INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

DOI: https://dx.doi.org/10.33289/IJRPC.12.1.2022.12(1)

A NOVEL RP-HPLC-DAD METHOD FOR THE SIMULTANEOUS ESTIMATION OF NINE WATER-SOLUBLE VITAMINS IN A PREMIX

M. Devendra Reddy*, Sundaram Ramachandran, Prakash Neswi Shivappa, Mohammed Azher, Rajendra Bojanala and Ilavarasu Arumugam

R& D Centre, Himalaya Wellness Company, Makali, Tumkur Road, Bangalore- 562 162, Karnataka, India.

ABSTRACT

The study was aimed to develop and validate a simple and rapid high-performance liquid chromatographic method for the simultaneous estimation of nine water-soluble vitamins (B_1, B_2, B_3, B_5, B_6, B_7, B_9, B_{12} and C) in a vitamin premix. The analysis was carried out on a C18columnusing a mobile phase consists of 25 mM sodium dihydrogen orthophosphate dihydrate in purified water (mobile phase A) and the mobile phase B consists of 950 ml of methanol and 50 ml of mobile phase A. The gradient elution was employed for the separation, the flow rate was maintained at 1.0 ml/minute. Ultraviolet detector at 210 nm was used to detect all the vitamins except vitamin B_{12} which was detected at 361 nm. The column temperature of 50°C and sample cooler temperature of 10°C were also maintained. Conclusively the method facilitated a good separation of nine water-soluble vitamins with superior performance characteristics and optimal system suitability characters. The selectivity, repeatability, intermediate precision, linearity, accuracy, robustness, standard and sample solution stability were performed as part of the method validation. The method validation parameters are found complying as per the acceptance criteria. Due to the high selectivity, the method shall be applied to a variety of commercial products including functional foodsfor the estimation of water-soluble vitamins.

Keywords: Water soluble vitamins, HPLC, Method development and Method validation.

INTRODUCTION

The World Health Organization (WHO) reinforces that proper dietary intake of vitamins arevital, as thevitamins are essential for normal growth, development, and health[1]. Their determination is of interest to biochemistry, pharmaceuticals and the food sciences. High Performance Liquid Chromatography (HPLC) was successfully applied by either gradient or isocratic elution techniques for the separation of vitamins in complex matrixes like food samples and human serum.

Vitamins are broadly distributed in natural food sources and can be easily introduced into the diets to persuade daily needs. Though vitamins are a group of organic compounds that have different structural and chemical properties, based upon their solubility they can be suitably categorized into two classes: water-soluble and fat-soluble vitamins. Water-soluble vitamins consisted of vitamin C and 8 B complex vitamins, namely thiamin (B1), riboflavin (B2), niacinamide (B3), pantothenate (B5), pyridoxine (B6), biotin (B7), folate (B9) and cyanocobalamin (B12) whereas the latter is composed of A, D, E, and K. Water-soluble vitamins are readily excreted in urine without considerable storage, so everyday consumption becomes crucial[2]. All water-soluble vitamins are useful as coenzymes or cofactors, supporting energy producing reactions and in the activity of important enzymes to progress normally[3,4]. Deficiencies can cause health problems and illnesses such as megaloblastic anemia, beriberi, and

pellagra due to their various specific functions. The requisite levels of vitamins are normally provided by a well-balanced diet. However, sufficient supplementation is required in the event of insufficient intake or elevated requirements[5,6].For these reasons, most commonly, multivitamin products are used in different dosage forms, available as medications or dietary supplements. The supply of B complex vitamins and vitamin C depends on diet; nevertheless, even foods that contain the essential water-soluble vitamins can have vitamin content in lesser quantity after processing, storage or cooking. Therefore, many people take B complex tablets or foods enriched or fortified with water-soluble vitamins to supplement their diet. Thus, the quality control assay plays a vital role to ensure that the labeled amount of nutritional facts is present in the formulations.

