavelength by HPLC in Food Supplement

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ABSTRACT

Background: Water-soluble B-complex vitamins are essential for energy metabolism and overall health. **Objectives:** To develop a simple, sensitive, accurate, validated and cost-effective reverse phase liquid chromatographic method for the simultaneous estimation of water-soluble vitamins (B1, B2, B3, B5, B6, B7, B9 and B12) in food supplement in a single run at single wavelength. **Materials and Methods:** The HPLC examination was done using a C18 Phenomenex Luna column (250x4.6 mm), 5 µ particle size. The mobile phase is a mixture of 0.1% orthophosphoric acid in HPLC grade water, 80% acetonitrile with 0.1% orthophosphoric acid, filtered and degassed. A Gradient solvent system at 0.8 mL/min flow rate with detection at 210 nm wavelength was used. **Results:** This method was validated per ICH guidelines, showing elution of vitamins in the order: B1, B3, B6, B5, B9, B12, B2 and B7. Linearity was established over a concentration range of 0.0005-0.6 mg/mL (5.0-600 ppm), which covered all the range of above vitamins in the sample with results r^2>0.99. LOD ranged from 0.101 to 1.25 ppm and LOQ from 0.305 to 3.79 ppm. Developed Formulation and market samples were also analysed by this method and results were found within the range, which shows the applicability of method. **Conclusion:** The developed in-house analytical method is simple, sensitive, accurate, fast and ICH-validated. It allows simultaneous analysis of vitamins B1, B2, B3, B5, B6, B7, B9 and B12 in a single run at single wavelength, enhancing throughput and reducing analysis time. This unique method also overcomes complications of existing approaches, sample preparation issues, complexity of mobile phase preparation (buffer system), matrix variability concerns and multiple runs for all vitamins, as it can be used for wide varieties of food matrix. It can be used for routine quality checks in various food products.

Keywords: Food supplement, RP-HPLC method, Simultaneous quantification, Single wavelength, Water-soluble vitamins.

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INTRODUCTION

Vitamins are necessary organic compounds that must be consumed in modest amounts for the biological system to work properly and support appropriate growth and health. The Greek word "VITAMINE," which meaning "vital for life," is where the name vitamin originates. Vitamin's importance was initially understood in early age, and they are crucial for the body for maintaining its normal growth and lack or loss of any vitamins may cause serious progressing diseases as age increases. To guarantee the quality of food items, it is equally crucial to determine the quantity and rate of vitamin degradation in the food products.

Hence analytical method development for the qualitative and quantitative analysis is required for analysing the concentration of vitamins in final food products during its storage and stability period (Ankali *et al.*, 2015).

Water-soluble and fat-soluble vitamins are the two groups into which they fall. Essential fat-soluble vitamins are A, D, E and K., while ascorbic acid and vitamin B complex, which includes thiamine-B1, riboflavin-B2, niacin- B3 pantothenic acid- B5, pyridoxine-B6, biotin-B7, folic acid-B9 and cyanocobalamin-B12, are water-soluble groups (Li and Chen, 2001).

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To find dietary references and daily values for the vitamins is very crucial and tedious for the health professional in early days. Now a days it is calculated by dose response curve and endpoint is detected by the ED_{50} (Effective Dose) or LD_{50} (Lethal Dose) and several toxicity studies. The FDA (Food Drug Administration) by using guidelines set daily values for the nutritional labelling as % DV for avoiding the toxic effect and differentiates in gender categories based on age.

Comprehensive pre-treatment techniques, such as physical, chemical, chromatographic, microbiological and biological techniques, are used in the extraction of vitamins. The material being analysed and its concentration determines which approach is best for vitamin analysis. Chromatographic techniques, however, are frequently insensitive to low vitamin concentration levels. For these low-level assays, microbiological techniques are used; however, they are slower, more costly and typically less precise. These days, there is less demand for delicate biological testing.

