Full Length Research Paper

HPLC analysis of nicotinamide, pyridoxine, riboflavin and thiamin in some selected food products in Nigeria

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Food fortification is an important tool for improving the health of the population. Motivated by this, the National Agency for Food and Drug Administration and Control (NAFDAC) has in the past few years embarked on a campaign to create this awareness. There has been varying degree of compliance to this, and so it is of utmost importance to know the food products that contribute significantly to the dietary vitamin B intake. This study investigates the amount of four Vitamin B compounds, namely nicotinamide, pyridoxine, riboflavin and thiamin, present in different packaged food items available in Lagos metropolis of Nigeria. The food items analyzed include dairy products, fruit juices and cereal products. These foods were chosen because of their widespread consumption in the area. The analysis was done using a high performance liquid chromatographic technique with a UV detector. The separation was carried out on a C18 column, using a mobile phase made up of 70% buffer (a solution of sodium salt of hexane sulphonic acid) and 30% HPLC grade methanol. The identification was based on retention time match against a standard while the quantitation was based on the peak area match against those of a standard. The calibration curves for the standards were linear with a linear regression coefficient close to unity.

INTRODUCTION

Vitamins in general, play a very important role in our health, even though they only make up a very small part of the food we eat each day (Olaniyi, 2000). Diets which do not contain adequate amounts of these vitamins often result in deficiency diseases including blindness and mental retardation, depending on the particular vitamin. For example, thiamine functions as a co-enzyme in the phosphogluconate pathway and a structural component of nervous system membranes (McCormick, 1996; 1997). Nicotinamide is essential for the metabolism of carbo-hydrates and for non redox adenosine diphosphate- ribose transfer reactions involved in DNA repair (Berger, 1985; McCormick, 1996; 1997). Pyridoxine plays an essential role in amino acid transamination (McCormick, 1996; 1997). Riboflavin functions as a co-enzyme for a wide variety of respiratory enzymes (McCormick, 1996) etc.

Deficiency diseases (McCormick, 1988a, b, c; 1994; Carpenter and Lewin, 1985; FAO/WHO, 1974) do not only occur in poverty stricken communities. In affluent societies, deficiency diseases also occur often as the result of poor choice of food or unhealthy eating habits, often coupled with different lifestyles. It is estimated that one in every three people in the world is at risk for one or more micronutrient deficiencies, thereby impacting on their nutritional status. Our nutritional status has an important impact on our health, productivity and quality of life in general. It is also commonly thought that women of childbearing age, young children, the elderly, and those recovering from illness are most at risk of developing micronutrient deficiencies.

In order to prevent and address the problem of micronutrient deficiencies, several strategies are used, one of which is food fortification in which specific and scientifically identified micronutrients are added to certain food vehicles. The type and amount of micronutrients added are determined by the nutritional status, and therefore

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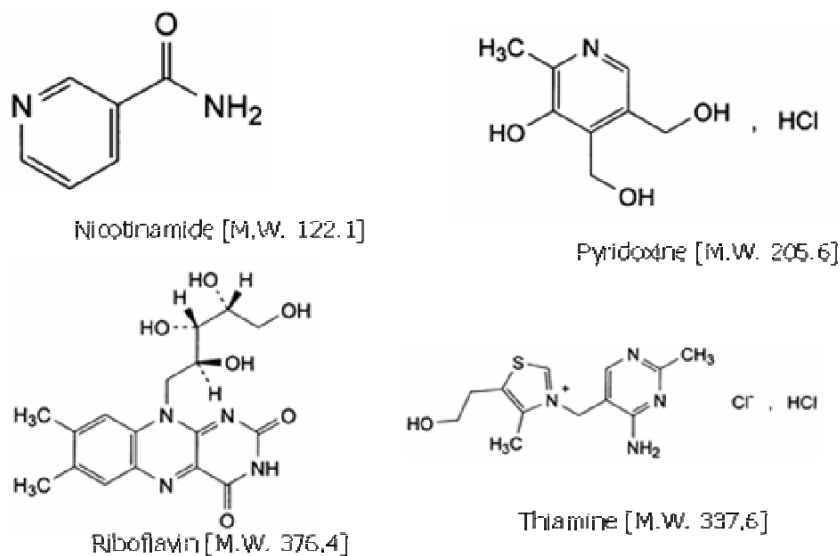
Figure 1. The chemical structures of the target compounds.

nutritional needs, of the population while the food to be fortified (food vehicle) depend on the eating habits of the population.