It is challenging to develop a single universal method for their simultaneous determination which is complicated by many factors like diverse chemical structures and properties of vitamins[7]. There are many instrumental methods available for the determination of B complex vitamins and vitamin C including capillary electrophoresis[8], electrochemical methods[9,10], normal phase and reversed phase TLC[11], spectrophotometry[12], derivative UV spectrophotometry[13], and spectrofluorimetry[14]. However, HPLC is considered as a selective and suitable technique for the simultaneous analysis of B complex vitamins and vitamin C. There are many research articles dealing with the determination of water-soluble vitamins, however there are fewer published research studies about simultaneous method development and validation of nine water-soluble vitamins by simple HPLC technique.

Several LC-MS, Capillary electrophoresis, electrochemical and TLC methods are reported as an alternate to HPLC-DAD methods for the systematic assessment of water-soluble vitamins[15-25].Nevertheless, LC-MS approaches are expensive, thus applying the same for the regular research or stability studies are becoming difficult.

This study also hypothesized that all main water-soluble vitamins which are commonly found in multivitamin products can be evaluated simultaneously by appropriately developed chromatographic technique.

EXPERIMENTAL

CHEMICALS AND REAGENTS

Reference standards of Vitamin B1 (Purity: 100%), B2 (Purity: 100%), B5 (Purity: 99.6%), B6 (Purity: 100%), B7 (Purity: 99.0%), B9 (Purity: 97.0%) was purchased from Sigma Aldrich. The reference standard Vitamin B3 (Purity: 99.78%) was purchased from Veer Chemie& Aromatics Private Ltd. The reference standard Vitamin B12 (Purity: 98.0%) and Vitamin C (purity: 99.7%) was purchased from Supelco and Rankem, respectively. Sodium dihydrogen orthophosphate dihydrate (HPLC grade), orthophosphoric acid (AR grade),methanol (HPLC grade) and sodium carbonate (AR grade) were procured from Rankem. A multivitamin premix containing eight B-group vitamins and vitamin C is supplied by Global Calcium Private Limited.

Instrumentation and Equipment

Separation was done by using Shimadzu NexeraX2i HPLC system equipped with a reverse phase Phenomenex Luna C18 column (250 x 4.6 mm, 5 µm particle size), LC-30AD low pressure gradient pump, a 100 µl injection loop, a SIL-30AC auto sampler, CTO-20AC column oven, DGU-20A5R degasser, SPD-M20A PDA detector and running on Shimadzu Chromatographic Software version 6.40 SP1. Mobile phases were degassed using solvent degasser andaconsort pH meter was used for pH adjustments.

Standard Preparation

The aqueous stock solutions of B complex vitamins were prepared by solubilizingthe respective vitamins in purified water. Vitamin B_9 solution was prepared by dissolving in 10 ml of 1.0% sodium carbonate solution followed by purified water. All solutions were prepared in amber color volumetric flasks and stored in a refrigerator to prevent the vitamins from photo oxidation. Working standard solutions were prepared from the stock solutions andthe final concentration of each vitaminsare as follows: B_1 (0.01 mg/ml), B_2 (0.008 mg/ml), B_3 (0.03 mg/ml), B_5 (0.05 mg/ml), B_6 (0.01 mg/ml), B_7 (0.005 mg/ml), B_9 (0.003 mg/ml), B_{12} (0.002 mg/ml) and C (0.5 mg/ml).

Sample solution preparation

Vitamin premix sample was homogenized with a trituration and 100mg of sample was weighed into a100 ml volumetric flask. The sample was solubilized using purified water with the aid of sonication. The sample solution was diluted with purified water to obtain a final concentration of 1mg/ml.

Chromatographic conditions

The separation was achieved using Phenomenex Luna C18, 250x4.6mm, 5µm column. The mobile phase A consists of 25 mM sodium dihydrogen orthophosphate dihydrate in purified water with adjusted pH to 2.5usingortho phosphoric acid.Mobile phase B

consists of 950ml of methanol and 50ml of mobile phase A. The elution was programmed in a gradient mode as in Table 1. The detection wavelength was set as 210nm for all the vitamins except B12, which was monitored at 361nm. Column oven temperature was maintained at 50°C.10 µL of standard and sample solutions were injected through autosampler and the total analysis time was maintained as 40 minutes.