To put forward a simple, reliable and economical technique for simultaneous recovery of water-soluble category vitamins from food supplement is of great importance in food industry and food manufacturer because vitamins are most unstable and easily deteriorate by the presence of light, heat, presence of oxygen. So, developing a validated method which can identify the exact amount of degradation of each vitamin during its storage. According to the findings of this study, it can be added in greater quantities to the finished product in order to maintain food product quality in accordance with FDA guidelines (Kucukkolbasti *et al.*, 2013).

Vitamin analysis is a tedious process due to its complex chemical structures and its complexity with the matrices. Each vitamin has its own unique properties and due to their unstable nature and most of its activity loses during processing and storage as a result of exposure to the high temperature, oxygen and light. Therefore, a method using HPLC or mass spectrometry can be developed for the rapid quantification of all water-soluble vitamins (Trang, 2013).

Different matrices have been used in literature research to provide appropriate methodologies for the assessment of individual or combined water-soluble vitamins. Among these reported methods most commonly used techniques spectroscopic, chromatographic, chemical or biological and hyphenated techniques like HPLC, GC, MS etc., even though hyphenated techniques like GC-MS is very expensive and sensitive, its use in developing stage is risky and required expert personalities for handling, so the HPLC is used worldwide as it involves less sample pre-treatment and easy to maintain, environment friendly and less complicated for the analysis of wide variety compounds.

Since these water-soluble vitamins can be analysed by many other types of method and by using different matrices depending upon the formulation and amount present in that formulation and even from blood plasma, urine etc. each method and each matrix have different condition for getting better resolution and pre-treatment process (Zafra:Gómez *et al.*, 2006).

Research Gap

There are many recent advances in analytical methods of Water-soluble vitamins uses HPLC/DAD, HPLC-MS/MS and RP-HPLC/ELSD. However, there are certain limitations still exist in the methods like few are not able to quantify all 8 vitamin B-complex range in single injection run and require multiple runs at different wavelength for quantification of all vitamins (Abdelfatah *et al.*, 2024; Wazed *et al.*, 2022; Saptarini *et al.*, 2022; Fatima *et al.*, 2019; Rakusa *et al.*, 2021; Faraz *et al.*, 2019; Klejdus *et al.*, 2004; Papadoyannis *et al.*, 1997; Matteva *et al.*, 2023) few methods use derivatization or solid phase extraction techniques which make them time consuming and even more complex, few techniques employ buffers as mobile phase components, resulting in longer processing times and shorter column lives (Zia *et al.*, 2023; Hosain *et al.*, 2021). HPLC-MS/MS are highly sensitive and reliable techniques but cost is the main factor while considering the routine analysis.

To address all these challenges this research aims to develop a method, which is simple, sensitive, robust and capable for simultaneous estimation of water-soluble vitamins (B_1, B_2, B_3, B_5, B_6, B_7, B_9 and B_{12}) in a single method at single wavelength (210 nm) utilising a PDA detector in formulated food product and validate as per ICH guideline. Three different brands of Food Products (HFD) samples were purchased from the local market and tested with the validated method to compare with label claim.

MATERIALS AND METHODS

Instrumentation

Analytical determination was held using Shimadzu HPLC system (LC-2030C3D) with Phenomenex C_{18} column (250x4.6 mm), 5 µ, Flow rate 0.8 mL/min, Wavelength used for detection 210 nm, Column temperature 30°C, pump consisting of five line degassing unit with gradient elution of four solvents with a maximum pressure of 2244 MPa, autosampler of needle injection mode of capacity 0.1-100 µL, oven temperature ranges from 4°C to 60°C and detector used is PDA detector of wavelength range from 190-700 nm, flow cell capacity of 12 µL, with low noise level base line detection.

Chemicals and reagents

The water-soluble standards for reference standard were procured from Sigma Aldrich Company and Supplier Company. The purity of thiamine mononitrate (B1) is 99%, riboflavin (B2) is 97.9%, niacinamide (B3) is 99.21%, calcium pantothenate (B5) is 100.0158%, pyridoxine (B6) is 99.70%, biotin (B7) is 99% folic acid (B9) is 97.10%, cyanocobalamin (B12) is 99.20%. These

reference standards are in powder form and stored in dark cool condition to prevent degradation from light and sunlight. Other than these vitamins, materials used include orthophosphoric acid (thermo Fischer), acetonitrile (Qualigens), milli-Q water.

