Investigation of Retention Mechanism of B_2 and B_3 Vitamins in Home-Made ZIC-HILIC Columns and Its Application for The Simultaneous Estimation in Multivitamin Tablets

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الخلاصة

في الدراسة الحالية، تم فحص سلوك الاحتجاز لإثنين من الفيتامينات (فيتامين ب2 و ب3) باستخدام تقنية كروماتوجرافيا السائل للتفاعل المحب للماء zwitterionic (ZIC-HILIC) على عمودين محليين الصنع، ونتيجة لذلك تم إنشاء طريقتين تحليليتين لهذه الفيتامينات. تم التحقيق بدقة في العديد من المعلمات الكروماتوغرافية وتحسينها. تم انجاز أفضل فصل على عمودين جديدين محليين الصنع (ZIC-1 و ZIC-3) باستخدام محلول الأسيتونيتريل/خلات الصوديوم كطور متحرك (40 ملي مولار، درجة الحموضة 4.75). تم الكشف عن الفيتامينات المستهدفة عند 254 nm. في أعمدة ZIC-1 و ZIC-3، كانت النطاقات الخطية للفيتامينات المستهدفة (ب2 و ب3) 0.06-11.0 و 0.08-7.0 ميكروغرام/مل على التوالي. لكل من الفيتامينات المستهدفة، كانت حدود الكشف (LOD) والتقدير (LOQ) للطرق المقترحة 0.011-0.055 و 0.033-0.166 ميكروغرام/مل، على التوالي. كما أظهروا دقة عالية (RSD ≤ 0.93) وخطية (r^2 ≥ 0.9988) واستعادية (بين 99.0 و 101.71%). تم التحقق من صحة الإجراءات المقترحة واختبارها على أقراص الفيتامينات، مما يثبت أنها مناسبة لتحليل المكملات الغذائية لهذه الأقراص ، وتم الحصول على نتائج مقبولة للدراسة.

Abstract

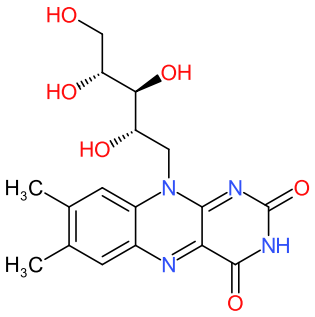
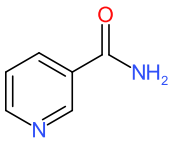
In the present study, the retention behavior of two vitamins (B_2 and B_3 vitamins) was investigated using a zwitterionic hydrophilic interaction liquid chromatography technique (ZIC-HILIC) on two home-made columns, and two analytical methods for these vitamins were established as a result. Several chromatographic parameters were carefully investigated and optimized. The best separation was achieved on two new home-made columns (ZIC-1 and ZIC-3) employing acetonitrile/sodium acetate buffer (95:5) v/v as the mobile phase (40 mM, pH 4.75). The target vitamins were detected at 254 nm. In the ZIC-1 and ZIC-3 columns, the linear ranges of the target vitamins (B_2 and B_3) were 0.06-11.0 and 0.08-7.0 µg/mL, respectively. For both target vitamins, the detection and quantitation limits of the suggested methods were 0.011-0.055 and 0.033-0.166 µg/mL, respectively. They also showed high precision (RSD ≤ 0.93), linearity (r^2 ≥ 0.9988), and recovery (between 99.0 and 101.71%). The suggested procedures have been validated and tested on multivitamin tablets, proving that they are suitable for analyzing food supplements for these tablets, and acceptable results were obtained for the study.

Keywords: riboflavin, vitamin B_2, nicotinamide, vitamin B_3, HILIC.

1. Introduction

Riboflavin (vitamin B_2) is a water-soluble vitamin essential for metabolism and energy production since it is the only source of the coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) in humans (1). Riboflavin participates in essential oxidation-reduction reactions in the form of coenzymes (2). Niacin (vitamin B_3) is an essential water-soluble vitamin that can be present in foods as either nicotinic acid or nicotinamide, the latter of which is the most commonly employed form for enriching foods for dietary supplementation (3). Because these vitamin forms are reciprocally converted in the body, their effectiveness is similar, but their effects and uses are different. As a precursor of NAD^+/NADH and $NADP^+$/NADPH, vitamin B_3 plays an important metabolic role in living cells. Vitamin B_3 in the form of dinucleotides is essential for energy metabolism in the body, including oxidative phosphorylation, protein, fat, and carbohydrate metabolism (4). Multivitamin tablets are a common and effective option for humans to avoid or delay a variety of illnesses caused by vitamin deficiency (5). Therefore, it is important to develop an effective analysis method for monitoring the quality of these commercial multivitamin tablets. **Table 1** summarizes the physicochemical characteristics and chemical structure of the selected vitamins (6).