Validation of the test procedure

The objective of validating the analytical method is to prove that it is appropriate for its intended purpose. A variety of criteria must be explored in order to verify the methodological procedures as per the existing pharmaceutical regulatory requirements.

Selectivity, repeatability, intermediate precision, linearity, accuracy, robustness, standard and sample solution stability are selected as a validation element.

RESULTS

All eight B complex vitamins and vitamin C were well resolved and did not interfere with other vitamins and diluent which was depicted in Figure 1, Figure 2 and Figure 3.

System Suitability

System suitability was executed by injecting working standard mixture of water-soluble vitamins in six replicates and the parameters like retention time, theoretical plates (N), peak asymmetry (As), & resolution were studied (Table 2). The results are found complying with the acceptance criteria as per USP 621.

Specificity

The Specificity was performed by injecting diluent (blank), working standard and sample preparation. The interference (if any) at the retention time of analyte was checked. It is established based on resolution factor (Table 2), peak purity index (Table 3).

System precision

System precision was executed for working standard solution by injecting five injections from the single preparation. The average area under curve (AUC) and percentage of relative standard deviation (%RSD) was recorded (Table 4).

Intraday precision (Repeatability)

The intraday precision of the method was checked by injecting three replicates of three different concentrations at 50%, 100% and 150% of proposed concentration of sample respectively on the same day. The mean assay and % RSD values were calculated (Table 5).

Interday precision

The inter day precision of the method was checked by injecting three replicates of three different concentrations at 50%, 100% and 150% of proposed concentration of sample respectively on the next day of intraday precision. The mean assay and % RSD values were calculated (Table 6). The comparison of %RSD between intra and inter day precision was recorded and tabulated (Table 7).

Linearity of assay

Linearity was performed by preparing five different concentrations of the sample ranging from 50% to 150% of proposed concentration (1mg/ml). The regression coefficient (R^2) was recorded and tabulated (Table 8).

Accuracy

It can be defined as exactness of an analytical method or the closeness of results. The accuracy was derived once precision, linearity and specificity have been established (option “c” as per the ICH guideline Q2 R1 for validation).

Robustness

Reliability of the method was done with respect to deliberate variations in method parameters like variation in the flow rate of mobile phase (±0.1 ml/min), variation in column temperature (±5°C) and change in buffer pH (±0.1)

The sample, standard and diluent was analyzed for small changes in method parameters by injecting two replicates of single preparation.

Solution Stability studies

Stability of the standard and sample solutions are established as a part this study. The solutions are found stable for at least 48 hours with %RSD values of not more than 15 (Table 10). This study results allows the usage of prepared standard and sample solutions up to 48 hours.

DISCUSSION

The study we have reported elucidates an analytical method and validation for the simultaneous detection and quantification of nine water-soluble vitamins in a vitamin premix. There are many research studies dealing with the determination of water-soluble vitamins, though there are fewer published studies about simultaneous method development and validation of nine water-soluble vitamins by a simple HPLC technique.

The optimal system suitability and validation criteria was achieved, the method shall be applied for the estimation of water-soluble vitaminsto a variety of commercial products including functional foods. The chromatographic conditions like buffer pH, column oven temperature and cooler temperature was identified as a critical parameter to uphold the better separation and solution stability during the analysis.

CONCLUSION

A simple, sensitive, precise, robust and reliable HPLC method was developed for the estimation of nine water-soluble vitamins in multivitamin premix. The compounds were individually characterized against individual authentic commercially available standards using retention time and UV spectral characteristics. The method was fully validated demonstrating its suitability, repeatability, linearity as per the ICH Q2 R1 guidelines for the validation of analytical methods and the results are complying as per the acceptance criteria. The method also demonstrated vitamin solution stability. This method can be employed in routine, stability indicating analysis where multivitamin analysis is necessary and low concentrations of vitamins are anticipated.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGMENT

Authors thanks to R & D Centre, Himalaya Wellness Company, Bangalore for the facilities offered.

