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RESEARCH ARTICLE

Quantitative Analysis of Thiamine, Riboflavin, and Niacin Vitamins by High-Performance Liquid Chromatography in Pharmaceutical Sample Tablets and Drops

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ABSTRACT

Levering cutting-edge high-performance liquid chromatography techniques with a C-18 column, we meticulously crafted a gradient of MeOH and glacial CH_3COOH (96–5%), pulsating through at a mesmerizing 1.2 ml/min. With ultraviolet detection at a precise wavelength of 245 nm, we unraveled the mysteries of these vital nutrients. Thiamine reveals itself at a retention time of 3.5 min, followed by Riboflavin at 8.1 min and Niacin at 10.8 min. The findings of this study were not just a spectacle of scientific powers but were also rigorously benchmarked against commercial drugs, ensuring accuracy and reliability in our analysis.

Keywords: High-performance liquid chromatography, Niacin, Riboflavin, Thiamine

Introduction

Vitamins are essential micronutrients that support human healthy by performing metabolic processes and preventing disease. They play a variety of functions, including hormone regulator, cellular signaling and differentiation mediators, and antioxidants.[1] These include the water-soluble vitamins B_1, B_2, and B_3, as well as thiamine, riboflavin, and niacin. Serious health conditions may result from a lack of these vitamins.[2]

Thiamine is essential for metabolism of carbohydrate as well as for the health of the immunological, neurological, muscular, and cardiovascular systems.[3] In contrast, riboflavin participates in several redox processes as a coenzyme.[4] It is a light-sensitive vitamin that helps produce niacin from amino acids.[3] Its functions include proliferation within biological systems, tissue regeneration, and oxidation-redaction reaction.[5]

Niacin is naturally synthesized in the body and transforms into nicotinamide adenine dinucleotide (NAD^+),[6] a essential component in several stages, including its reduction to nicotinamide adenine dinucleotide + hydrogen (H^+) (NADH). NAD and NADH are essential for the synthesis of cholesterol, fat, DNA and in energy production, and repair.[7] Due to there are many multivitamin, multidrug formulations and group B vitamin-based products from various manufacturers necessitates meticulous oversight

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over their production, quality control, shelf life, and medical use is required. The search for innovative analytical techniques for group B vitamin analysis across variety of substances has increased as a result.[8] HPLC is unique among these techniques due to its ease of use, precision, specificity, high sensitivity, and use of various detectors.[1,9] Given the variety of pharmacological effects of vitamins, it's imperative that the vitamin content of multivitamin formulations be subjected to strict quality control. The manufacture and storage instability of vitamin drugs highlight the significance of careful content monitoring in commercial formulations.[2] A key component of multivitamin analysis acknowledges that every vitamin has a distinct structure that effects its physicochemical characteristics, which frequently presents difficulties in simultaneous multivitamin mixture analysis.[10] This study aimed to developing a simple, gradient, stability-indicating reverse phase high performance liquid chromatography (HPLC) method to precisely measure essential vitamins thiamine (B_1), riboflavin (B_2), and niacin (B_3) in both tablet and liquid drop formulations of pharmaceutical drugs.

Experimental part

Equipment and reagents

HPLC Autosampler SyKNM from Germany S5200 pump model S1122 Column oven S4011, and a UV detector S3240 set at wavelength 254 nm with a C-18 column. The authors employed a hotplate stirrer of the LMS 1003 type from Daihan Labtech and an ultrasonic device (ultrasonicator) made in Germany for sample dissolution. The measurements were conducted using a Sartorius handy 4-digit analytical balance. Standard vitamins B1, B2, and B3 were sourced from Sigma, while the tablets and drops were obtained as commercial drugs. The remaining solvent and chemicals were procured from BDH.

Procedure

Preparation of standard vitamins

Creating a standard solution began with dissolving 10 mg of each of vitamins B_1, B_2, and B_3 in methanol to form a 100 parts per million (ppm) stock solution, diluted to 100 ml. This stock solution was further diluted to prepare other standard solutions by subsequent dilution of the stock solutions.