Table 1: **The physicochemical properties and structure of the targeted vitamins**

Vitamin name	Structure	$LogP_{ow}$	pK_a	IP
B_2 (riboflavin)		-0.917	5.97	3.36
B_3 (nicotinamide)		-0.045	3.9	4.47

The lack of polar molecule retention in the traditional reversed-phase (RP) mode led to the development of the relatively new chromatographic technique known as hydrophilic interaction liquid chromatography (HILIC) (7). Water-soluble vitamins can be difficult to separate using RP; they are highly polar molecules with varying extents of hydrophilicity and, as a result, can be poorly retained utilizing RP separation mechanisms (8). The HILIC stationary phase is a polar stationary phase, like as silica, diol, amino, amide or ZIC-HILIC, while the mobile phase is formed of polar solvents, commonly acetonitrile and water, with a higher percentage of the

organic solvent (9). The separation mechanism in HILIC mode depends on the differential distribution of the injected analyte molecules solute between the water-enriched layer adsorbed onto the hydrophilic stationary phase and an acetonitrile-rich mobile phase (10–12). The previously published theory assumed that HILIC retention is due to partitioning. Recent studies have shown that the process of separation in HILIC is not only dependent on the partition between the aqueous layer collected at the solid surface and a highly organic mobile phase (13). Other interactions that have a significant impact include adsorption via hydrogen bonding, dipole-dipole, and electrostatic interactions with bound ionic groups (14). The complexity of this mechanism can be seen in the different selectivity of various HILIC phases. The literature data contains some information regarding the usage of HILIC modes such as diol and amide stationary phases for the study of selectivity behavior and retention mechanism of water-soluble vitamins (15–18).

Zwitterionic stationary phases are relatively novel types of polar stationary phases that have been used successfully in HILIC because they have a highly hydrophilic surface layer and contain both positive and negative charges in a single molecule on the stationary phase to enhance the ion exchange selectivity (19,20). Zwitterionic ion chromatography (ZIC) is a development in ion-exchange liquid chromatography that separates anions and cations simultaneously using zwitterionic stationary phases (21). Strong ion exchange interactions are not promoted because of the zwitterionic nature, but rather weaker electrostatic contributions from the charged groups (22). These characteristics make zwitterionic stationary phases especially suitable for HILIC separations. zwitterionic stationary phases under HILIC mode (ZIC-HILIC) is a new separation technique that is rapidly becoming popular. It is appropriate for the separation of ionic and hydrophilic compounds. Some studies have successfully used zwitterionic stationary phase in the HILIC mode to determine and optimize water-soluble vitamins (22–25).

In our study, we used two home-made types of these zwitterionic stationary phases: ZIC-1 (4-vinylbenzyl-dimethylammonio methanesulfonate–PS/DVB) and ZIC-3 (4-vinylbenzyl-dimethylammonio propanesulfonate–PS/DVB). The numbers 1 and 3 in stationary phases refer to methylene groups between the charged groups (inner quaternary amines and outer sulfonic acids) in sulfobetaine monomers. ZIC-HILIC-1 and ZIC-HILIC-3 columns have been used in a wide range of investigations and applications by Rasheed et al (26–29).

The aim of our study is to investigate the B_2 and B_3 vitamins separation characteristics and estimate simultaneously on zwitterionic stationary phases with various chain lengths between the charged functional groups in HILIC conditions conjugated with UV detector to achieve baseline separation and acceptable peak shapes of these vitamins. Additionally, the effects of various chromatographic parameters, such as water content, pH, and buffer concentration of salt in the mobile phase on retention behavior were studied. Finally, analytical methods for

simultaneous estimation of B_2 and B_3 vitamins in three types of commercial multivitamin tablets were established.

2. Experimental

2.1 **Reagents and Materials**

Riboflavin (vitamin B_2, ≥ 98%) and Nicotinamide (vitamin B_3, 95.5%) were purchased from BDH and Fluka AG respectively. Acetonitrile (ACN) for HPLC (gradient grade, ≥99.9%) from Merck (Germany). Acetic acid and sodium acetate were purchased from Carl Roth (Germany). The multivitamin tablets were obtained from a local pharmacy for various companies: A-Z VITL® (hansal, Germany), MADDOVIT® (Maddox Pharma Swiss, Switzerland), Centrum® (GSK Consumer Healthcare, USA). Distilled deionized Milli-Q water (Millipore, Bedford, MA, USA) was used to prepare all reagents and sample solutions, as well as for eluents and system rinsing.