Table 1: Gradient program

Time (Minutes)	Mobile phase (B %)	Mobile phase (A %)
0.01	00	100
6.00	00	100
25.00	35	65
30.00	35	65
33.00	00	100
40.00	00	100
40.01	Stop	-

Table 2: System suitability testing for method

Name of the vitamin	Retention time (minutes)	Theoretical plates	Peak asymmetry	Resolution
Vitamin B1	3.00	53416	1.33	----
Vitamin C	4.17	90654	1.06	8.4
VitaminB3	5.51	102797	1.15	8.3
VitaminB6	7.66	118802	1.03	10.5
VitaminB5	17.98	1045614	1.01	50.1
VitaminB9	22.80	1682479	1.06	26.5
VitaminB12	23.35	1683247	1.06	-----
VitaminB7	24.84	1690151	1.04	10.7
VitaminB2	26.34	1859842	1.05	7.4

Table 3: Peak purity index of the method

Name of the vitamin	Standard		Sample	
	Peak purity index	Single point Threshold	Peak purity Index	Single point threshold
Vitamin B1	1	0.999	0.999	0.999
Vitamin C	1	1	1	1
VitaminB3	1	0.999	1	1
VitaminB6	0.999	0.999	1	0.999
VitaminB5	0.999	0.999	1	0.999
VitaminB9	0.999	0.999	0.999	0.999
VitaminB12	0.999	0.999	0.999	0.999
VitaminB7	0.999	0.999	0.999	0.990
VitaminB2	1	0.999	0.999	0.999

Table 4: System precision of the method

Name of the vitamin	% RSD
Vitamin B1	0.08
Vitamin C	0.06
VitaminB3	0.06
VitaminB6	0.07
VitaminB5	0.04
VitaminB9	0.13
VitaminB12	0.39
VitaminB7	1.16
VitaminB2	0.11

Table 5: Intraday precision of method

Name of the vitamin	Mean assay (%w/w)	% RSD
Vitamin B1	0.83	4.86
Vitamin C	55.11	2.97
VitaminB3	4.39	2.15
VitaminB6	0.86	3.28
VitaminB5	5.89	2.63
VitaminB9	0.20	2.60
VitaminB12	0.012	5.92
VitaminB7	0.076	8.62
VitaminB2	1.08	1.57

Table 6: Inter day precision of method

Name of the vitamin	Mean assay (%w/w)	% RSD
Vitamin B1	0.84	3.90
Vitamin C	54.47	1.58
VitaminB3	4.36	0.66
VitaminB6	0.91	1.80
VitaminB5	5.86	2.15
VitaminB9	0.25	1.55
VitaminB12	0.014	13.36
VitaminB7	0.060	3.20
VitaminB2	1.13	1.18

Table 7: Comparison of intraday and inter day precision

Name of the vitamin	%RSD
Vitamin B1	4.25
Vitamin C	2.39
VitaminB3	1.58
VitaminB6	3.01
VitaminB5	2.35
VitaminB9	2.39
VitaminB12	12.00
VitaminB7	13.93
VitaminB2	2.56

Table 8: Linearity of method

Name of the vitamin	Regression equation	Regression coefficient (R^2)
Vitamin B1	Y= 2,236.7444x - 7,164.5149	0.997
Vitamin C	Y= 71,430.301x - 170,328.420	0.999
VitaminB3	Y= 28,567.6944x + 4,569.7547	0.999
VitaminB6	Y= 8,391.1951x + 2,690.5656	0.999
VitaminB5	Y= 3,986.7750x + 551.8292	0.999
VitaminB9	Y= 1,298.8553x- 124.1468	0.999
VitaminB12	Y= 24.1662x - 30.9625	0.998
VitaminB7	Y= 62.6830x + 19.5548	0.998
VitaminB2	Y= 4,745.8637x + 3,596.4145	0.999