Preparation of supplement formulation

Ingredients

Malt extract (liquid form), Sugar powder form, Puffed rice powder, Soya flour, Casein, coco butter, Vitamin premix, Mineral premix (salt form).

Procedure

To prepare the diskettes formulation with the above-mentioned ingredients, oil is heated and to that small quantity of water is added. To this oil water mixture all the powder ingredients like soya flour, casein, vitamin premix, mineral premix. Then add crispy items like puffed rice powder and sugar powder. Mixed well and passed it through 8 mesh size for producing granules. These granules are dried in oven by keeping at 60°C for 2 or 3 hr. Again, pass it through small mesh size to produce fine powder. Finally added flavours and transferred this powder mass into punching machine. The final diskettes are dried in oven at 60°C.

Chromatographic conditions

The mobile phase contains mixture of two water miscible solvents-0.1% orthophosphoric acid (Fisher scientific HPLC grade) in water (A) and 80% acetonitrile (Qualigens HPLC grade) with 0.1% orthophosphoric acid (B). The gradient programme was optimized-A/B-98: 02 for 5 min, ramped to A/B-72: 28 in 17 min and held constant at A/B-72: 28 for 3 min. Total run time were 35 min for all 8 vitamins.

Preparation of working standard

Each standard vitamin (B_1, B_2, B_3, B_5, B_6, B_7, B_9 and B_{12}) was prepared by putting around 5 mg in 100 mL flask separately and 50 mL of HPLC grade water was added then the standard was sonicated for 5 min and volume was made up-to 100 mL by using same diluent. To identify individual peaks of vitamins these individual standards were injected in HPLC.

After the retention time identification of each of individual vitamins, mixed standard solution of all vitamins was used further.

The mixed stock solution was made with 100 mg of each standard vitamin in 100 mL flask and HPLC grade water to get 1 mg/mL concentration and different dilution were made by using the stock solution. To prepare glass vials for HPLC analysis, supernatant was directly poured into them.

Preparation of sample solution

The diskette sample was made powder by using pestle and mortar, the powdered sample was weighed approximately 1 g in a 100 mL volumetric flask, to which 50 mL of diluent (HPLC grade water) was added. Sample was then sonicated for 5 min, the volume was adjusted up-to 100 mL and filtered through a 0.45 µm PVDF syringe filter in glass vial and injected into chromatographic system (HPLC).

Validation of Method

Validation of Method was done using the ICH recommendations' metrics, which included robustness, system suitability test, Limit of Quantification (LOQ), Limit of Detection (LOD), specificity, range, linearity, precision and accuracy.

Linearity

An analytical procedure is deemed linear by the ICH criteria if it is able to yield test results that are precisely proportionate to the analyte concentration in the sample. By injecting the sample in duplicate and by making a calibration curve by graphing concentration vs. area, the linearity of water-soluble vitamins was ascertained. By ensuring that the correlation coefficient r^2 was more than 0.99, linearity of the method was verified.

Precision

According to the ICH precision guideline, the analytical procedure expresses how closely several measurements taken from several sample under the given conditions agree with one another. There are different types of precision: intraday and interday and repeatability. By injecting samples 6 times and comparing the results to the standard, the precision can be ascertained. The 6 injections' RSD was computed.

The laboratory's intra-day precision was represented within a day variation, demonstrating results that are in close proximity to a set of measurements derived from several samplings. Precision across same condition is expressed by repeatability. Repeatability is achieved by injecting the 6 runs of standard and samples. Intermediate precision can be demonstrated by conducting an experiment in a separate lab by a different individual at a different time. Through the use of different instruments for the same experiment, the intermediate precision demonstrates the method's robustness. The acceptance criteria for all, was maximum 3.0%. RSD.