The preparation of standard tablet vitamin solutions of approximately 1000 parts per million (ppm) was made using vitamins from five different manufacturers. Each brand's single tablet of vitamins B1, B2, and B3 was ground and thoroughly mixed. A 100 mg sample of this mixture was dissolved in a small quantity of methanol, filtered, and then diluted to 50 ml with methanol.[11]

Analysis of samples

All the prepared drugs and standard vitamin solutions were chromatographically analyzed with a C-18 column, using methanol and glacial CH3COOH (95:5) isocratic mobile phase. The flow rate remained constant at 1.2 ml/min, and detection was performed at 245 nm using UV technology.[12]

Results and discussion

This study was carried out to analyze vitamins using HPLC. The vitamins B_1, B_2, and B_3 were chromatographed using the C-18 column (25 $*$ 0.4 cm). The effect of the changed percentage of mobile phase for separation vitamins was studied. The percentage results showed that 5% gives the best capacity factor Ḱ and separation factor α, as listed in Table 1. The Ḱ values for these three vitamins, B3, B1, and B2, ranged from 1.01–1.43, 1.42–1.99, 2.10–3.22 and 4.11–04.83 at 5%, 10%, 15% and 20%, respectively. The separation factor (α) values ranged from 1.19–1.18, 1.25–1.26, 1.09–1.21 and 1.24–1.29 at 5%, 10%, 15% and 20%, respectively. The capacity and separation factors for vitamins were evaluated across a range of acid percentages, varying from 5% to 25% in methanol. The findings suggest a strong competitive interaction between these vitamins and the stationary phase, highlighting the optimal performance achieved with the ideal mobile phase composition, with vitamin B_3 showing the weakest retention, while vitamins B_1 and B_2 exhibit progressively stronger interactions with the stationary phase.

Table 1. Variation of capacity (Ḱ) and separation (α) factors with changing mobile phase composition for vitamins.

	Percentage of Glacial acetic acid in the mobile phase									
	5%		10%		15%		20%		≤25%	
Compounds	Ḱ	α	Ḱ	α	Ḱ	α	Ḱ	α	Ḱ	α
Vitamin B_3	1.01	–	1.42	–	2.10	–	4.11	–	–	–
Vitamin B_1	1.21	1.19	1.64	1.25	2.76	1.09	4.44	1.24	–	–
Vitamin B_2	1.43	1.18	1.99	1.26	3.22	1.21	4.83	1.29	–	–

Table 2. Retention time (tr), capacity (Ḱ) and separation (α) factors, resolution, and peak asymmetry for vitamins using c-18 column, eluent gradient 5% glacial acetic acid-95% methanol, flow rate 1.0 ml per min and detection wavelength 245 nm.

Vitamins	Retention time (tr)	Capacity (Ḱ)	Separation factors (α)	Resolution	Peak asymmetry
Vitamin B_3	3.5	1.01	–		
Vitamin B_1	9.1	1.21	1.19	1.11	0.98
Vitamin B_2	10.96	1.43	1.18	1.23	1.01

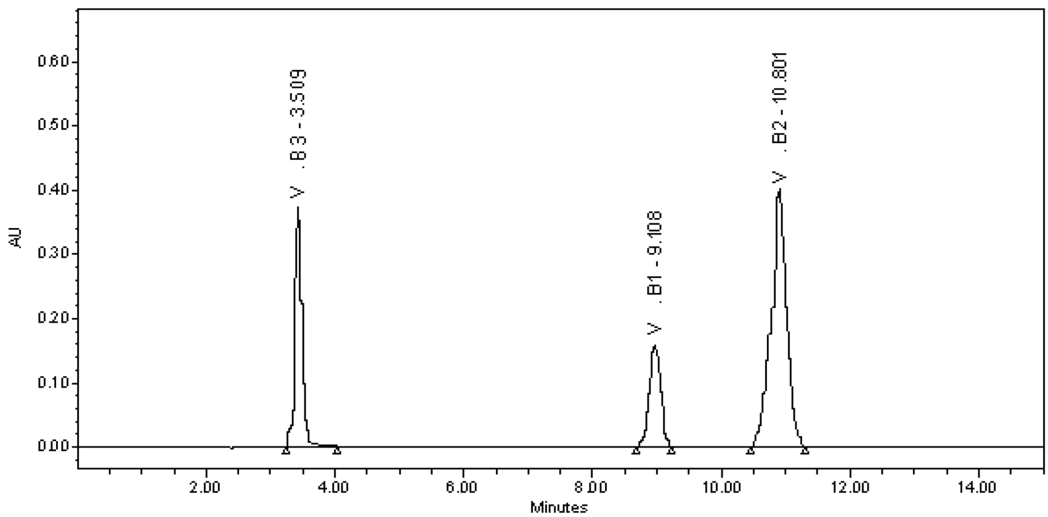

Fig. 1. Chromatograms of mixture standard of 2.0 ppm B_3, B_1, and B_2, C-18 column, eluent gradient 5% glacial acetic acid-95% methanol, flow rate 1.0 ml per min and detection wavelength 245 nm.

The separation factors and resolution values indicate effective separation, particularly between vitamins B_1 and B_2. The ideal mobile phase composition (5% acetic acid, 95% methanol) and flow rate contribute to achieving optimal performance, as shown by the effective separation and acceptable peak asymmetry values as show in Table 2.