Table 9: Comparison between assay of robustness and intraday precision

Robustness variables	Results Assay (%w/w)	%RSD	Comparison % RSD between assay of robustness and intraday precision
Vitamin B1			
Change in Flow rate (0.9 ml/minute)	0.85	0.83	1.68
Change in Flow rate (1.1 ml/minute)	0.84	1.68	0.84
Column temp: 45°C	0.84	1.68	0.84
Column temp: 55°C	0.82	0.86	0.85
Buffer pH: 2.4	0.79	5.37	3.49
Buffer pH: 2.6	0.83	5.11	0.00
Vitamin C			
Change in Flow rate (0.9 ml/minute)	56.21	0.14	1.40
Change in Flow rate (1.1 ml/minute)	51.75	0.53	4.45
Column temp: 45°C	51.36	0.45	4.98
Column temp: 55°C	50.65	0.53	5.96
Buffer pH: 2.4	55.90	1.06	1.01
Buffer pH: 2.6	58.45	0.38	4.16
Vitamin B3			
Change in Flow rate (0.9 ml/minute)	4.35	0.81	0.82
Change in Flow rate (1.1 ml/minute)	4.37	0.65	1.14
Column temp: 45°C	4.38	0.65	1.30
Column temp: 55°C	4.47	0.63	2.74
Buffer pH: 2.4	4.54	0.62	3.84
Buffer pH: 2.6	4.53	0.78	3.68
Vitamin B6			
Change in Flow rate (0.9 ml/minute)	0.86	0.82	0.82
Change in Flow rate (1.1 ml/minute)	0.86	0.82	0.82
Column temp: 45°C	0.85	0.00	0.82
Column temp: 55°C	0.85	0.00	0.00
Buffer pH: 2.4	0.88	0.80	2.44
Buffer pH: 2.6	0.88	0.80	2.44
Vitamin B5			
Change in Flow rate (0.9 ml/minute)	6.14	0.81	2.94
Change in Flow rate (1.1 ml/minute)	5.87	0.72	0.24
Column temp: 45°C	5.91	0.72	0.24
Column temp: 55°C	5.89	0.72	0.00
Buffer pH: 2.4	5.81	0.85	0.97
Buffer pH: 2.6	5.77	0.86	1.46
Vitamin B9			
Change in Flow rate (0.9 ml/minute)	0.23	3.07	0.82
Change in Flow rate (1.1 ml/minute)	0.22	3.21	0.82
Column temp: 45°C	0.22	3.21	0.82
Column temp: 55°C	0.21	3.37	0.00
Buffer pH: 2.4	0.19	0.00	2.44
Buffer pH: 2.6	0.20	3.54	3.54
Vitamin B12			
Change in Flow rate (0.9 ml/minute)	0.012	0.00	5.46
Change in Flow rate (1.1 ml/minute)	0.012	0.00	5.46
Column temp: 45°C	0.012	0.00	5.46
Column temp: 55°C	0.013	5.46	0.00
Buffer pH: 2.4	0.013	5.46	0.00
Buffer pH: 2.6	0.013	5.46	5.46
Vitamin B7			
Change in Flow rate (0.9 ml/minute)	0.067	3.16	3.07
Change in Flow rate (1.1 ml/minute)	0.077	3.68	6.69
Column temp: 45°C	0.071	2.99	1.00
Column temp: 55°C	0.081	4.37	10.24
Buffer pH: 2.4	0.083	2.52	12.86
Buffer pH: 2.6	0.076	2.79	5.81
Vitamin B2			
Change in Flow rate (0.9 ml/minute)	1.11	0.64	1.93
Change in Flow rate (1.1 ml/minute)	1.08	1.31	0.00
Column temp: 45°C	1.10	0.64	1.30
Column temp: 55°C	1.08	0.00	0.00
Buffer pH: 2.4	1.16	0.61	5.05
Buffer pH: 2.6	1.16	0.61	5.05

Table 10: Stability of standard and sample preparation

Name of the vitamin	Standard preparation		Sample preparation	
	% RSD of Area (12 hours)	% RSD of Area (48 hours)	% RSD of Area (12 hours)	% RSD of Area (48 hours)
Vitamin B1	0.45	1.65	0.54	1.82
Vitamin C	0.51	2.91	2.95	7.26
VitaminB3	0.19	1.18	0.48	1.81
VitaminB6	0.21	0.83	0.76	2.62
VitaminB5	0.17	0.68	0.60	0.60
VitaminB9	0.36	1.66	0.91	2.51
VitaminB12	0.37	1.45	4.04	12.04
VitaminB7	1.80	7.21	7.56	13.56
VitaminB2	0.45	1.43	1.31	5.15

