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DETERMINATION OF RIBOFLAVIN IN MULTIVITAMIN CAPSULES USING HPLC-DAD DETECTOR

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Abstract
The concentrations of vitamins contained in many drugs are unrealistic, and this leads to the fact that after taking the recommended drugs, avitaminosis develops. This study was conducted to determine and quantify riboflavin (VB2) in multivitamin capsules using high performance liquid chromatography (HPLC). Analysis was performed using an analytical reversed-phase chromatographic HPLC (Agilent 1260 Quad Pump Infinity Compartment) equipped with a Zorbax ODS column (5 µ, C18, 4.6 × 250 mm ID) operating at 30 °C. The mobile phase consisted of 5 % acetonitrile as solvent (A), 95 % water and 0.05 M formic acid as solvent (B), and autosampler injections were performed at 10.0 µl in 12 min at a flow rate of 1.0 mL/min. UV detection at 280 nm of riboflavin, which is present at various concentrations in actual samples. Compounds were identified by comparing their retention and UV spectra with those of vitamin standards. Results showed that VB2 (riboflavin) was detected in the range of 2.9-0.7 mg/g. The actual concentration of riboflavin in the multivitamin capsule differ with the concentration written on the label. The vitamin concentrations of some of the multivitamins used in this study are above the Recommended Daily Allowance (RDA), while others are below. (RDA).
Keywords; Riboflavin, Multivitamins, HPLC

Introduction

Most vitamins function in the body as part of enzymes (coenzymes). Enzymes catalyze biochemical reactions which take place in the body and are needed for normal functioning of Cells and tissues (Lewis and Stone, 2023). Riboflavin is a precursor to the coenzymes, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). These coenzymes are important for a wide range of biological reactions, including those involved in carbohydrate, fat, and protein metabolism (Buehler, 2011; Thakur *et al*., 2017). Riboflavin is a type of water-soluble vitamin that are carried to the body tissues and are absorbed in the small intestine and excreted in the urine (Abano and Rosemond, 2013). It is an essential nutrient for human health, also known as vitamin B2. It plays an important role in energy production and metabolism of fats, drugs and steroids. It also helps maintain healthy skin, eyes and nerve function.

Food's rich in riboflavin include organ meats, lean meats, eggs, green leafy vegetables, milk and dairy products and fortified grain products (Saedisomolia, *et. al.,* 2018). A lack of riboflavin can lead to ariboflavinosis, a condition marked by symptoms such as a sore throat, redness and swelling of the lining of the mouth and throat, cracks or sores on the outside of the lips (lip sores) and in the corners of the mouth (angular stomatitis), inflammation and redness of the tongue (magenta tongue) and moist, scaly skin inflammation known as seborrheic dermatitis (Buehler, 2011). Deficiencies may be especially common in populations with limited access to different foods or with specific dietary restrictions (Buehler, 2011). Genetic factors that may affect the metabolism, absorption, and activity of riboflavin in the body are attracting increasing interest (O'Callaghan *et al*., 2019). Research has

been conducted on the role of riboflavin in lowering blood pressure, particularly in people with a specific genetic profile "MTHFR 677TT genotype" (Wilson *et al.,* 2013). Research by Thompson and Saluja in 2017 and another research by Maizels and colleagues in 2004, suggests that riboflavin may be effective in preventing migraines, possibly by improving mitochondrial function.

The European Food Safety Authority stated in 2017 that recommended daily intakes vary, but general guidance recommends around 1.1 mg/day for women and 1.3 mg/day for men. Recommended daily allowance (RDA) varies by age, gender, and specific life stages (eg, pregnancy). This is why many people take multivitamins to supplement their diet.

Hence this research aims to determine the actual amount of riboflavin present in some multivitamin capsules sold in kano with respect to the amount stated on the label. Although the ingredients list of these products is available on the label but to ensure that these drugs contain the indicated amounts of vitamin, even in the absence of quality control testing as well as to investigate counterfeiting of these vitamins. Quality control testing for these vitamins is therefore required.

MATERIALS AND METHODS

Reagents and Apparatus

HPLC and analytical grade reagents, HPLC grade water milli-Q Gradient, acetonitrile HPLC gradient and formic acid used were purchased from Sigma Aldrich. All solutions were prepared with HPLC grade water and glass wares used were carefully cleaned and dried. Vitamins Standard Riboflavin, Ammonium hydroxide solution and Sodium hydroxide pellet.

All measurements were taken using analytical balance. HPLC (Agilent 1260 quad pump infinity compartment), Ultrasonic bath Branson 5510, Vortex mixer Vortexer S0100A clever scientific, Centrifuge 80-2, Weighing balance mettler Toledo, Spatula, Measuring cylinder, Pestle and mortal, Volumetric flask, Beaker, Vials, glass Vials, Rubber Test-tube, Micropipette, Glass rod and Test tube rack.