2.2 **Instruments**

The experiment was carried out with a Merck-Hitachi HPLC system (20 µL injection loop) equipped with a separation center T-6300 (injection valves and column oven) and an L-4200 UV/Vis detector and L6200 gradient pump. Ultrasonic water bath (Fisherbrand-CPXH, USA) and pH 740 (WTW) were employed. N2000 Photographic Data Workstation Module Integrator was used to collect and analyse the data. Chromatographic separation was performed using home-made stationary phases (ZIC-1 and ZIC-3) that Rasheed and co-workers prepared according to the references (21,30). Sulfobetaine monomers were grafted onto polystyrene-divinylbenzene (PS/DVB) with 4.6 µm particle sizes to prepare the ZIC-1 and ZIC-3 stationary phases. Using a down fill slurry technique and a 50 MPa head pressure, the ZIC-1 and ZIC-3 stationary phases were packed onto polyether ether ketone (PEEK) columns (100 mm × 4 mm I.D.).

2.3 **Chromatographic Conditions**

The solvent (A) in the mobile phase was ACN, and the solvent (B) was acetate buffer (40mM, pH 4.75). In the presence of an acetate buffer, the gradient program started at 60% ACN and increased to 95% ACN. Ultrasonication was used to degas the mobile phase before usage. The column was run at a temperature of 25 ℃. The injection volume was 10 µL, and at the 254 nm wavelength was employed to detect the chromatographic peaks of chosen vitamins, while the mobile phase had a flow rate of 0.5 ml/min.

2.4 **Standard Solutions**

Individual vitamin stock solutions (100 µg/mL) were prepared fresh weekly by dissolving appropriate amounts of riboflavin and nicotinamide in ACN-water (1:1 v/v) and water, respectively. Stock solutions were kept refrigerated (4 ℃) in opaque

vials after being sonicated in a water bath for 5 minutes. Working standard solutions were prepared on a daily by diluting concentrated stock solutions with the mobile phase. Before injection, each solution was filtered using a 0.45-µm syringe filter.

2.5 **Sample Solutions**

In order to analyze multivitamin/ multimineral food supplement tablets, fourteen tablets were precisely weighed and crushed to a fine powder. The average mass of one tablet was placed into a 50 ml volumetric flask, and a 50% (v/v) ACN–water mixture was added, which resulted in excellent recoveries for all vitamins. The mixture was sonicated for 10 min, diluted to the mark with the same solvent, then centrifuged at 5000 rpm for 15 min. 1 ml of this solution was placed in a 20 ml volumetric flask, diluted using the same solvent to the mark, and filtered with a 0.45 µm PTFE syringe filter. Following that, the sample solutions (10 µL) were directly injected into the HPLC system for analysis.

2.6 **Method Validation**

The approaches were validated according to International Conference on Harmonization (ICH) criteria (31), and they include linear range, LOD, LOQ, accuracy, and precision. To investigate method linearity, standard working solutions of B_2 and B_3 vitamins at concentrations of 0.06-11.0 and 0.08-7.0 µg/mL, respectively were created using 100 µg/mL pure ingredient standard stock solutions. Three injections of each concentration were examined under the same conditions. The least squares linear method was used in linear regression analysis to estimate the linearity of the calibration curve. Recovery experiments with three different concentrations (2.5, 3.5, and 4.5 µg/mL), involving six replicates (n = 6), were used to evaluate the accuracy of the proposed approaches. In order to determine the precision of the suggested approaches, RSD % values of three concentrations (2.5, 3.5, and 4.5 µg/mL) were utilized, with six replicates (n = 6) for every concentration injected.

2.7 **Development of The ZIC-HILIC Methods**

This work used two home-made ZIC-HILIC-1 and ZIC-HILIC-3 columns (100 mm × 4.6 mm I.D.) with gradient mobile phase contents to investigate the retention behaviors of chosen vitamins (B_2 and B_3). Significant factors were improved to construct an effective ZIC-HILIC system with high resolution and separation efficiency. Different mobile phase mixes containing sodium acetate buffer as the aqueous phase and ACN as the organic phase were employed to study the separation mechanism. The retention behavior of the selected vitamins in the under-researched mobile phases was investigated in relation to the levels of organic modifier, pH, and ionic strength.

3. Results and Discussion

3.1 The Impacts of Different Chromatographic Conditions on Retention Behavior

In HILIC mode, several experimental conditions including water percentage, pH, and buffer salt concentration in the mobile phase influence the retention of a solute on a column.