Accuracy

Agreement degree between the value that is recognised as either an acceptable reference value or a conventional actual value is expressed by the accuracy of an analytical method. After spiking the sample with 50%, 100% and 150% of a specified amount of standard, the percentage recovery of the vitamins was used to calculate the method's accuracy. In order to determine the ultimate RSD and percentage recovery, three runs of unspiked samples, 50% spiked samples, 100% spiked samples and 150%

spiked samples were examined. An accuracy acceptance criterion was 80-120% with maximum 3.0% RSD.

Detection Limit

A specific analytical method's detection limit is the lowest concentration of analyte in a sample that can be detected but not accurately measured. There are numerous methods for figuring out the detection limit, including visual inspection, the noise to signal ratio and standard error graphing with a standard calibration curve.

$$\text{Detection limit} = \frac{3.3\,\sigma}{S}$$

σ: Standard deviation derived from response,

S: Slope derived from calibration curve,

(ICH Q2 R1 guidelines).

Quantification Limit

In analytical method, the limit of quantification is the lowest practical concentration of an analyte in a sample that can be quantitatively measured with adequate precision and accuracy. A parameter of quantitative testing called the limit of quantification is particularly helpful for identifying contaminants and/or degradation products at low chemical concentrations in sample matrices.

$$\text{Quantification limit} = \frac{10\,\sigma}{S}$$

σ: Standard deviation derived from response,

S: Slope derived from calibration curve,

(ICH Q2 R1 guidelines).

Range

The range of the method is the range of analyte concentrations in the sample between which the analytical procedure has been shown to have an appropriate degree of linearity, accuracy and precision. The sample concentration was determined by taking its 25% to 125% (2.5-15 mg/mL). The standard concentration was 0.0005 mg/mL to 0.6 mg/mL. The range was determined by comparing the maximum high concentration and lowest concentration of the standard to the concentration that will be present in the finished product.

Robustness

Indicating its dependability under typical operating conditions, robustness is a measure of its ability to withstand little but intentional variations in method parameters. We evaluated the method's robustness in this instance by varying the vitamin's wavelength and flow rate. Though the retention period has altered, the approach still yields the same results.

Ruggedness

By minimally altering the main procedure portion without altering the solvent or analyte, ruggedness checking can be carried out.

System suitability

In the developing stage, system suitability tests are carried out to demonstrate that the system can carry out the suggested technique for the identification of specified standards and samples. Theoretical plates, Capacity factors, Tailing Factor and Resolution are among the key parameters for system suitability.

Specificity

The purpose of the specificity test is to quantify the analyte of interest in the presence of other components that may be anticipated to exist. These typically contain matrixes, degradants and contaminants. The specificity of a method is assessed using a placebo and solvent.

RESULTS

Linearity

The correlation coefficient was identified by plotting the area v/s concentration. The linear regression and correlation coefficient were found to be >0.99 for all the water-soluble vitamins.

LOD and LOQ

The most common technique for determining the LOD and LOQ is the Signal to Noise ratio (S/N), which is 3:1 for the LOD and 10:1 for the LOQ (Table 1). Other methods include visual evaluation, standard deviation of response and slope. Software can also be used to calculate LOD and LOQ and report results.

Table 1: LOD and LOQ of vitamins.

Vitamins	LOD (ppm)	LOQ (ppm)
B1	0.356	1.08
B2	0.101	0.305
B3	0.238	0.722
B5	0.938	2.843
B6	0.413	1.251
B7	1.25	3.79
B9	1.222	3.702
B12	0.415	1.259

Accuracy

By adding a known quantity of standard to the sample, one can obtain reliable results by measuring the percentage of recovered data using the accuracy parameter. The range used for detection was 80-120% and the accuracy within the concentration range

met the acceptance criteria with a percentage RSD inside the limit.

Precision

Intraday was performed by injecting 6 samples replicates on the same day whereas interday was done on a different day. All results were found within max. 3.0% RSD.

System suitability criteria

System suitability ensures that system is suitable to perform the quality testing without any interference. The result found passes the system suitability criteria (Table 2).