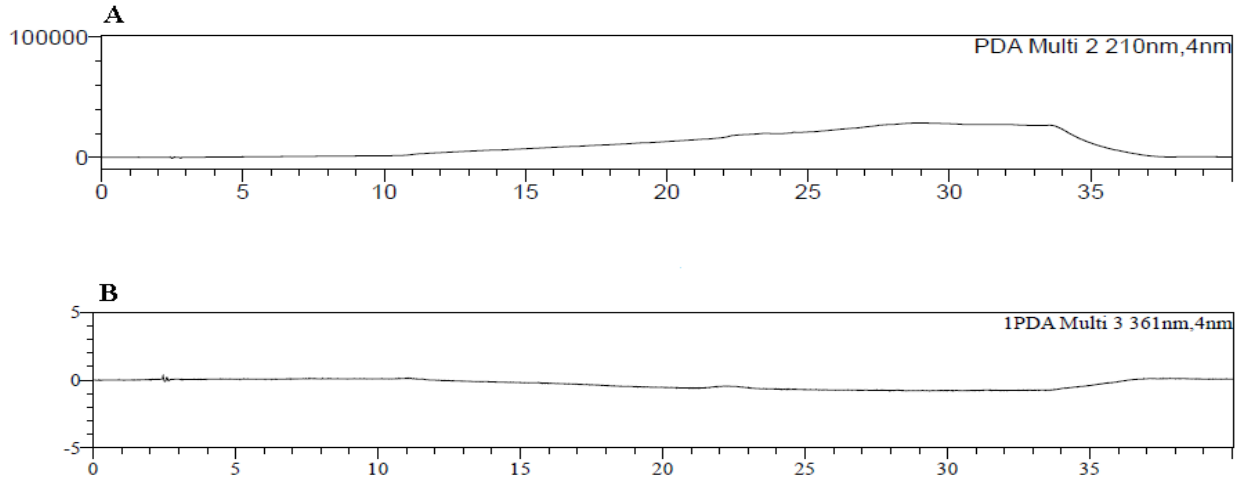

Fig. 1: Blank (water) chromatogram: A- Chromatogram of blank at 210 nm, B- Chromatogram of blank at 361 nm