The increasing interest in good eating habits has provided a greater awareness of the vital role that micro-nutrients play in growth and health. The National Agency of Food and Drug Administration and Control (NAFDAC), in conjunction with the micronurient Initiative (MI) has contributed immensely to the creation of this awareness in Nigeria. However, since there is still varying degree of compliance to this, it is therefore of utmost importance to know the food products that contribute significantly to the dietary micronutrient intake. In the present work, the determination of dietary water-soluble vitamins namely nicotinamide, pyridoxine, riboflavin, and thiamin (Figure 1) in selected food products in Lagos metropolis of Nige-ria is carried out using high performance liquid chromato-graphic method (HPLC). The food items analyzed include dairy products, fruit juices, and cereal product, which were chosen because of their widespread consumption in the area. The results show a relatively high level of these compounds in the analyzed samples.

EXPERIMENTAL

Equipment

Analysis was carried out using a Varian HPLC with manual inject-tion. The system comprises a Varian 9012 pump, a Varian 9050 U.V- Vis detector, and a DELL computer. The HPLC column used was a reversed-phase C_{18} (250 x 4.6 mm, 5 μm, ODS2) from waters. Data acquisition was done with Varian Star Software.

Chemicals and reagents

All solvents were HPLC grade, and were employed as supplied by manufacturers. Deionized water was used in all procedures, and

deionization was carried out by means of a Millipore deionizer. A buffer solution comprising of hexane sulphonic acid sodium salt (BDH, England), deionized water, and glacial acetic acid (M and B, England) was used in sample preparation and separation of the vitamins. The buffer solution was prepared by weighing 1.8822g of hexane sulphonic acid sodium salt into a 2l volumetric flask and dissolved with a minimum amount of deionized water. This was followed by the addition of 1500 ml of deionized water to the solution; 20 ml of glacial acetic acid was then added to keep the medium acidic, and finally making up with deionized water, which helped to dissolve the water soluble vitamins. The mobile phase, which comprised of methanol (BDH England) and buffer (made of hexane sulphonic salt of sodium), was properly sonicated prior to analysis. The food samples were obtained from a local market in Lagos metropolis.

Chromatographic conditions

Chromatographic separation and analysis of the vitamins were done using a reversed-phase Nova-Pack C_{18} (250 x 4.6 mm, 5 μm) column (Waters,) at room temperature. The mobile phase composi-tion used was 70% buffer (that is, sodium salt of hexane sulphonic acid) and 30% methanol (HPLC grade). The analysis was carried out in isocratic mode at a flow rate of 1ml/min, with column effluent being monitored at 254 nm wavelength.

Preparation of standard solutions

Pure standards of nicotinamide, pyridoxine, riboflavin and thiamine (Figure 1), obtained from BDH Biochemicals (England), were used as external standards in the analysis. Different concentrations of the standards were used based on the range required to plot a suitable calibration curve. The calibrants were all carefully weighed into a 25 ml volumetric flask, using a digital weighing balance (Mettler Toledo) . About 5ml of buffer solution was added into the volumetric flask afterwards. This was then properly vortexed to ensure proper dissolution, after which the flask was made up to 25 ml with buffer, and filtered. Different concentrations of these stan-dards were analyzed using the same chromatographic conditions as those of the target compounds and a calibration curve generated for each of the standards.

Table 1. Analytical characteristics of standards.

Compound	Mol. Wt.	tr	r^2
Nicotinamide	122.1	3.86	0.995
Pyridoxine	205.6	6.34	0.996
Riboflavin	376.4	11.56	0.995
Thiamin	337.3	17.98	0.982

Sample preparation

Solid food samples were prepared by weighing out 50 g of the sample in a volumetric flask. To which 1000 ml of buffer (a solution of sodium salt of hexane sulfonic acid) was added. Dissolution was achieved by vortexing the mixture and volume made up to 1000 ml with the same buffer. The dissolution was done at room tempe-rature. Prior to sample injection the solution was filtered through a Millipore filter (0.22 μm) to remove any undissolved particle. Liquid samples were prepared by pipetting out 25 ml of the sample and making it up to 1000 ml with buffer. In the same way prior to sample injection the solution was filtered through through a Millipore filter (0.22 μm).

RESULTS AND DISCUSSION Analytical

characteristics of standards

Calibration curves for the standards (nicotinamide, pyri-doxine, riboflavin and thiamin) were obtained using a series of concentrations of these compounds at range of 0.05 to 0.25 mg/ml. The calibration curves for the four standards were linear. The regression coefficients ranged from 0.982 to 0.996 as shown in Table 1. The relative standard deviations also shown in Table 1 for the four compounds were all below 8%. Figures 2 – 5 show the calibration curve for the four standards.