Fig. 1 displays the chromatograms of vitamins B_3, B_1, and B_2 using a C-18 column, a 10 μl sample loop, and a flow rate of 1ml/min. The retention time sequence for these drugs on the column is $B_3 > B_1 > B_2$, indicating their respective interactions with the stationary phase, primarily driven by hydrogen bonding between their functional groups and the column's stationary phase. Gradient elution programming was employed to enhance the separation of these vitamins in the mixture. The quantitative analysis involved construction calibration curves for vitamins B_1, B_2, and B_3 on the C-18. The resulting linear calibration curves are presented in Table 3.

The slopes of the linear calibration curves using the C-18 column varied depending on the type of vitamins, ranging from 3450.6 to 3778.2. The correlation coefficient ranged from 0.9996 to 0.9998, and the detection limit spanned from 0.5 to 1.0 ppm.

Standard solutions were injected thrice under the same condition on the C-18 column to ensure accuracy. The %RSD values were 0.849% and 0.811%, with recoveries falling between 98.0% and 99.0%. Relative errors ranged from 1.00% to 2.0% for B_1, $B_{2,}$ and B_3, as detailed in Table 4.

The vitamins were determined in tablets and drops under the same column to analyse real solutions, as shown in Fig. 2. The recovery value for vitamins ranged from 96.24–99.77%, as in Table 5.

The commercial pharmaceutical market revolves around various forms of drugs, such as capsules, tablets, drops, powders or granules. There is an ongoing demand to create new or improve existing analytical methods for efficient analysis of

Table 3. Linear equation, correlation coefficient, and detection limits for vitamins.

Vitamins	Linear equation	Concentration range	R	Detection limit
Vitamin B_3	Y = 3778.2x–274.8	0.5–100	0.9996	0.5
Vitamin B_1	Y = 3450.6x–262.2	0.5–100	0.9996	0.5
Vitamin B_2	Y = 3567.4x–254.1	0.5–50	0.9998	1.0

Table 4. Recovery and percentage of relative error of vitamins eluted on column using gradient eluent 5% glacial acetic acid.

Vitamins	Concretion injected (mg/ml)	Concentration founds (mg/ml)	Recovery	Relative error%
Vitamin B_3	100	99.94	98.5	1.5
Vitamin B_1	100	99.97	99.0	1.0
Vitamin B_2	20	19.91	98.0	2.0

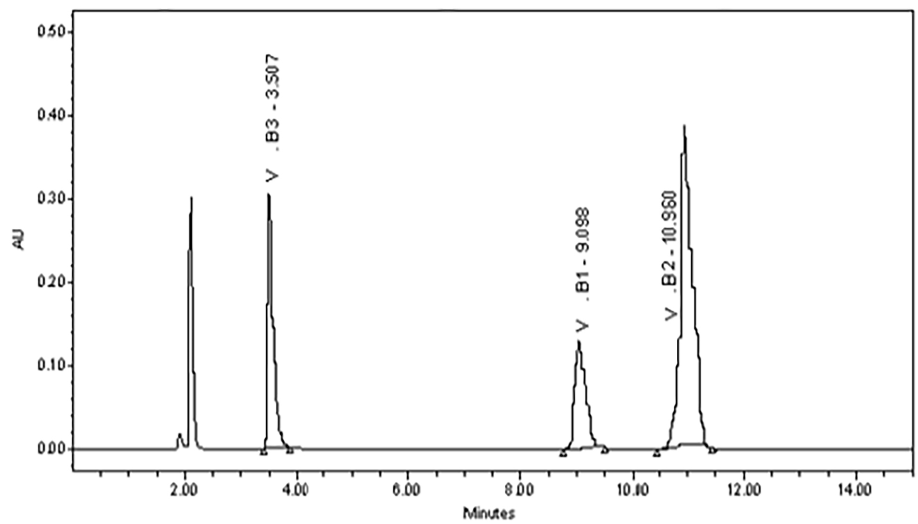

Fig. 2. Chromatograms of real samples B_3, B_1, and B_2, C-18 column, eluent gradient 5% glacial acetic acid- 95% MeOH, flow rate 1.0 ml/min and detection wavelength 245 nm.

Table 5. Analysis of vitamin B_1 (100 mg), vitamin B_2 (100 mg) and vitamin B_3 (20 mg) in tablets using the same conditions of procedure.

Types of drugs	Vitamin B_1	Vitamin B_2	Vitamin B_3
Tablets			
Wt.(mg) injected	100.00	100.00	20.00
Wt.(mg) calculated	94.98	99.95	19.95
Recovery %	99.25	99.77	99.77
Drops			
Wt.(mg) injected	50.00	50.00	2.00
Wt.(mg) calculated	48.84	49.78	1.98
Recovery %	96.24	98.28	98.28

multi-component solid mixtures. This has seen growth with continuous development and evolution in spectroscopic methods. Recent trends show a shift from traditional transmission measurement to various reflection techniques, gaining more popularity.