Sample Preparation/Extraction

Different brands of multivitamin supplements were purchased from Abubakar Rimi market in Kano metropolis. The multivitamins were coded as follows: AMM, ASM, HAM, HEM, FIM, MMM, ZMV.

The samples were grinded into fine powder using a mortar and pestle. 50 mg of each sample was then dissolved in 10 ml of HPLC grade water and homogenized in a vortex for 1 min. The homogenized samples were then degassed in an ultrasonic bath for 15 min. The mixture was then centrifuged for 5 mins at 3000 rpm, and then stored at 4^{o}C for later use.

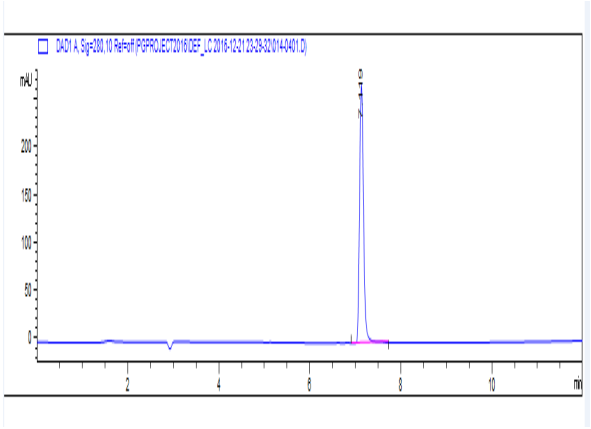
In a sample prepared by Moreno and Salvado in 2000, 0.1 g of Sodium hydroxide was accurately weighed and dissolved in 100 ml of HPLC grade water in 100 ml volumetric flask to make (0.1%) Sodium Hydroxide Solution. To prepare the Stock VB2 (Riboflavin) Standard Solution; Riboflavin Standard (50 mg) was accurately weighed and dissolved in to 10 ml of HPLC grade water in 100 ml volumetric flask to make a solution of 5mg/ml. Working standards was prepared from the stock standards on the day of use by dilution with HPLC grade water from the concentration of 0.005 mg/ml-0.12 mg/ml. The standard solution was kept at 4^{o}C and was prepared fresh daily.

Chromatographic condition/Injection

The extracted sample that was stored at 4^{0}C were put in HPLC viles and then arranged in the sample tray which is attached to the HPLC, the samples are automatically injected into the auto sampler injector after setting the chromatographic condition The chromatographic condition for the Reversed-phase Chromatographic HPLC with Zorbax ODS Column (5 µ, C18, 4.6x250 mm) was used: the mobile phase was acetonitrile 5 % as Solvent (A) and water 95 % and 0.05 % formic acid as Solvent (B). The column was operated at 30^{o}C. The flow rate was 1.0 ml/ min, and the auto injection volume was at 10.00 µl for 12 min. Detection was performed with UV-DAD detector at 280 nm for riboflavin (Antakli *et al*., 2015).

RESULTS AND DISCUSSION

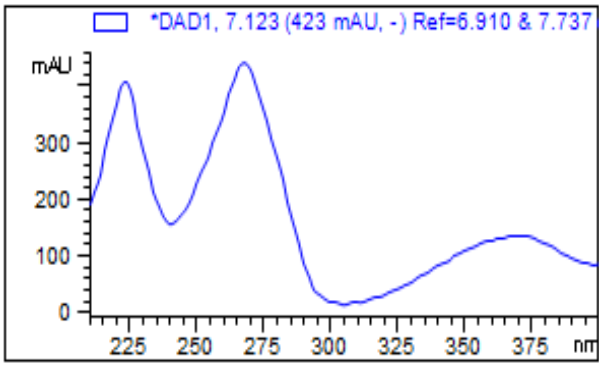
In this study, Vitamin B2 (riboflavin) was selected, which is considered necessary for cellular metabolism especially carbohydrate



metabolisms. The target vitamin was identified using retention time match against those of the

Table 1 Riboflavin (VB2) of different multivitamin samples showing consistency of retention (time) and also separate at 280 nm.

Sample	Concentration (mg/g)	Retention time (mir		
AM-M	1.5	7.1		
AS-M	3.0	7.1		
HA-M	2.0	7.1		
HE-M	1.0	7.1		
FI-M	3.0	7.1		
M-M	1.0	7.1	280	50
Z-M	1.0	7.1	280	50



(a)

(b)

Fig 1: (a) UV-Spectrum of VB2 (Riboflavin) chromatogram, (b) UV-Spectrum of VB2 (Riboflavin) Pure Standard chromatogram

calibration standards, while quantification was performed by means of peak area match against those of Standards.