Figure 6 shows the total ion chromatogram for the mixture of the standards for the four target compounds. The same separation conditions were employed for the analysis of the samples from the Lagos metropolis. The target compounds were identified using retention time match against those of the calibration standards while quantitation was performed by means of peak area match against those of the standards. Due to the nature of the samples analyzed there was little or no interference with the target compounds. This made identification and quantitation much easier. The detection wavelength (254 nm) of the UV contributed a lot to this. Even though the analysis involved the separation, detection and quanti-tation of multiple target compounds, the retention times of the target compounds were sufficiently far apart to enable detection and quantitation.

Extraction

There was minimum sample work up prior to HPLC analysis. The liquid samples were just diluted to proper concentration using a buffer (sodium salt of hexane sul-

phonic acid, glacial acetic acid and deionized water). For the solid samples, the samples were dissolved in buffer solution and by vigorous shaking. Hexane sulphonic acid in the buffer served as a deproteinating agent, removing the bulky proteins in the food, hence fostering the proper dissolution of the vitamins. The solution was filtered and the filtrate diluted to mark. The target com-pounds are all water soluble hence also soluble in the buffer.

Target compounds distribution in samples

Table 2 gives the distribution of nicotinamide, riboflavin, thiamin and pyridoxine in the studied samples and Figure 7 shows the graphical distribution. The compounds were found to be in the samples at very varied concentrations, between zero and over 1000 μg/ml. As shown in Figure 7, there is no particular trend followed in food fortification, and variations occur even within the same food product. This indicates that different manufacturers follow their own plan in food fortification. The food drinks were found generally to contain most of the compounds at very low concentrations.

Implication of results

Some of the samples contain these compounds in very high concentrations (Table 2). Nicotinamide occurred in a very high concentration in one of the studied samples with a concentration of up 854.05 μg/ml recorded, even though some of the samples contained no nicotinamide. The mean concentration was found to be 336.4 μg/ml or μg/g as the case may be. Also Riboflavin concentration of over 1400 μg/ml was recorded. But as with the case in the distribution of Nicotinamide, some samples did not contain any Riboflavin. Thiamin and pyridoxine were generally in much lower concentration than the nicotina-mide and riboflavin.