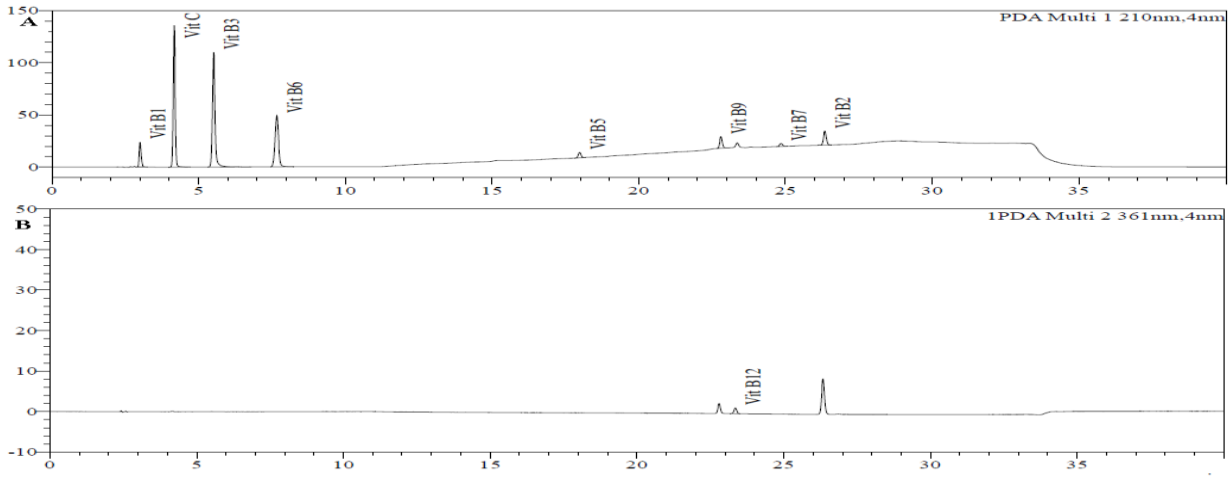

Fig. 2: Chromatogram of Standard: A- Chromatogram of 7 B-vitamins and vitamin C at 210 nm, B- Chromatogram of B_{12} at 361 nm

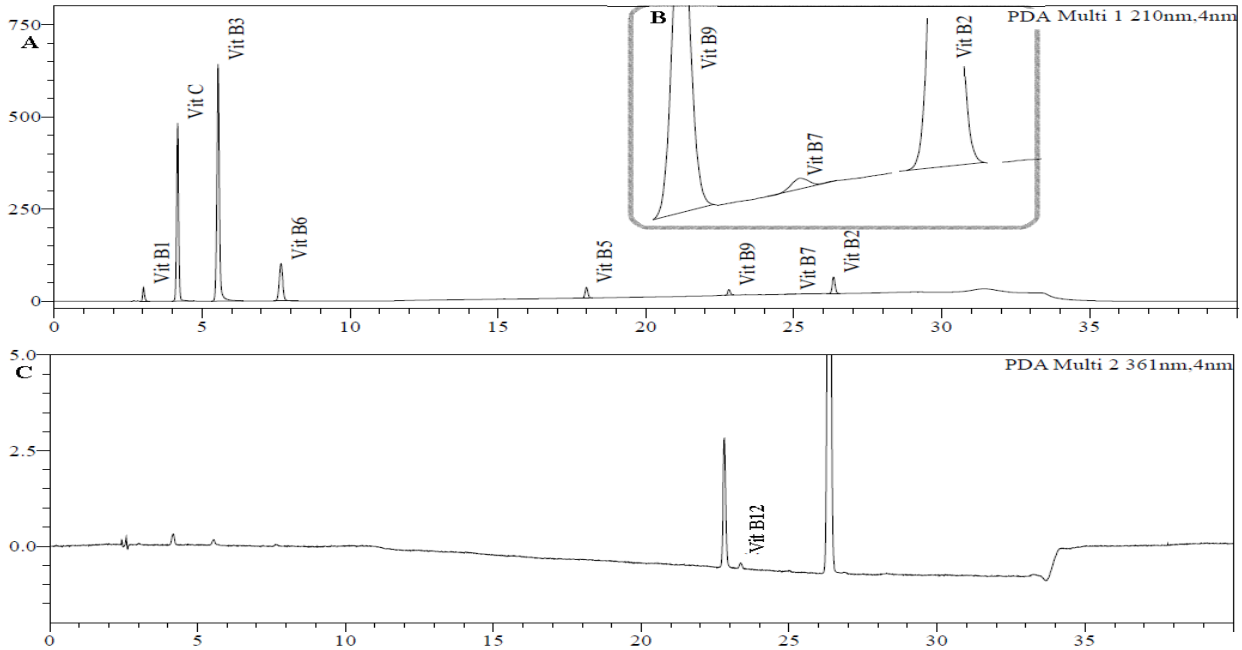

Fig. 3: Chromatogram of Sample: A- Chromatogram of 7 B-vitamins and vitamin C at 210 nm, B- B_7 peak at 210 nm with retention time of 24.84 minutes, C- Chromatogram of B_{12} at 361 nm.