Robustness

The validation parameter is crucial, because despite our deliberate changes to the parameters, the outcome ought to be the same. The wavelength and flow rate per minute are the two most often changed parameters in the process. Wavelength change was performed at 208 and 212 nm whereas change in flow rate was performed at 0.7 mL/min and 0.9 mL/min. The RSD of all robustness parameters was found to be under the 3.0% limit. Validation results (Tables 3 and 4).

Peak of individual vitamin in the sample was confirmed by comparing with Retention Time (RT) by overlying with standard

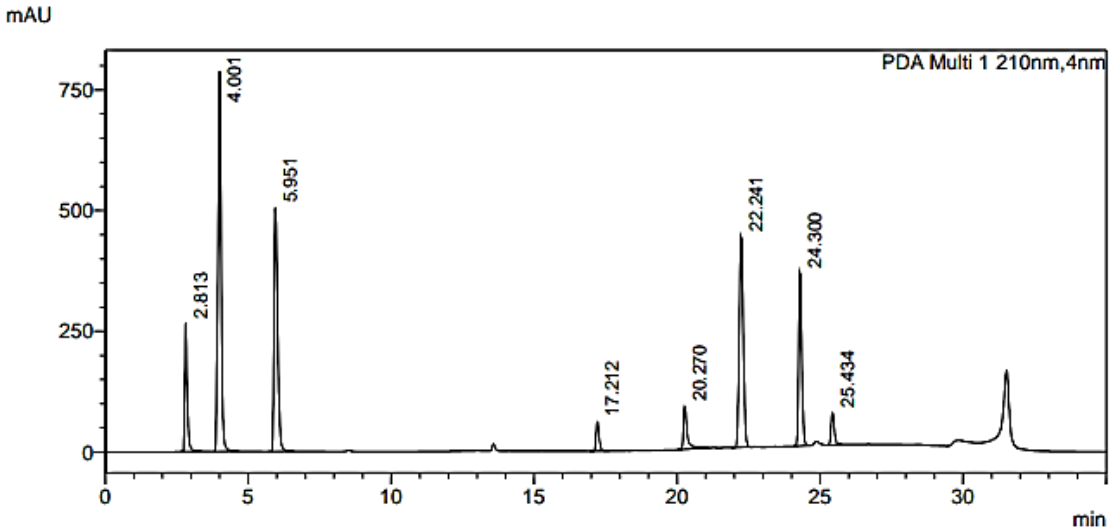

Figure 1: HPLC Chromatogram of standard water soluble vitamins 1) B1 (RT 2.813 min) 2) B3 (RT 4.001 min) 3) B6 (RT 5.951 min) 4) B5 (RT 17.212 min) 5) B9 (RT 20.270 min) 6) B12 (RT 22.241 min) 7) B2 (RT 24.300 min) 8) B7 (RT 25.434 min).

Table 2: System suitability parameters of the vitamins.

Vitamins	Retention Time (Min)	Theoretical plate (USP)	Tailing-factor	Resolution (USP)	Capacity Factor (k')
Vitamin B1	2.844	3209	1.45	--	1.84
Vitamin B3	3.989	5073	1.11	5.393	2.989
Vitamin B6	6.005	11903	1.28	9.075	5.005
Vitamin B5	17.226	111039	1.28	52.569	16.226
Vitamin B9	20.255	165126	1.26	14.914	19.255
Vitamin B12	22.26	106180	1.11	8.482	21.26
Vitamin B2	24.318	204540	1.24	8.432	23.318
Vitamin B7	25.45	216069	1.2	5.212	24.45

Table 3: Validation results for standard vitamins.

Parameters	B1	B2	B3	B5	B6	B7	B9	B12
Range (mg/mL)	0.000535-0.107	0.000545 -0.08175	0.000555-0.604	0.00054-0.108	0.000515- 0.103	0.001- 0.100	0.000515-0.0515	0.000535-0.107
Linearity	y=2E+07 x-1741.1	y=3E+07 x+4483	y=4E+07 x+6843.1	y=4E+06 x-843.12	y=3E+07 x+14333	y=2E+07 x+4880.4	y=2E+07 x+62657	y=4E+07 x-3074.6
Correlation coefficient (r^2)	0.9999	0.9997	0.9999	0.9996	0.9999	0.999	0.991	0.9999

In linearity, "x" is concentration of Vitamins in mg/mL and "y" is peak area at 210 nm.