Assuming 50 ml or grams daily consumption of any of these food products, there will be an average daily consumption of 9.2, 2.6, 10.8 and 0.6 mg/day for nicotinamide, pyridoxine, riboflavin and thiamin respect-tively. Numerous studies have shown minimum require-ment for most of these vitamins. Andersen et al (1985) concluded in their study that to avoid deficiency disease, a minimum of 1.0 and 1.2 mg/day intake of thiamin for adult men women and men respectively are required. Other studies have also found out that aging may increase thiamin requirement (Nichols and Basu, 1994). Studies on Nicotinamide have suggested that that 5.6 mg/day is the minimum sufficient intake for adults (Pat-terson et al., 1980; Shibabta and Matsuo, 1989). Ribo-flavin and Pyridoxine minimum requirements have also been put at around 1.3 mg/day (FAO/WHO, 2002). Based on several of these studies, the World Health Organi-zation has compiled and recommended minimum bodily daily requirements and toxic upper limits for these com-
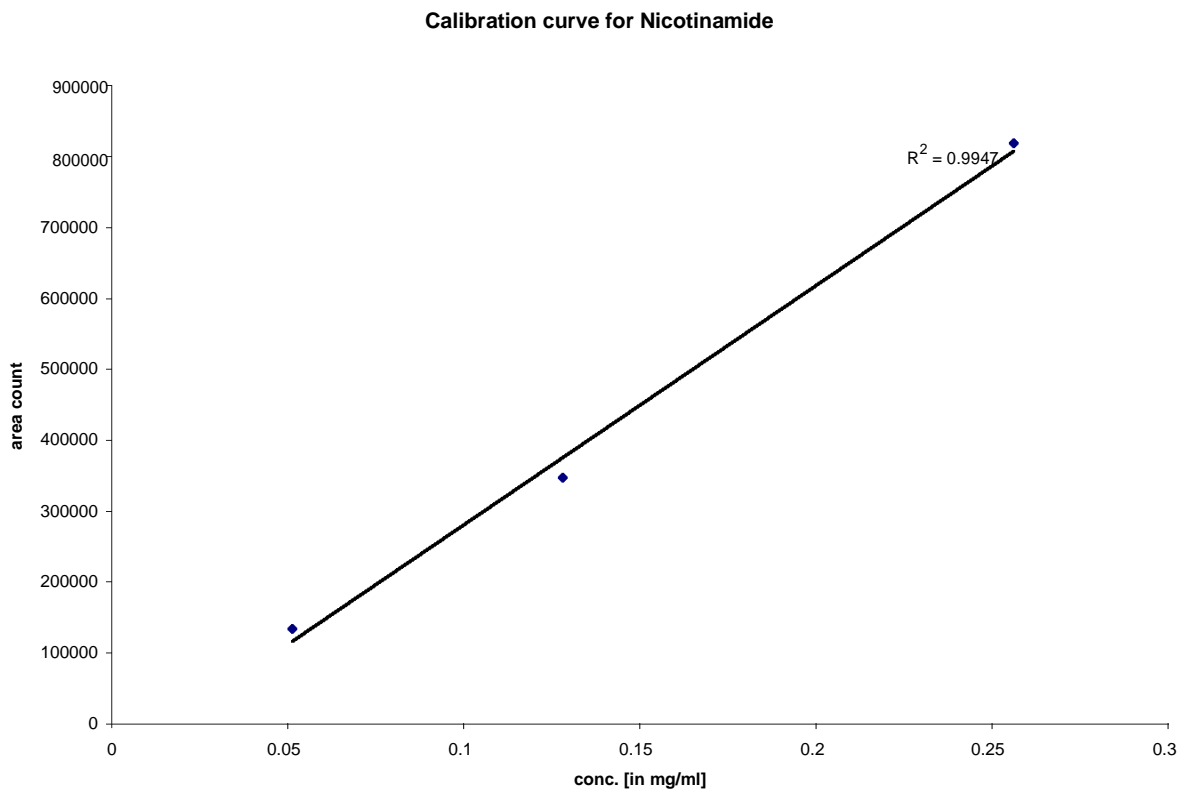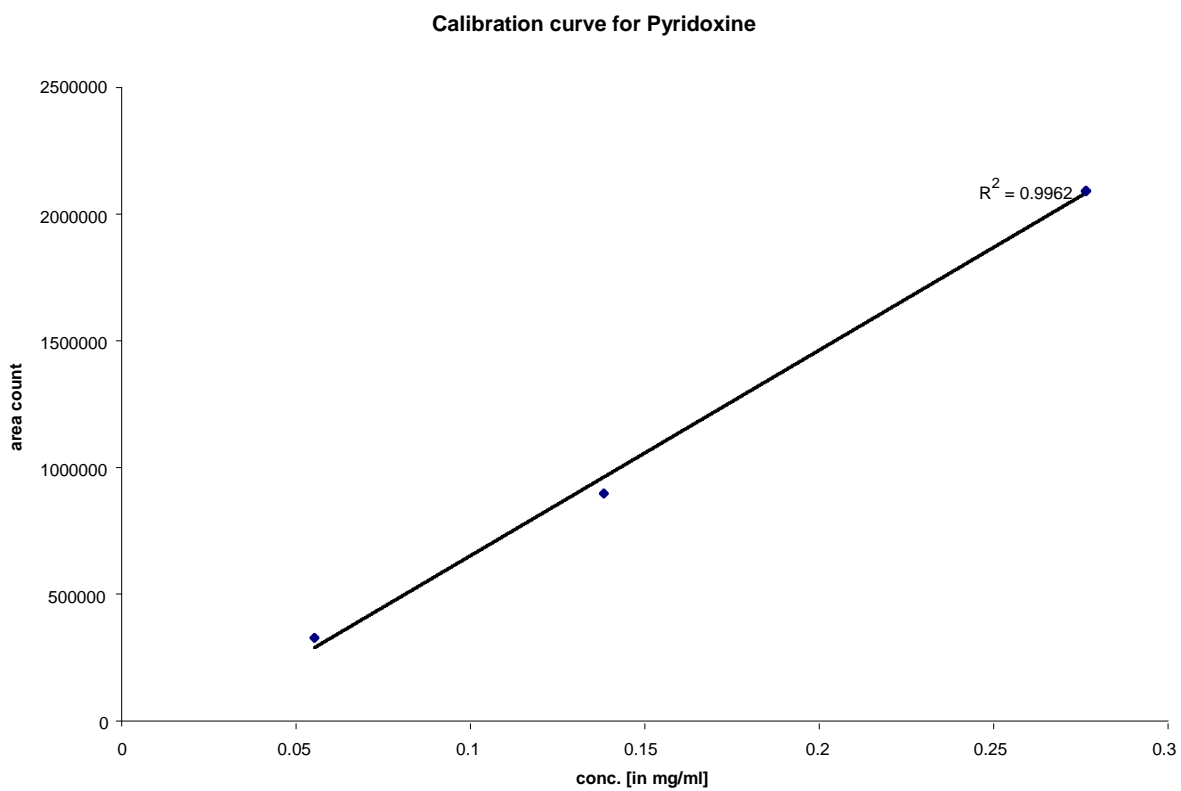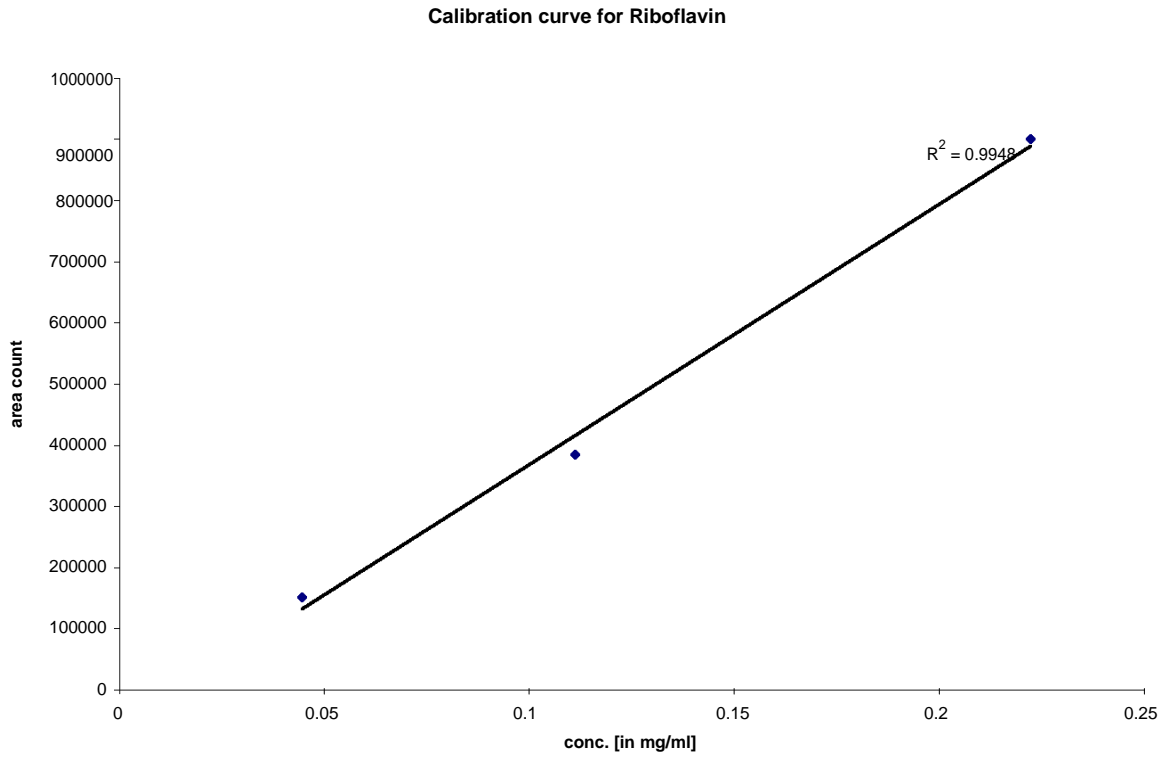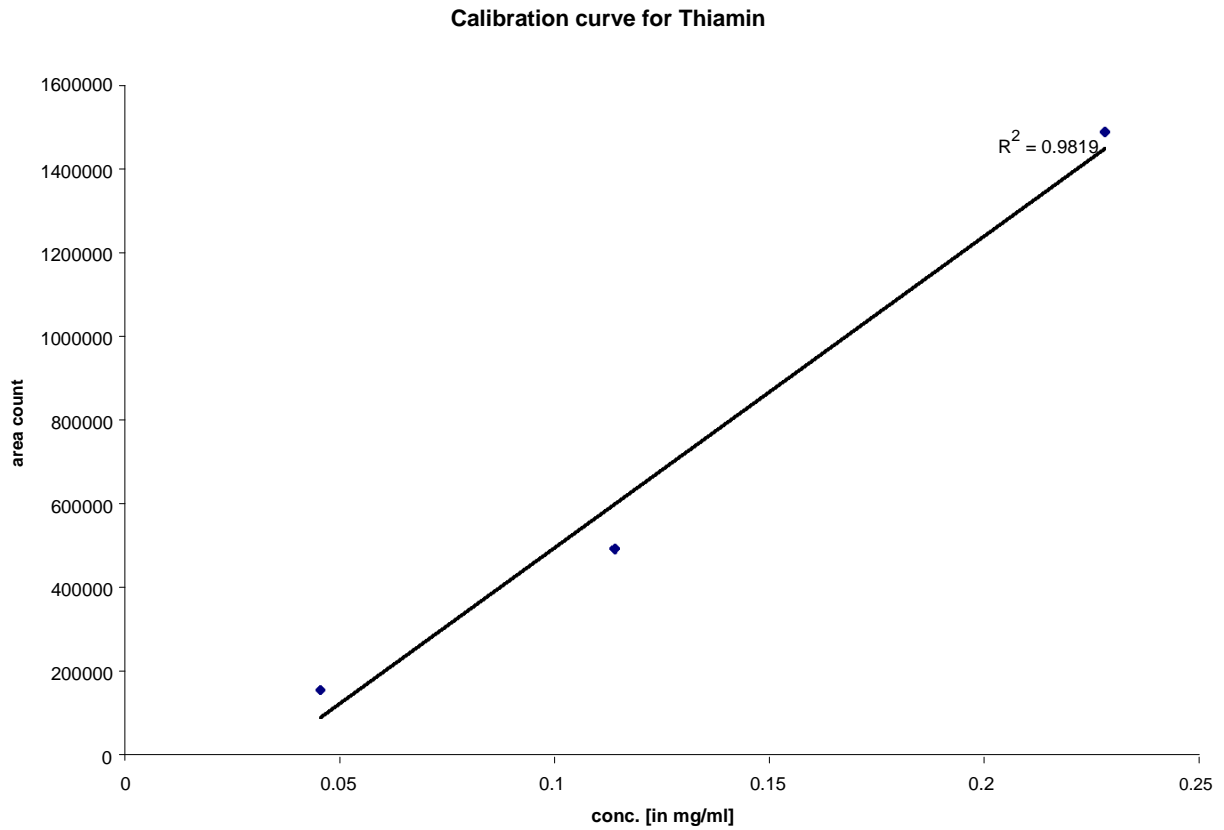
Calibration curve for Nicotinamide