The typical chromatograms of pure Standard and UV-Spectrum of VB2 was absorbed at 280nm for UV-DAD detector as presented in Fig 1. This demonstrated the excellent seperation of VB2 in 12.0 mins. The value of retention times was 7.1 mins for VB2. Table 1 shows the presence of riboflavin in all the multivitamin capsule as they were all absorbed at 280 nm with retention time of 7.1 as in the standard riboflavin.

Table 2: Mean Concentration of VB2 (riboflavin) in human multivitamin capsules

S/N	Sample	WT+CAP (mg)	WT-CAP (mg)	VB2L Conc. (mg)	VB2A Conc. (mg)
1.	AM-M	1,724.4	0.8154	1.5	1.45
2.	AS-M	434.7	358.05	3.0	2.9
3.	HA-M	541.2	438.3	2.0	2.1
4.	HE-M	1,644.5	608.8	1.0	0.8
5.	FI-M	570.75	470	3.0	2.6
6.	M-M	346.75	275.6	1.0	0.9
7.	Z-M	834.9	600	1.0	0.7

Table 3: Sample of various brands of multivitamin supplement with their label concentration and Actual concentrations

S/N	SAMPLE CODE	MEAN CONC. ± STD VB2A mg/g	P-Value	F-Value
1	AMM	1.45 ± 0.212		
2	ASM	2.9 ± 0.141		
3	HAM	2.1 ± 0.141		
4	HEM	0.8 ± 0.141	0.001	52.85
5	FIM	2.6 ± 0.424		
6	MM	0.9 ± 0.283		
7	ZM	0.7 ± 0.141		

Weight of multivitamin +capsule (WT+CAP), Weight of multivitamin without capsule (WT-CAP), concentration (Conc.), milligram (mg). Riboflavin Label concentration (VB2L), Riboflavin Actual concentration (VB2A)

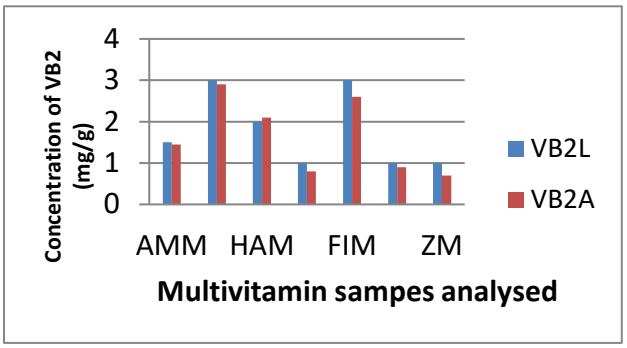


Fig 2 shows the graphical representation of table 2 showing the concentration of riboflavin presented on the label and the actual concentration of riboflavin present in the drug.

The statistical analysis used in this research is One way ANOVA. Table 2 shows the statistical results for the mean concentrations ± Standard deviation of VB2A (riboflavin) in the human being multivitamin capsule analyzed with p value 0.001. From the table it can be observed that the sample of multivitamin coded (ASM) had the highest concentration of VB2A (2.9±0.141 mg/g) among the various samples analyzed. This is followed by sample (FIM) (2.6±0.424 mg/g) and sample coded (ZM) has the least concentration of VB2A (0.7±0.141 mg/g) compared to the other samples analyzed. Table 1 shows the mean concentration of Riboflavin present in each coded drug. Table 2 shows the difference in concentration of the actual concentration of VB2A in the samples of the multivitamin capsule analyzed compared to the concentration written on the label. The analysis shows that these companies hike the amount of the concentration by 0.1 mg to 0.4 mg as compared to the actual concentration present in the multivitamin capsule. Some of these concentrations fall below the daily recommended dose while others fall above the daily recommended dose. The general recommended dose for adult woman is about 1.1 mg/day and 1.3 mg/day for men.

CONCLUSION

This analysis shows that the actual concentration present in the multivitamin was found to vary with the concentration on the label. Some were found to be above while others were found to be below the recommended daily allowance (RDA). Hence there is need for quality assurance testing on all the pharmaceutical companies to ensure that, what is written on the label is the actual concentration present in the multivitamin.

In this research HPLC was used because it can be used for detection and quantification, unlike UV spectrophotometer which can only detect while gravimetric method is time consuming and in this type of method injection of sample is only done manually which increases error and contamination. HPLC is automatic it saves time, reduces error and it injects the sample automatically thereby reducing contamination. Other vitamins were detected and quantified using the HPLC but for the purpose of this article only riboflavin was reported. This research only involved detection and

quantification of the said vitamin

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