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DETERMINATION OF NIACIN FROM FOOD SUPPLEMENTS

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ABSTRACT
Adequate intake of all vitamins is important, since they play a vital role in many biochemical functions in the human body and are essential components for maintaining optimal health. Niacin is found in animal and vegetable aliments, its amounts being higher in unprepared foods compared to processed. In addition to serving as cofactors in biochemical reactions, the representatives of the vitamin B complex are vital for normal body growth and development, healthy skin, the proper function of nerves and heart, as well as red blood cell formation. Vitamin deficiency can be compensated with food supplements. Literature surveys has revealed various analytical methods for the determination of vitamins from pharmaceutical formulations in combination with other drugs, such as: RP- HPLC, HPTLC, UV-Spectroscopy, or LC–MS/MS. In the present study, the UV spectrophotometric method was used to determine Niacin (Vitamin B3) content in food supplements and to validate the selected method. Niacin was dissolved in ethanol as a solvent and from its UV spectra the max was determined. Solutions of different concentrations were prepared in order to obtain the calibration curve. After the linearity calibration curve was obtained at 262 nm, preliminary concentration determinations on different food supplements were performed.
KEY WORDS: *niacin, vitamin B3, food supplements, UV-Spectroscopy*

INTRODUCTION

The discovery of vitamin B3, as a vitamin, resulted from the urgent need to treat pellagra, which devastated many people in the 20th century. The Spanish physician, Casal, was the first who described the new disease in 1735. Due to the widespread incidence of pellagra and the establishment of numerous hospitals for victims of the disease, there was painstaking documentation of its symptoms. These include diarrhea, dementia, dermatitis and eventual death (Penberthy & Kirkland, 2020).

Vitamin B3 is the third member of water-soluble B vitamins. Niacin (also known as nicotinic acid or pyridine-3-carboxylic acid) is a form of B3 vitamins besides nicotinamide (pyridine-3-carboxamide) and nicotinamide riboside (Hrubša et al., 2022).

The main dietary sources of niacin include meat, whole grains, and dairy products. Peanuts, legumes, nuts, fish,mushrooms and yeast are rich in niacin too (Hrubša et al., 2022).

For several decades niacin has been used as pharmacotherapeutics, in the treatment of dyslipidemia and pellagra (Hrubša et al., 2022). Vitamin B3 is necessary in cellular respiration and helps in the release of energy and in the metabolism of carbohydrates, fats and proteins; proper circulation, maintenance of healthy skin, functioning of the nervous system and normal secretion of bile and gastric fluids. It is used in the treatment of schizophrenia and other mental disorders and as a memory enhancer. Niacin administered in certain doses improves the cholesterol profile in the blood and thus is used in the treatment of cardiovascular diseases (Chand & Savitri, 2016). Unmetabolized niacin or very high niacin intakes are eliminated through the urine (Lenglet et al., 2016).

The recommended daily dose of niacin for adults over the age of 19 is 16 mg for men and 14 mg for women (Institute of Medicine, 1998), but in the case of vitamin deficiency, food supplements can be administered (Bates, 2012). In this study we tried to determine the concentration of niacin in food supplements, using a simple and economical method, namely the UV-VIS spectrophotometric method. Niacin was dissolved in ethanol, then we determined its spectra and found out the maximum absorbance.

MATERIALS AND METHODS

Instruments. A T90+ UV/VIS Spectrophotometer PG Instruments Ltd, an analytical balance (Partner), magnetic stirrers, ultrasonic bath (Aqua Wave 9375, Barnstead Lab-Line), and micropipettes of various volumes (Easy 40+ Elite) were used.

Materials. Pure niacin (purity 99%) was purchased from Fluka (Germany) and was used without any other purification. Ethanol was purchased from Merck KGaA (Germany). Three Niacin food supplements containing 100 mg Niacin (manufactured by Vitaking and Swanson), respectively 250 mg Niacin (manufactured by GNC) were purchased from a local pharmacy, in Timișoara (Romania).

Determination of the maximum absorption wavelength. A 100 mL of 100 μg/mL Niacin stock solution was prepared using ethanol as a solvent. 0.5 mL of stock solution was diluted to 25 mL with the same solvent to obtain a reference solution (2 μg/mL). The reference solution was analyzed in the spectral region 230- 300 nm.