$R^2 = 0.9947$

Figure 2. Calibration curve for nicotinamide
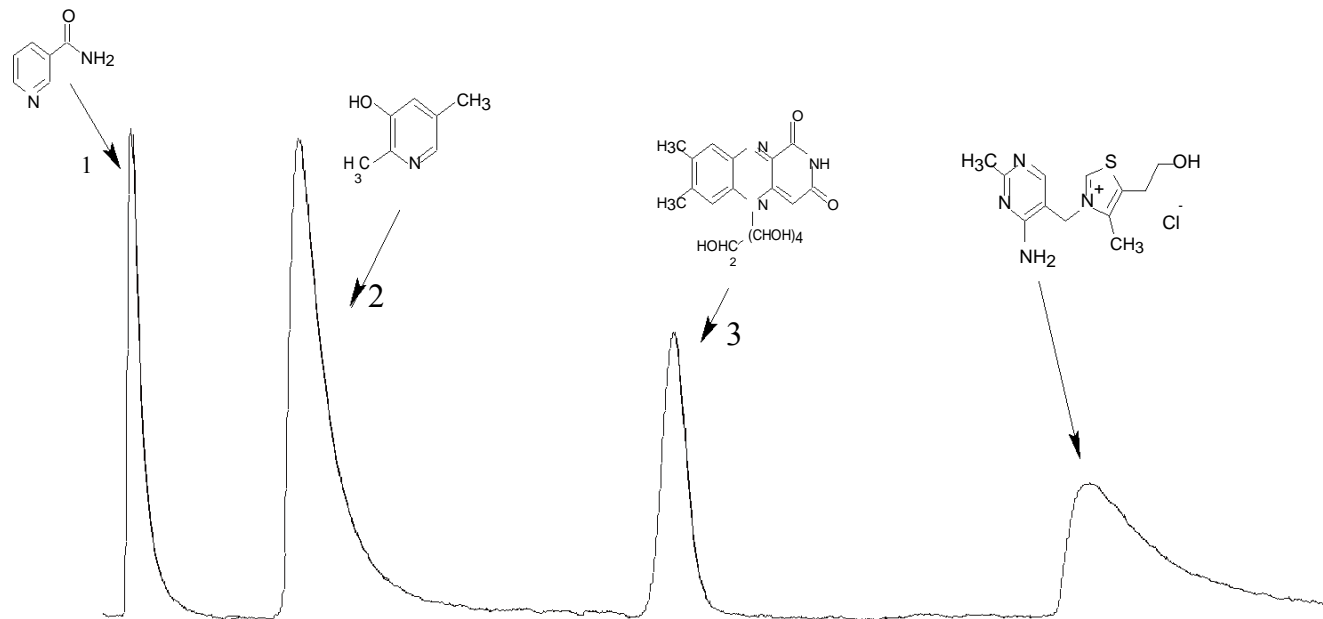
Calibration curve for Pyridoxine

$R^2 = 0.9962$

Figure 3. Calibration curve for pyridoxine.