3.1.1 The Impact of ACN Content on Retention Behavior

Acetonitrile, a polar aprotic solvent, has proven to be the best solvent to utilize in HILIC mode (32), and it is also miscible with water in all ratios. When acetonitrile was employed as the mobile phase, no peak broadening was observed since it is an aprotic solvent that doesn't form hydrogen bonds (33). The retention changes of tested vitamins with ACN contents were investigated in the mobile phase at 60-95% ACN with a constant buffer salt concentration of 40 mM and buffer pH of 4.75. Retention time versus ACN volume fractions in the mobile phase are plotted in **Figure 1**. B_2 and B_3 vitamins displayed an increase in retention on the ZIC-HILIC-1 and ZIC-HILIC-3 columns as the ACN percentage in the mobile phase increased; this indicated a typical HILIC retention mechanism. According to **Table 1**, the Log P_{ow} values explain the reason for the hydrophilicity behavior of two B vitamins.

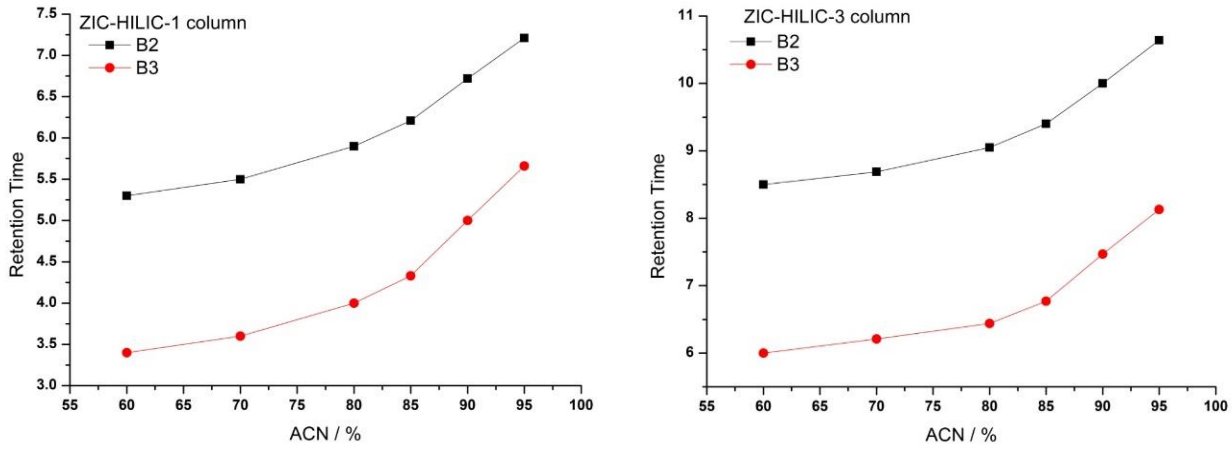


Figure 1: Influence of ACN% on the retention behavior for ZIC-1 and ZIC-3 columns

3.1.2 The Impact of Buffer pH on Retention Behavior

The separation of charged solutes in ZIC-HILIC mode depends significantly on the pH of the mobile phase, which can affect the ionization of the solutes and charged groups of the stationary phase (34). As shown in **Figure 2**, the pH of sodium acetate aqueous solutions was adjusted with acetic acid to various values (pH from 4.0 to 5.5) before mixing with ACN in order to investigate the impact of mobile phase pH. In other terms, the pH of the mobile phase refers to the pH of the stock sodium

acetate solution before mixing with ACN. The sodium acetate concentration was kept constant at 40 mM and the acetonitrile content was maintained at 95%. The retention behavior was explained according to **Table 1**, which displays the structures, IP, and pK_a values of the vitamins under investigation.