Preparation of different concentration solutions for the linearity curve. Twenty dilutions (0-20 μg/mL) were prepared using the previously obtained Niacin stock

solution and ethanol as solvent. The absorbance of these solutions was measured at 262 nm and the obtained data was used to plot the linearity calibration curve.

Analysis of niacin content in the food supplement. The tablets were weighed and grinded in order to obtain a fine powder. The powder was dissolved in 100 mL of ethanol. Niacin was dissolved using the magnetic stirrer for 20 min, then the solutions were transferred to the ultrasonic bath for another 20 min. The obtained solutions were filtered 5 times using filtraTECH filter paper. 0.25 mL filtrate was diluted with ethanol in 25 mL volumetric flask (theoretical concentration of 10 µg/mL). The absorbances of these solutions were measured after 3, 4, respectively 5 filtrations and, from the calibration curve, the concentration of niacin was determined. The niacin content of the tablets was calculated.

RESULTS AND DISCUSSIONS

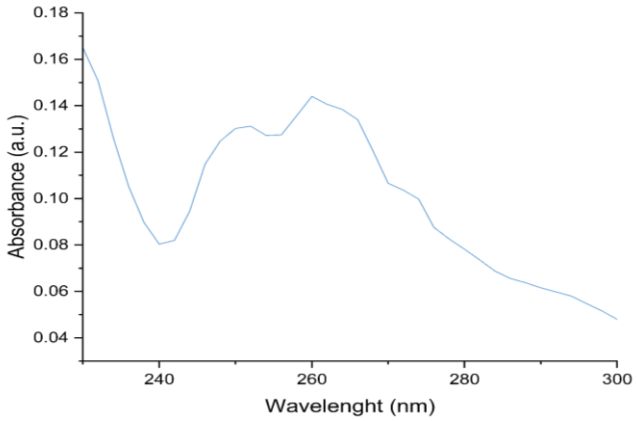

FIG. 1. Spectrum of Niacin solution (2 µg/mL) in the wavelength range 230-300 nm

The obtained UV spectrum revealed a series of absorption peaks. These were compared with literature data and λ_{max} = 262 nm was determined (Chanda et al., 2017) (FIG. 1).

Using the UV/VIS spectrophotometer, the absorbance values of the 20 Niacin solutions of different concentrations were determined at 5 different wavelengths in order to determine the maximum absorbance. In this way comparing the data in TABLE. 1 with the spectrum from FIG. 1 λ_{max} was established.

TABLE. 1. The results of the experimental determinations for the 20 niacin solutions of different concentrations made with the UV/VIS spectrophotometer

Solution number	Concentration (μg/mL)	Absorbance (a.u.)				
		λ=270 nm	λ=264 nm	λ=262 nm	λ=260 nm	λ=250 nm
1	0	0,005	0,008	0,001	0,007	0,002
2	1	0,037	0,044	0,045	0,039	0,027
3	2	0,057	0,66	0,074	0,062	0,051
4	3	0,077	0,107	0,109	0,097	0,086
5	4	0,103	0,124	0,136	0,124	0,113
6	5	0,153	0,154	0,166	0,154	0,143
7	6	0,185	0,192	0,195	0,183	0,172
8	7	0,195	0,218	0,232	0,22	0,209
9	8	0,224	0,243	0,257	0,245	0,234
10	9	0,227	0,281	0,284	0,272	0,261
11	10	0,241	0,301	0,314	0,302	0,291
12	11	0,273	0,343	0,348	0,336	0,325
13	12	0,27	0,37	0,384	0,372	0,361
14	13	0,313	0,396	0,412	0,4	0,389
15	14	0,342	0,418	0,448	0,436	0,425
16	15	0,371	0,437	0,47	0,458	0,447
17	16	0,414	0,471	0,5	0,488	0,477
18	17	0,433	0,506	0,536	0,524	0,513
19	19	0,478	0,522	0,552	0,54	0,529
20	18	0,509	0,546	0,576	0,564	0,553
21	20	0,588	0,611	0,641	0,629	0,618

The calibration curve obtained using the experimental data for the Niacin solution at 262 nm is shown in FIG. 2. In FIG. 2 it is observed that Niacin shows a linear dependence of absorbance in relation to the concentration in the concentration range 0-20 μg/mL, for which a correlation coefficient $R^2 = 0.9985$ was obtained.