Calibration curve for Riboflavin

Figure 4. Calibration curve for riboflavin.

Calibration curve for Thiamin

Figure 5. Calibration curve for thiamin.

Figure 6. Standard vitamins. Nicotinamide (3.857 min), pyridoxine (6.345 min), riboflavin (11.563 min) and thiamin (17.9781min) respectively analyzed at different concentrations employing the chromatographic conditions explained above.

Table 2. The distribution of the target compounds in the samples.

Sample name	Concentration (μg/ml(g)			
	Nicotinamide	**Pyridoxine**	**Riboflavin**	**Thiamin**
Sample 1	506.235	59.960	228.855	6.740
Sample 2	525.690	54.915	1433.055	6.940
Sample 3	0.065	0.732	0.459	0.025
Sample 4	64.588	23.108	234.766	2.248
Sample 5	156.745	50.975	283.295	0
Sample 6	854.053	108.532	72.036	11.868
Sample 7	34.059	120.764	210.554	0
Sample 8	0	0	62.131	121.055
Sample 9	0	0	0	2.377
Sample 10	0	0	0	5.549
Sample 11	0	5.4	0	0
Sample 12	45.25	189.659	85.585	3.12

compounds (FAO/WHO, 2002). Figure 8 compares the results from this study with the World Health Organization's recommended daily intake. Even though only the estimated average daily intake for Riboflavin andpyridoxine fell above the minimum requirement, some of the samples individually considered fell far above the recommendation but quite a number fell below this recommended daily intake. Nevertheless we can still say that the effort of the agency is working since these food samples taken are not the only sources of these compounds' intake by this population. They just serve as a supplement to traditional sources of vitamins B's. Therefore the concentrations in the analyzed samples are very adequate for complementing other sources of these compounds in the body.

We can conclude that even though the food fortification campaign is working, a more precise recommendation should be pursued to stop having such a huge variation as discovered in this study. For instance nicotinamide in 12 samples taken at random ranged from 0 ug/ml to 854.05 ug/ml with a mean value of 182.22 μg/ml. Pyridoxine has a mean value of 51.17 μg/ml but ranged from 0 to 189.659 μg/ml. Riboflavin with a mean value of 217.56 μg/ml ranged from 0 to 1433.055. Thiamine which ap-peared generally in lowest concentration ranged from 0 to 121.055 μg/ml with a mean value of 13.33 μg/ml.