Table 4: Validation results for sample.

Validation Results						
Parameters	**Acceptance criteria**	**B1**	**B2**	**B3**	**B5**	**B6**
Linear range (mg/mL)-Sample.	-	2.5-15				
Correlation coefficient (r^2)- Sample.	0.99	0.992	0.9928	0.9981	0.9988	0.9985
Intraday precision % RSD.	3.0 % Max n=6	2.536	1.474	0.269	2.513	2.867
Interday precision % RSD.		2.5845	2.89	0.75	2.16	2.61
Accuracy 50% level.	80-120% (Mean±SD) n=3	101.49±0.035	110.25±0.822	101.79±1.024	84.58±1.01	118.89±2.082
100% level.		91.73±0.506	115.57±0.035	99.12 ±1.751	97.65± 0.056	104.52±0.453
150% level.		91.01±0.618	109.27±0.042	107.52±0.039	117.53±0.044	104.98±0.052
Robustness (a) Change in flow rate (0.7 mL/min), % RSD.	3.0% Max n=3	0.953	0.399	0.216	1.963	0.179
(b) Change in flow rate (0.9 mL/min), % RSD.		2.352	0.494	0.585	1.846	0.676
(c) Change in wavelength (208 nm), % RSD.		1.747	1.379	0.054	2.577	0.734
(d) Change in wavelength (212 nm), % RSD.		1.336	1.418	0.101	0.040	0.594

Table 5: Vitamin quantification results in Formulated Food Supplement Sample.

Formulated Food Supplement Sample	B1%	B2%	B3%	B5%	B6%
	0.0075	0.0018	0.0493	0.0140	0.0214

Table 6: Vitamin quantification results in Market Samples.

Sample	B1%	B2%	B3%	B5%	B6%
Sample 1 (Brand-A)	0.00405	0.00385	0.052	0.0041	0.0023
Sample 2 (Brand-B)	0.00473	0.00489	0.05	0.0043	0.0026
Sample 3 (Brand-C)	0.0034	0.00379	0.044	0.0022	0.0016

vitamin and by comparing with standard UV spectra. The peak purity angles of all the peaks were found to be lower than their respective peak threshold, confirming the purity of peaks.

Vitamins quantification in Formulated food sample and in Market sample are shown in Tables 5 and 6 respectively.

DISCUSSION

This analytical technique is validated according to ICH guidelines Q2(R1) for all the criteria. The developed method can elute all 08 vitamins B1, B3, B6, B5, B9, B12 B2 and B7 as shown in Figure 1. However, in the sample due to absence of vitamin B7, B9 and B12, the rest of the vitamins validation was carried out. The formulated food sample as well as market samples were tested as per the procedure mentioned earlier at the concentration of 10 mg/mL as shown in Figure 2 for which values were found within 90-110%