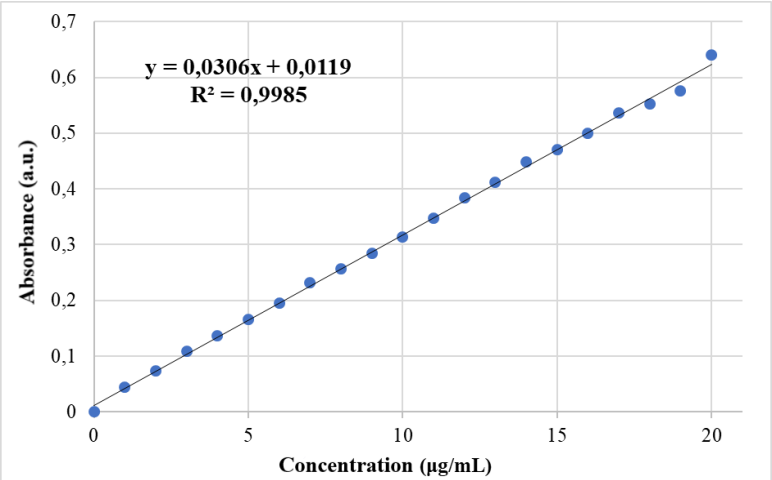

FIG. 2. Linearity curve of Niacin at 262 nm

TABLE. 2. Niacin concentration in samples filtered 3, 4, respectively 5 times.

Sample name	Filtration number	Absorbance (262 nm)	Concentration of Niacin from sample (µg/mL)	Concentration of Niacin from sample (mg/mL)	Amount of niacin in the tablet (mg/tablet)
Vitaking Niacin 100 mg	3	0,326	10,26	1,02	102,65
Vitaking Niacin 100 mg	4	0,338	10,66	1,06	106,57
Vitaking Niacin 100 mg	5	0,321	10,10	1,01	101,01
Swanson Niacin 100 mg	3	0,253	7,88	0,78	78,79
Swanson Niacin 100 mg	4	0,277	8,66	0,86	86,63
Swanson Niacin 100 mg	5	0,316	9,94	0,99	99,38
GNC Niacin 250 mg	3	0,619	19,84	1,98	198,40
GNC Niacin 250 mg	4	0,671	21,53	2,15	215,39
GNC Niacin 250 mg	5	0,556	17,78	1,77	177,81

The final test results are consistent with the tablet leaflets (TAB. 2). Magnetic stirring and ultrasonication of the niacin solutions were performed to solubilize the niacin, and filter paper with the same porosity was used for filtration.

The Vitaking tablet after 3 filtrations presented the amount of niacin of 102.65 mg/tablet, close to the value of 100 mg/tablet mentioned in the leaflet.

The Swanson sample contains 99.38 mg of niacin/tablet close to the 100 mg of niacin/tablet according to the package insert after 5 filtrations.

The sample from the GNC tablet equivalent to 250 mg niacin/tablet showed after 4 filtrations the value of 215.39 mg/tablet.

CONCLUSIONS

The concentration of niacin in three food supplements containing 100 mg niacin/tablet from two manufacturers Vitaking and Swanson and 250 mg/tablet from the manufacturer GNC was experimentally determined.

The Vitaking and Swanson supplements after the determinations method (after 5 filtrations) have a content of 102.65 mg niacin/tablet and 99.38 mg niacin/tablet, respectively. As for the GNC tablet, the closest value to that in the package insert was after 4 filtrations (215.39 mg niacin/tablet), and after the fifth filtration the value of niacin content decreased to 177.81 mg niacin/tablet.

The UV-VIS spectrophotometric method is an effective method for niacin dosage, but the solubilization method, the stirring time and the number of filtrations of the solution must be considered in order to reach correct results.

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