THE DISTRIBUTION OF THE TARGET COMPOUNDS IN THE STUDIES SAMPLES
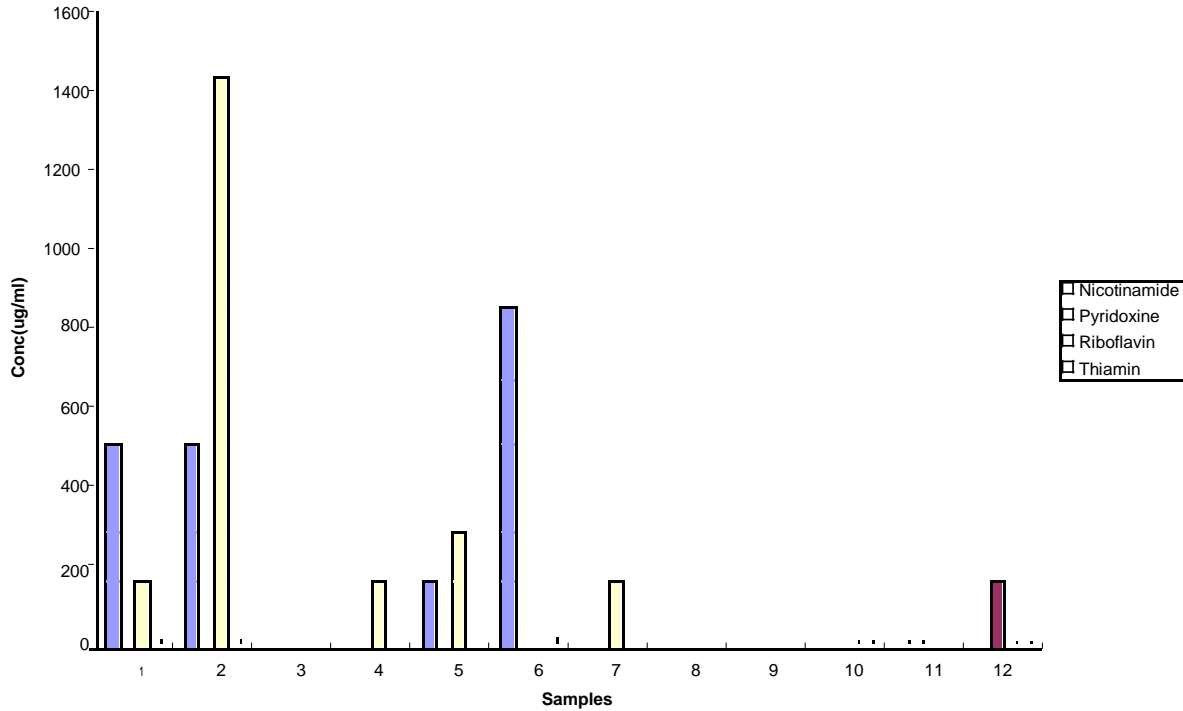

Figure 7. The distribution of the target compounds in the studied samples
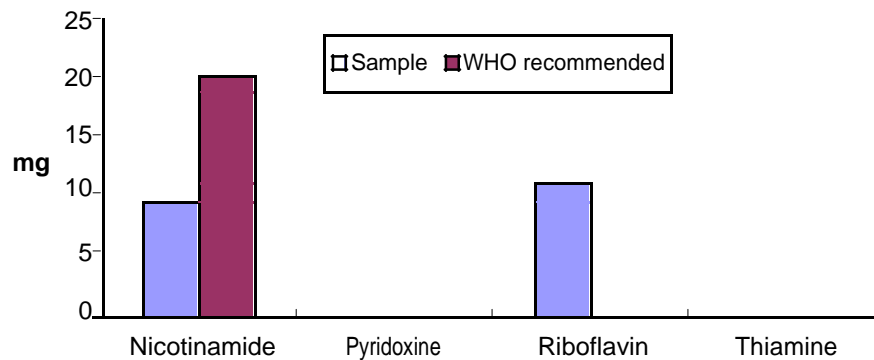

Figure 8. Comparison of the target compounds detected in the samples analyzed with the WHO recommendation [assuming 50 ml (g) daily consumption

REFERENCES

Anderson SH, Charles TJ, Nicol AD (1985). Thiamine deficiency at a district general hospital: report of five cases. Q. J. Med. 55: 15-32.

Berger NA (1985). Poly (ADP-ribose) in the cellular response to DNA damage. Radiat. Res. 101: 4-15.

Carpenter KJ, Lewin WJ (1985). A reexamination of the composition of diets associated with pellagra. J. Nutr. 115: 543-552.

FAO/WHO (1974). Handbook on Human Nutritional Requirements, eds. Passmore R, Nicol BM, Rao M, Narayana Beaton GH, de Mayer EM, FAO Nutritional Studies WHO Monograph Series, Rome, Italy. 6 : 28

FAO/WHO (2002). Human Vitamin and Mineral Requirements, WHO, Rome, Italy.

McCormick DB (1988). Thiamin. In: Modern Nutrition in Health and Disease, 6th edition. Shils ME, Young VR, eds. Philadelphia: Lea and Febiger, pp. 355-361.

McCormick DB (1988). Vitamin B6. In: Modern Nutrition in Health and Disease, 6th edition. Shils ME, Young VR, eds. Philadelphia: Lea and Febiger. pp. 376-382.

McCormick DB (1988). Niacin. In: Modern Nutrition in Health and Disease, 6th edition. Shils ME, Young VR, eds. Philadelphia: Lea and Febiger. pp.370-375.

McCormick DB (1994). Riboflavin. In: Modern Nutrition in Health and Disease, 8th edition. Shils ME, Olson JA, Shike M, eds. Philadelphia: Lea and Febiger. pp. 366-375.

McCormick DB (1996). Co-enzymes, Biochemistry of. In: Encyclopedia of Molecular Biology and Molecular Medicine, Meyers RA, ed. Weinheim: VCH. 1:396-406.

McCormick DB (1997). Co-enzymes, Biochemistry. In: Encyclopedia of Human Biology 2nd edition. Dulbecco R, ed.-in-chief. San Diego: Academic Press, pp. 847-64.

Nichols HK, Basu TK (1994). Thiamin status of the elderly: dietary intake and thiamin pyrophosphate response. J. Am. Coll. Nutr. 13: 57-61.

Olaniyi AA (2000). Essential Medicinal Chemistry, 2nd Edition, Shaneson, Ibadan, p. 324.

Patterson JI, Brown RR, Linkswiler H, Harper AE (1980). Excretion of tryptophan-niacin metabolites by young men: effects of tryptophan, leucine, and vitamin B6 intakes. Am. J. Clin. Nutr. 33: 2157-67.

Shibata K, Matsuo H (1989). Effect of supplementing low protein diets with the limiting amino acids on the excretion of N1-methylnicotinamide and its pyridones in rat. J. Nutr. 119: 896-901.