Accurate measurement of multivitamin in biological fluids, foods, and tablets is crucial due to their nutritional importance, making it a significant area of research. In literature, HPLC coupled with DAD or ultraviolet (UV) is widely utilized for developing methods to analysis multivitamin,[13] Porada et al. presented an eco-friendly sensor-based electroanalytical method to quantify vitamins B_9, B_2, B_3, and B_{12} in various food samples,[14] while Zhang et al. extensive review on methods for quantifying vitamins in food,[3] most documented HPLC coupled with UV/DAD methods do not encompass the complete range of major B vitamins commonly present in natural food or used for fortification purposes.[2] Additionally, Bakhsh et al., discussed a simultaneous volumetric technique for determining vitamin C and B_6 using modified electrodes in sample of food.[15] Sasaki and Hosain successfully used HPLC to determine water-soluble vitamins in food supplements and premix of veterinary feed, respectively.[16,17] These studies collectively demonstrated the feasibility and reliability of HPLC-based methods for analyzing B complex vitamins in various sample type.

Researchers have often employed phosphate or acetate buffers in various studies to regulate the mobile phase's pH, as documented.[18] However, this approach comes with a compromise regarding effective chromatographic separation of water-soluble vitamin analysts, such as vitamin C, vitamin B_1, and vitamin B6; running the analysis using a significantly high percentage of aqueous mobile phase becomes necessary. Poongothai et al. analyzed B_1 in multivitamin capsules using liquid chromatography-UV detection by pretreated simple with ultrasonic-assisted extraction (UAE) filtration.[19] Bendryshev et al. and Patil et al. analyzed water-soluble vitamins in vitamin tablets using HPLC_UV, where the sample was pretreated with UAE. However, this high salt content can harm the integrity of the HPLC system, leading to significant silting of columns and tubing.[20,21] Additionally, methods employing these buffer salts are incompatible with mass spectrometry detectors due to the non-volatile nature of these salts.[8] The result in this study indicate faster run times and efficient comparison with pervious methods[22] that take over 30 min to separate riboflavin while in this method takes only 8 min to separate it, so it cost-effective for routine pharmaceutical analysis. Also the method in current study can analyzed both liquid and solid formulations, providing a broder applications compared to some methods that focus on just one type formulation.[8,17,23]

Conclusion

The current study successfully employed advanced reverse phase HPLC-UV method, utilizing a meticulously optimized gradient of MeOH and glacial acetic acid (95–5%). Method in this study has been meticulously crafted to precisely measure essential vitamins B_1, B_2, and B_3 in both tablet and liquid drop formulations of pharmaceutical drugs. This method simplifies the process and ensures the stability and integrity of current study analysis findings.

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Authors' declaration

- Conflicts of Interest: None.
- We hereby confirm that all the tables in the manuscript are ours.
- No animal studies are present in the manuscript.
- No human studies are present in the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at University of Technology.

Authors' contribution statement

S.A.A and N.M.A contributed to conceptualization and design of the study, and performed critical revision of manuscript, S.A.I Investigation, resources, data validation and visualization, N.M.A conducted the chromatography assay, method optimization, and review of results. A.S.A did the sample preparation, and assisted in the manuscript final writing and editing, M.Y.H participated in experimental design, drafted the initial manuscript, and contributed to data analysis. All authors read and approved the final manuscript.

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التحليل الكمي للفيتامينات الثيامين و الرايبوفلافين والنياسين بواسطة كروماتوغرافيا السائلة عالية الاداء في اقراص وقطرات العينات الصيدلانية

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الخلاصة

بالاستفادة من تقنيات كروماتوغرافيا السائلة عالية الاداء مع العمود C-18 قمنا بتطوير تدرج تدقيق من الميثانول وحامض الخليك الثلجي (96-5%) كطور متحرك. حيث تم تمريره بمعدل يبلغ 1.2 مل في الدقيقه. وباستخدام الكشف بواسطة الاشعة فوق البنفسجية بطول موجي 245 نانوميتر كشفنا عن العناصر الغذائية المهمه حيث تم الكشف عن الثايمين في زمن احتباس 3.5 دقيقه يليه الرايبوفلافين عند 8.1 دقيقه والنياسين عند 10.8 دقيقه. لم تكن النتائج التي توصلنا اليها مجرد مشهد علمي قوي وانما تم قياسها بدقة مقارنة بالادوية التجارية مما يضمن الدقة والموثوقية في تحليلنا.

الكلمات المفتاحية: كروماتوغرافيا السائلة عالية الاداء، النياسين، الرايبوفلافين، الثايمين.