REFERENCES

1. World Health Organization. Vitamin and mineral requirements in human nutrition. World Health Organization. 2004.
2. Combs Jr GF and McClung JP. The vitamins: fundamental aspects in nutrition and health. Academic press. 2016; Dec 15.
3. Miyake Y. Maternal B vitamin intake during pregnancy and childhood behavioral problems in Japan: The Kyushu Okinawa maternal and child health study. Nutritional neuroscience. 2020 Sep 1;23(9):706-13.
4. Simpson JL. Micronutrients and women of reproductive potential: required dietary intake and consequences of dietary deficiency or excess. Part I–folate, vitamin B12, vitamin B6. The Journal of Maternal-Fetal & Neonatal Medicine. 2010;23(12):1323-43.
5. Peechakara BV and Gupta M. Vitamin B2 (riboflavin). StatPearls [Internet]. 2020.
6. Rembe JD. Effects of vitamin B complex and vitamin C on human skin cells: is the perceived effect measurable? Advances in skin & wound care. 2018;31(5):225-33.
7. Trang HK. Development of HPLC methods for the determination of water-soluble vitamins in pharmaceuticals and fortified food products.
8. Dinelli G and Bonetti A. Micellar electrokinetic capillary chromatography analysis of water- soluble vitamins and multi- vitamin integrators. Electrophoresis. 1994;15(1):1147-50.
9. Škrovánková S, Mlček J, Sochor J, Baroň M, Kynický J and Juríková T. Determination of ascorbic acid by electrochemical techniques and other methods. International Journal of Electrochemical Science. 2015.
10. Antal IP. Electrochemical methods for determining group B complex. Journal of Analytical Chemistry. 2013;68(7):565-76.
11. Mohammad A. Amino acid and vitamin determinations by TLC/HPTLC. Review of the current state. Open Chemistry. 2012;10(3):731-50.
12. Ghasemi J and Abbasi B. Simultaneous spectrophotometric determination of group B complex using parallel factor analysis: PARAFAC. Journal of the Chinese Chemical Society. 2005;52(6):1123-9.
13. Aberásturi F. UV-visible first-derivative spectrophotometry applied to an analysis of a vitamin mixture. Journal

of Chemical Education. 2001;78(6):793.
14. Mohamed AM. Spectrofluorimetric determination of some water-soluble vitamins. Journal of AOAC International. 2011;94(6):1758-69.
15. Vidović S. Simultaneous determination of some water-soluble vitamins and preservatives in multivitamin syrup by validated stability-indicating high-performance liquid chromatography method. Journal of chromatography A. 2008;1202(2):155-62.
16. Chen P and Wolf WR. LC/UV/MS-MRM for the simultaneous determination of water-soluble vitamins in multi-vitamin dietary supplements. Analytical and bioanalytical chemistry. 2007;387(7):2441-8.
17. Rakuša ŽT. 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. 337:127768.
18. Kakitani A. Simultaneous determination of water-soluble vitamins in beverages and dietary supplements by LC-MS/MS. Food Additives & Contaminants: Part A. 2014;31(12):1939-48.
19. Sim HJ. A systematic approach for the determination of b-group vitamins in multivitamin dietary supplements by high-performance liquid chromatography with diode-array detection and mass spectrometry. Journal of AOAC International. 2016;99(5):1223-32.
20. Wang J. Simultaneous analysis of water-soluble vitamins in vitamin-enriched beverages and multivitamin dietary supplements by UHPLC-MS/MS. Thermo Scientific. 2000;294:1-5.
21. Simionato AVC. Separation of Water Soluble Vitamins by Micellar Electrokinetic Capillary Chromatography in Pharmaceutical Samples. Journal of liquid chromatography & related technologies. 2006;29(3):349-363.
22. Schreiner M. Determination of water soluble vitamins in soft drinks and vitamin supplements using capillary electrophoresis. Food/Nahrung. 2003;47(4):243-247.
23. Fotsing L. Multivariate optimization approach for the separation of water-soluble vitamins and related compounds by capillary electrophoresis. Biomedical chromatography: BMC. 2000; 14(1):10-1.
24. Fotsing L. Determination of six water-soluble vitamins in a pharmaceutical formulation by capillary electrophoresis. Journal of pharmaceutical and biomedical analysis. 1997;15(8): 1113-1123.
25. Delgado-Zamarreno M. Separation and simultaneous determination of water-soluble and fat-soluble vitamins by electrokinetic capillary chromatography. Journal of Chromatography A. 2002;953(1-2):257-262.