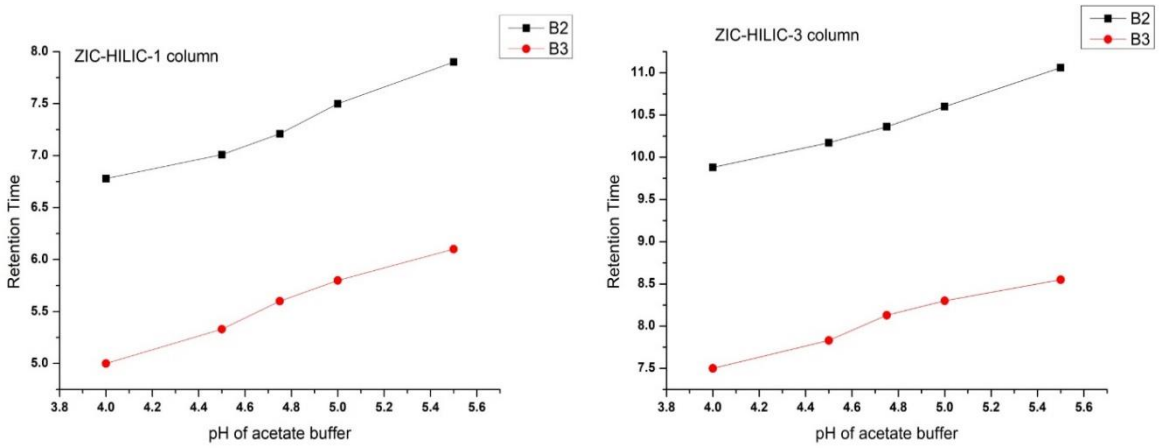


Figure 2: Influence of buffer pH on the retention behavior for ZIC-1 and ZIC-3 columns

In an acidic to neutral mobile phase, the amino/amide groups become protonated and acquire a positive charge (15). Increasing pH (from 3.0 to 6.0) reduces the positive charge in amino/amide groups (17). The retention time of amphoteric vitamins B_2 and B_3 increased due to a reduction in electrostatic repulsion interaction with increasing pH (5). Besides that, the protonated amino/amide groups and the sulfonate groups may interact more strongly through hydrogen bonding or electrostatic adsorption. At pH 4.75, the targeted vitamins may achieve baseline separation. At pH 4.75, appropriate symmetry factors were obtained. So sodium acetate with a pH of 4.75 was selected for further investigation.

3.1.3 The Impact of Buffer Salt Concentration on Retention Behavior

In general, the addition of buffer salts to the mobile phase would increase peak symmetry and enhance separation efficiency. Increased buffer strength should reduce intramolecular ion pairs, promoting linearization of the stationary phase's functional groups even though the presence of acetonitrile (35). This enhances the creation of a semi-immobilized aqueous adsorption layer on the zwitterionic stationary phases, which increases the partitioning capacity on the enriched water layer. In the current study, the effect of various sodium acetate concentrations on retention in the range of 20-80 mM was examined while maintaining a constant pH of 4.75 and acetonitrile content of 95 % (v/v). **Figure 3** shows the retention behaviors when Log *k* is plotted versus Log buffer concentration. The negative slope of these compounds is due to anion or cation exchange interactions (36). The cation exchange of vitamins B_2 and B_3 with the sulfonate group is most likely the cause of this behavior. There would be lesser active sulfonate groups available to interact with B_2 and B_3 because of an increase in counter-ions (sodium) in the mobile phase as a result of an increase in

buffer concentration, which would result in a decrease in retention time. Vitamins B_2 and B_3 could achieve baseline separation at the examined salt concentration. Additionally, at a 40 mM buffer concentration, satisfactory symmetry factors were obtained. So, a 40 mM salt concentration was used for the purpose of the investigation.

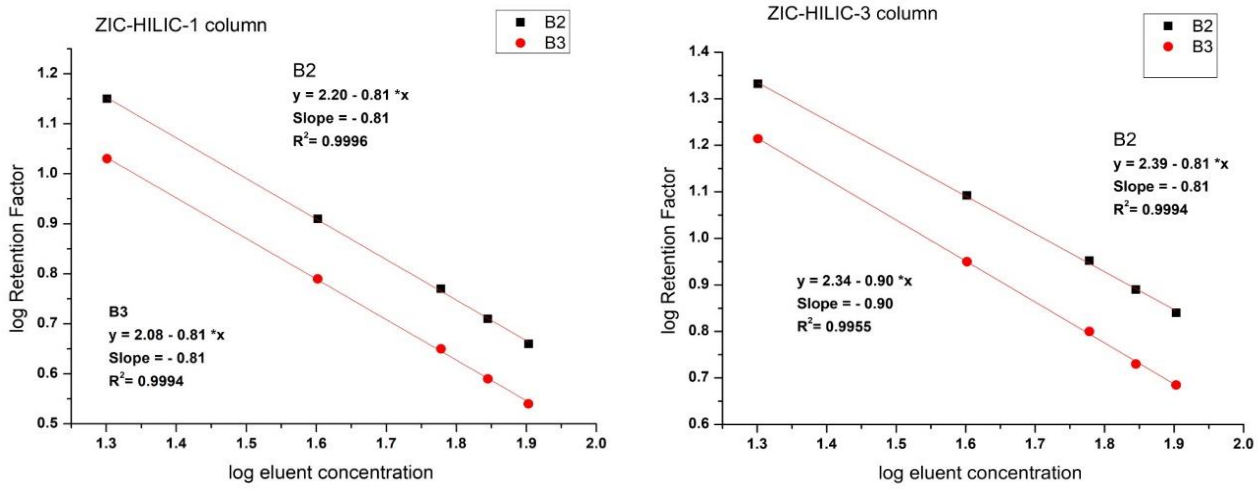


Figure 3: Influence of buffer concentration on the retention behavior for the two columns