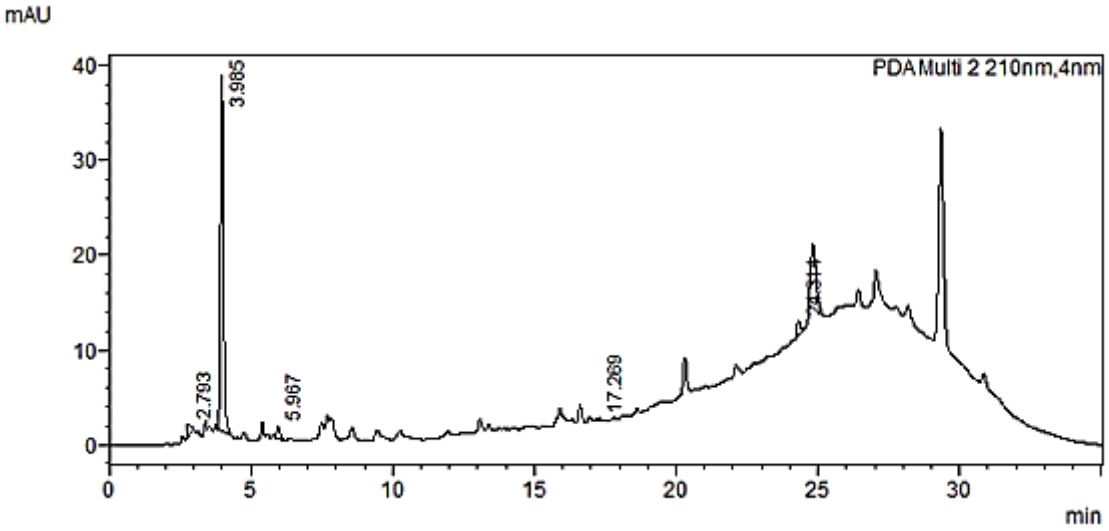

Figure 2: HPLC Chromatogram of vitamins (water soluble) in Formulated Food Supplement. B1 (RT 2.793 min) 2) B3 (RT 3.985 min) 3) B6 (RT 5.967 min) 4) B5 (RT 17.269 min) 5) B2 (RT 24.314 min).

of label claim. This demonstrated the applicability of proposed method for analysing the water-soluble B-Complex vitamins in different finished food products.

This research addressed the limitation of the available methods as we were able to analyse all vitamin B-complex in a single method at single wavelength. We have simplified the process of sample preparation by using only purified water as a diluent compared to complex sample preparation like solid phase extraction or derivatization. The current method has been developed and validated using the simple mobile phase that does not contain any buffer system, which makes the process time saving and extending column life compared to existing method. With recovery rates of 80-120%, the method ensures high accuracy in the food matrix. This research removes barriers to wider applications by demonstrating that the approach is applicable to a variety of food matrices and is not just restricted to designed food products. Stress degradation study can be explored further to improve the scope of the method in terms of stability indicating under various conditions.

CONCLUSION

The RP-HPLC method was successfully developed and validated for the determination of all B complex water-soluble vitamins. The method developed was validated by ICH guidelines in terms of accuracy, precision, linearity and specificity. This method shows good resolution between all vitamins in single run and single wavelength which makes it economical as multiple runs can be minimized. The food and pharmaceutical industries could benefit from this method's increased utility in the future by investigating additional optimization for other complicated matrices or extending its application to liquid and pharmaceutical samples.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

ABBREVIATIONS

HPLC: High Performance Liquid Chromatography; **UV-DAD:** Ultraviolet Diode Array; **FLD:** Fluorescence detector; **HPLC:** High Performance Liquid Chromatography; **MS/MS:** Mass Spectrometry/Mass Spectrometry; **RP:** Reversed Phase; **ELSD:** Evaporative Light Scattering Detector; **ICH:** International Council for Harmonisation; **HFD:** Health Food Drinks; **C_{18}:** Carbon 18; **mm:** Millimetre; **mg:** Milligram; **mL:** Millilitre; **GC:** Gas chromatography; **MPa:** Megapascal; **nm:** Nanometre; **µL:** Microliter.

REFERENCES

Abdelfatah, R. M., Abd Elhalim, L. M., Darwish, H. W., Ayoub, B. M., Tony, R. M., & Gamal, M. (2024). A stability: indicating HPLC assay of ten different vitamins in a food supplement: Appraisal of the method's greenness, whiteness and blueness. Talanta, 277, Article 126324. https://doi.org/10.1016/j.talanta.2024.126324

Antakli, S. A. A. D., & Sarkees. (2015). Determination of water: soluble vitamins B1, B2, B3, B6, B9, B12 and C on C18 column with particle size 3µM in some manufactured food products by HPLC with UV: DAD/FLD detection. N.A.Z.E.E.R.A. and Sarraf, T.E.R.E.Z.A. International Journal of Pharmacy and Pharmaceutical Sciences, 7(6), 219, 224.