3.2 **Separation of B_2 (Riboflavin) and B_3 (Nicotinamide)**

The optimum conditions for separating the studied vitamins were established in the HILIC mode using a 95:5 proportion mixture of acetonitrile and acetate buffer (40 mM, pH 4.75), each of which was detectable by UV at 254 nm. خطأ! لم يتم العثور على مصدر المرجع. shows the chromatograms of chosen vitamins.

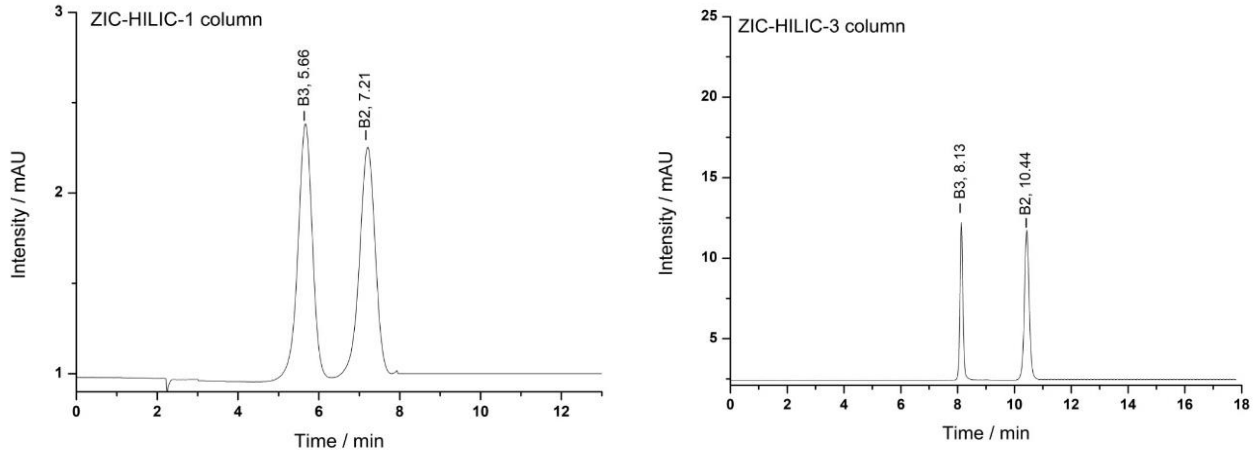


Figure 4: Chromatograms of B2 and B3 vitamins for ZIC-HILIC-1 and ZIC-HILIC-3 columns

Vitamins B_3 (t_R= 5.66 and 8.13 min) and B_2 (t_R= 7.21 and 10.44 min) eluted out in that order for (ZIC-HILIC-1 and ZIC-HILIC-3) columns, respectively. The

primary forces for retention that controlled the final elution order of the B vitamins might be electrostatic interaction (attraction or repulsion) and hydrogen bonding between the functional groups of ZIC-HILIC and the targeted vitamins. It is generally known that a highly polar compound has a longer retention time on the HILIC columns. Since vitamin B_2 has the greatest polarity ($LogP_{ow}$ = -0.917) than vitamin B_3 ($LogP_{ow}$ = -0.045), it was predicted that it would elute last.

In HILIC conditions, the zwitterionic stationary phases exhibit similar behavior. Column ZIC-3 showed the highest selectivity and retention for the test mixture when compared to column ZIC-1. The length of the methylene chain between the charged groups in ZIC-HILIC columns is the most likely cause. The highest retention of the target vitamins in the ZIC-3 column is often caused by the geometric arrangement of the sulfobetaine groups. Because ZIC-1 has the lowest polarity compared to ZIC-3, it has a slightly more inflexible geometry, which causes the charges to be offset intramolecularly. This behavior may be explained by this property (37,38).

3.3 **Calibration Graphs**

The calibration graphs for vitamins B_2 and B_3 are constructed in optimal circumstances by plotting the peak area against the concentrations of the selected vitamins and show the range of concentrations (0.06-11.0 and 0.08-7.0 µg/mL, respectively) of the ZIC-HILIC-1 and ZIC-HILIC-3 columns, as illustrated in **Figure 5**.