Farag, A. M., & El Rizk, M. S. (2019). Fast and efficient RP: HPLC method for simultaneous determination of water: soluble vitamins in some nutraceutical supplements. Bassel, H.A. and Youssif, M.H. Egyptian Journal of Chemistry, 62(12), 2195, 2211.

Fatima, Z., Jin, X., Zou, Y., Kaw, H. Y., Quinto, M., & Li, D. (2019). Recent trends in analytical methods for water: soluble vitamins. Journal of Chromatography. A, 1606, Article 360245. https://doi.org/10.1016/j.chroma.2019.05.025

Gómez, A., & Garballo, A., Morales, J. C., and García: Ayuso, L. E. (2006). Simultaneous determination of eight water: soluble vitamins in supplemented foods by liquid chromatography. Zafra. Journal of Agricultural and Food Chemistry, 54(13), 4531, 4536.

Hosain, M. Z., Islam, S. M. S., & Kamal, M. M. (2021). Development of a rapid and reliable high: performance liquid chromatography method for determination of water: soluble vitamins in veterinary feed premix. Veterinary World, 14(12), 3084–3090. https://doi.org/10.14202/vetworld.2021.3084-3090

Klejdus, B., Petrlová, J., Potěšil, D., Adam, V., Mikelová, R., Vacek, J., and Kubáň, V. (2004). Simultaneous determination of water: and fat: soluble vitamins in pharmaceutical preparations by high: performance liquid chromatography coupled with diode array detection. Analytica Chimica Acta, 520(1: 2), 57, 67.

Kucukkolbasti, S., Ires, N., & Kara, H. (2013). Development of method to simultaneous determination of some water and fat soluble vitamins in feeding additives. Journal of Selçuk University Natural and Applied Science, 1(4), 30.

Li, H. B., & Chen, F. (2001). Simultaneous determination of twelve water:and fat: soluble vitamins by high:performance liquid chromatography with diode array detection. Chromatographia, 54(270), 273.

Mateeva, A., Kondeva: Burdina, M., Peikova, L., Guncheva, S., Zlatkov, A. and Georgieva, M. (2023). Simultaneous analysis of water: soluble and fat: soluble vitamins through RP: HPLC/DAD in food supplements and brewer's yeast. Heliyon, 9(1).

Papadoyannis, I. N., Tsioni, G. K., & Samanidou, V. F. (1997). Simultaneous determination of nine water and fat soluble vitamins after SPE separation and RP: HPLC analysis in pharmaceutical preparations and biological fluids. Journal of Liquid Chromatography and Related Technologies, 20(19), 3203, 3231.

Saptarini, N. M., Ratriningrum, S. M., & Holik, H. A. (2022). Simultaneous analysis method of ten water: soluble vitamins with high: performance liquid chromatography in fish feed additives. Rasayan Journal of Chemistry, 15(3), 1735–1743. https://doi.org /10.31788/RJC.2022.1536990

Temova Rakuša, Ž. T., Grobin, A., & Roškar, R. (2021). A comprehensive approach for the simultaneous analysis of all main water: soluble vitamins in multivitamin preparations by a stability: indicating HPLC: DAD method. Food Chemistry, 337, Article 127768. https://doi.org/10.1016/j.foodchem.2020.127768

Trang, H. K. (2013). Development of HPLC methods for the determination of water: soluble vitamins in pharmaceuticals and fortified food products (Master's thesis, Clemson University).

Wazed, M. A., Hasan, M., Halim, M. A., Akhtaruzzaman, M., Ahmed, M., & Mozumder, N. R. (2022). Quantitative analysis of vitamins B2, B3, B5 and B6 by HPLC: UV in selected non: leafy vegetables. International Journal of Vegetable Science, 28(5), 493, 499.

Zia, H., Fischbach, N., Hofsommer, M., & Slatnar, A. (2023). Development and validation of HPLC:MS/MS method for simultaneous analysis of B vitamins present naturally or after fortification in fruit juices. LWT, 184, Article 115103. https://doi.org /10.1016/j.lwt.2023.115103

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