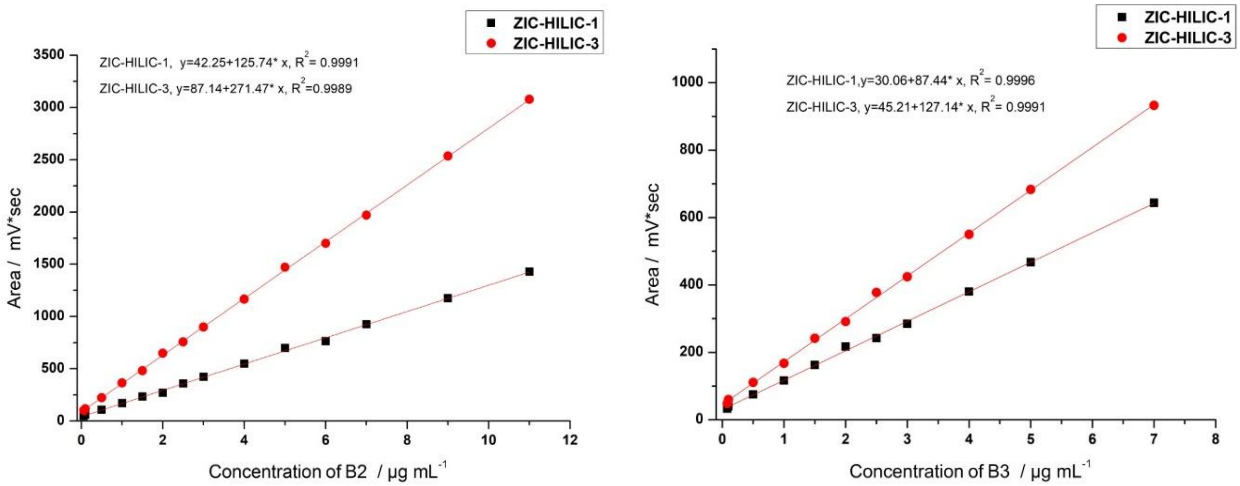


Figure 5: Calibration curves of B_2 and B_3 vitamins

3.4 **Statistical Data Analysis**

The direct calibration curves and statistical data for the ZIC-HILIC approach to measuring targeted vitamins are shown in **Table 2**. The accuracy (recovery %) and precision (RSD %) of two zwitterionic stationary phases determined during the same day (intra-day) as well as between days (inter-day) are shown in **Table 3**. The high recovery worth and low standard deviation indicate that the proposed method is acceptable.

Table 2: Calibration curve parameters of selected vitamins

Parameter	ZIC-HILIC-1	ZIC-HILIC-3	ZIC-HILIC-1	ZIC-HILIC-3
	B_2		B_3	
Conc. range (ppm)	0.06-11.0	0.06-11.0	0.08-7.0	0.08-7.0
r^2	0.9991	0.9989	0.9996	0.9991
LOD (ppm)	0.023	0.011	0.055	0.041
LOQ (ppm)	0.069	0.033	0.166	0.124

Table 3: Accuracy and Precision of the suggested methods

Same-Day Analysis (n = 6)				Day-to-Day Analysis (n = 6)		
ZIC-HILIC-1						
B_2 added (ppm)	**B_2 obtained (ppm)**	**Rec.%**	**RSD%**	**B_2 obtained (ppm)**	**Rec.%**	**RSD%**
2.50	2.511	100.44	0.65	2.513	100.52	0.68
3.50	3.488	99.66	0.42	3.474	99.26	0.34
4.50	4.464	99.20	0.52	4.470	99.33	0.58
B_3 added (ppm)	**B_3 obtained (ppm)**	**Rec.%**	**RSD%**	**B_3 obtained (ppm)**	**Rec.%**	**RSD%**
2.50	2.475	99.00	0.89	2.479	99.16	0.74
3.50	3.560	101.71	0.93	3.541	101.17	0.85
4.50	4.477	99.49	0.71	4.470	99.33	0.74
ZIC-HILIC-3						
B_2 added (ppm)	**B_2 obtained (ppm)**	**Rec.%**	**RSD%**	**B_2 obtained (ppm)**	**Rec.%**	**RSD%**
2.50	2.508	100.32	0.32	2.508	100.32	0.44
3.50	3.478	99.37	0.87	3.470	99.14	0.78
4.50	4.490	99.78	0.22	4.485	99.67	0.32
B_3 added (ppm)	**B_3 obtained (ppm)**	**Rec.%**	**RSD%**	**B_3 obtained (ppm)**	**Rec.%**	**RSD%**
2.50	2.490	99.60	0.65	2.487	99.48	0.60
3.50	3.544	101.26	0.54	3.532	100.91	0.45
4.50	4.477	99.49	0.71	4.477	99.49	0.73

3.5 **Determination of B_2 and B_3 Vitamins in Multivitamin Tablets**

The analysis of vitamins has been successful using three different types of commercial multivitamin pills that contain the target vitamins. **Table 4** summarizes the information obtained.

Table 4: Application of the suggested methods for the determination of B_2 and B_3 vitamins in multivitamin tablets.

Trade Name	Started conc. (ppm)	Get it (ppm)	%Rec.	%RSD n = 5	Get it (ppm)	%Rec.	%RSD n = 5
	ZIC-HILIC-1				ZIC-HILIC-3		
MADDOVIT complete A-Z							

B_2	1.10	1.084	98.55	1.11	1.089	99.00	0.97
B_3	1.4	1.392	99.43	0.95	1.389	99.21	0.90
Centrum Advance							
B_2	1.75	1.768	101.03	0.43	1.75	100.00	0.43
B_3	2.0	1.980	99.00	0.22	1.980	99.00	0.27
A-Z Vital							
B_2	1.75	1.741	99.49	0.63	1.733	99.03	0.73
B_3	2.0	1.984	99.20	0.71	1.984	99.20	0.51

The results of the ZIC-HILIC-1 and ZIC-HILIC-3 procedures were compared with those of the official method (39) using the student t-test and the variance F test with a 95% confidence level in order to evaluate their competence and efficiency. The computed t and F values were not greater than the theoretical values (**Table 5**), demonstrating that there was no observable difference in the accuracy and precision of the two approaches used to analyze the vitamins in multivitamin pills.

Table 5: Results of the t-test and F-statistical analysis comparing the suggested methods with the official method for the studied vitamins.

Multivitamin tablets	ZIC-HILIC-1*	ZIC-HILIC-3**	Official Method	t_{cal}	F_{cal}
B_2	Rec.%	Rec.%	Rec.%		
MADDOVIT	98.55	99.00	98.90	0.167^{*}	4.657^{*}
Centrum Advance	101.03	100.00	100.00	0.455^{**}	1.040^{**}
A-Z Vital	99.49	99.03	99.77		
B_3					
MADDOVIT	99.43	99.21	99.15	0.177^{*}	7.937^{*}
Centrum Advance	99.00	99.00	99.30	1.188^{**}	2.406^{**}
A-Z Vital	99.20	99.20	99.25		

Note: the tabulated value of the t-test is 2.7764 (95%) and the F-test is 19.000 (95%).

4. Conclusions

In this research, the ability of two unique home-made stationary phases (ZIC_1-HILIC and ZIC_3-HILIC) to study the retention behavior of B_2, and B_3 vitamins under UV-compatible conditions were investigated. Besides, the two ZIC-HILIC columns were compared in order to separate the targeted vitamins. Investigated was the impact of the mobile phase (organic solvent %, pH, and ionic strength of the buffer eluent) on the ZIC-HILIC retention mechanism. It was noticed that the retention of selected vitamins increased in both ZIC-HILIC columns when mobile phase water concentration decreased. In the same way, the retention time of vitamins increased as the buffer pH increased. In contrast, as the acetate buffer concentration increases, the retention time of vitamins drops. The ZIC-HILIC columns exhibited mixed HILIC-ion-exchange mechanisms for retention, when the water content was low (20%), the typical HILIC mechanism was observed. In addition to hydrophilic interactions (partitioning), the HILIC separation method also involves hydrogen bonds and

electrostatic interactions between the solute and stationary phase. The separation of the two vitamins performed best and with the greatest retention on the ZIC_3-HILIC column.

For the analysis of B_2 and B_3 in multivitamin pills, two ZIC-HILIC approaches were developed. The ZIC-HILIC approaches constructed use of a two ZIC home-made column made of PEEK (100 mm × 4 mm I.D., 4.6 µm), a mobile phase composed of ACN and a sodium acetate solution (95:5, v/v) at a concentration of 40 mM (pH 4.75) and a flow rate of 0.5 mL/min with a detection wavelength of 254 nm. The linearity and sensitivity of these techniques were good for both standard solutions and spiked samples. The methods were successfully used to identify the chosen vitamins in dietary supplements. The procedures may be used in the routine evaluation of multivitamin pills since the results of the validation show that they are straightforward and accurate.

5. References

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