Thiamine and Riboflavin in Pleurotusostreatus Mushrooms Assaying Using a Simplified, Specific HPLC Method in Different Substrate

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Abstract

This study investigates the quantification of thiamine (vitamin B1) and riboflavin (vitamin B2) in Pleurotusostreatus (oyster mushrooms) cultivated on various lignocellulosic substrates using a simplified specific High-Performance Liquid Chromatography (HPLC) method. The substrates included paddy, soya bean, and red gram. Mushroom samples were cultivated, harvested, dried, and ground before being analyzed. The HPLC system used Agilent TC-C18 columns with a mobile phase ratio of acetonitrile and water (90:10) and detection wavelengths of 245 nm for thiamine and 268 nm for riboflavin. Results indicated consistent retention times and accurate concentration measurements, with thiamine concentrations ranging from 3.003 µg/ml to 3.0229 µg/ml and riboflavin concentrations showing more variability from 7.22 µg/ml to 11.050 µg/ml. The method demonstrated reliability and precision, suggesting its suitability for analyzing these vitamins in similar sample matrices. Future research could explore the causes of variability in riboflavin concentrations and extend the method to other types of samples.

Keywords: Mushroom; Edible fungi; Vitamin B1 & B2

1. Introduction:

Mushrooms have been eaten as a source of nutrition for many years. While mushrooms can be found in various cultures for eating and healing, they are commonly used as a functional food in oriental cultures, such as China, with a history of thousands of years. The oyster mushroom, popular in Japan and other Asian nations, is now the second most cultivated fungus globally.

Asians have a long history of using mushrooms for medical purposes, going back over 2,000 years, in addition to their habit of eating them (Chang, 1996). After revision, Sadler (2003) came to the conclusion that some mushroom species have been utilized for over 2000 years due to their medicinal properties; research has even suggested that these

species may play a significant therapeutic function in the treatment of cancer and other ailments.

Mushrooms have been part of human diet for thousands of years and recently their consumption has been increasing, involving a great number of species apart from the popular oyster mushroom. More than 2000 edible species of mushroom are known, but only 25 are commercially cultivated. In India the main species cultivated are Agaricusbisporus, Volvariellavolvacea and Pleorotus spp., which according to ICAR-DMR, Solan. The annual report published in 2022 by ICAR- Directorate of Mushroom Research (DMR), Solan states that in 2016 India produced 129.782 metric tons of mushrooms which increased to 280.36 metric tons in 2022 and it is estimated that by 2030 it will further rise up to 3.31 lakh tons.

Food nutrition information is becoming more and more significant for both consumers and professionals working in the food and health industries. Consumers are growing more concerned about the nutritional value of the foods they eat or may include in their diets. However, there is limited information available regarding the nutritional content of the edible mushrooms grown in India. Because vitamins play such important roles in the bodies of humans and animals, their content is quite useful. According to Breene (1990), mushrooms can be a good source of vitamin B1, B2, niacin, and biotin. This study set out to quantify the amounts of vitamins B1 and B2 in Indian-grown mushrooms.

2. Materials and methods:

2.1 Samples–

The mushroom species analyzed in this study was: Pleurotusostreatus (Oyster mushroom). This mushroom species was cultivated on different lignocellulosic substrates like paddy, soya bean and redgram. Seed for cultivation was brought from IGKV Raipur and cultivated in mushroom cultivation room in Bio Resource Centre of Govt. V. Y. T. PG Autonomous College Durg. D.M.R. Solan's manual was followed for cultivation. The methodology was validated using about 250 grams of oyster mushrooms harvested from the production area and dried at 45°C for 72 hours. The dried mushrooms were collected and ground into small particles with the help of a blender and stored in desiccators to be analyzed. The sample code of mushroom taken is P1 for Paddy substrate, S1 for Soya bean substrate and R1 for Red gram substrate

2.2 Regents–

Sigma Chemical Co., USA provided the riboflavin (B2) and thiamine (B1) standards. In 0.1 N HCl, the usual vitamin solutions were made. The source of the enzyme takadiastase was Fluka, Switzerland. The other reagents were analytical quality, while the organic solvents utilized as the mobile phase in liquid chromatography were of chromatographic grade. The mobile phases were prepared using Milli-Q (Millipore) purified water. Filtration was performed on all mobile phases using membranes that had pore sizes of 0.22 µm.

2.3 Equipment–

The vitamins were determined using an HP model 1220 Lc system VL high performance liquid chromatograph, which included a temperature control compartment for the analytical column, a quaternary pump, an automatic injection system (0–100 lL), a diode array, and fluorescence detectors connected in series. Multiple wavelengths could be detected simultaneously thanks to the detection system. The openLab-ChemStation software managed the data gathering and treatment system in addition to controlling the entire system. Acquired weight information from the ME204 Digital Mettler Analytical Balance.

2.4 Stock solution of standard –

Weighed accurately 2 mg of standard powder and dissolved in 2 ml of methanol. From the Stock solution, working standard solution of standard was prepared by appropriate dilution with methanol. Standard calibration curve was prepared with 3-point calibration of concentrations 12.5, 25, 50 µg/ml. Stock solution was diluted to obtain desired concentrations of working solutions by serial dilution method.

2.5 Preparation of Sample –

The sample code of mushroom taken is P1 for Paddy substrate, S1 for Soya bean substrate and R1 for Red gram substrate. Transferred 5µg an accurately weighed quantity of extract in micro centrifuge tube, added about 5ml of water and sonicated for 15min, then filtered it with 0.22µm 25mm nylon membrane filters before injecting in the machine.

2.6 Extraction of Vitamin–

Firstly the sample was homogenized and was then weighed (5gm). Then the weighed sample was taken in a reagent bottle and then the extraction solvent (here distilled water) was poured into the bottle, keeping the ratio as 1:10 (i.e. for 5gm sample 50 ml water). Then the bottle was kept on rotary flask shaker for at least 48 hrs. After that the mixture

in reagent bottle was filtered in a pre-weighed beaker, using whatman filter paper, so that there were no sample remains in the extract. Then the filtered solvent was kept for drying at 90°C for 3hrs. After drying, the extract obtained was weighed (for yield), and then was dissolved in desired solvent and used for analysis on HPLC.

2.7 Chromatographic Conditions –

Stationary phase Agilent TC-C18 (2), 4.6x250mm, 5um. Mobile phase: Acetonitrile: Water. Mobile phase ratio: 90:10. Detection wavelength: 245 nm (Thiamine) and 268nm (Riboflavin). Flow rate: 0.4 ml/min. Sample volume: 20µl. Test method temperature: Ambient. LC System: Agilent test system and OpenLab CDS2.

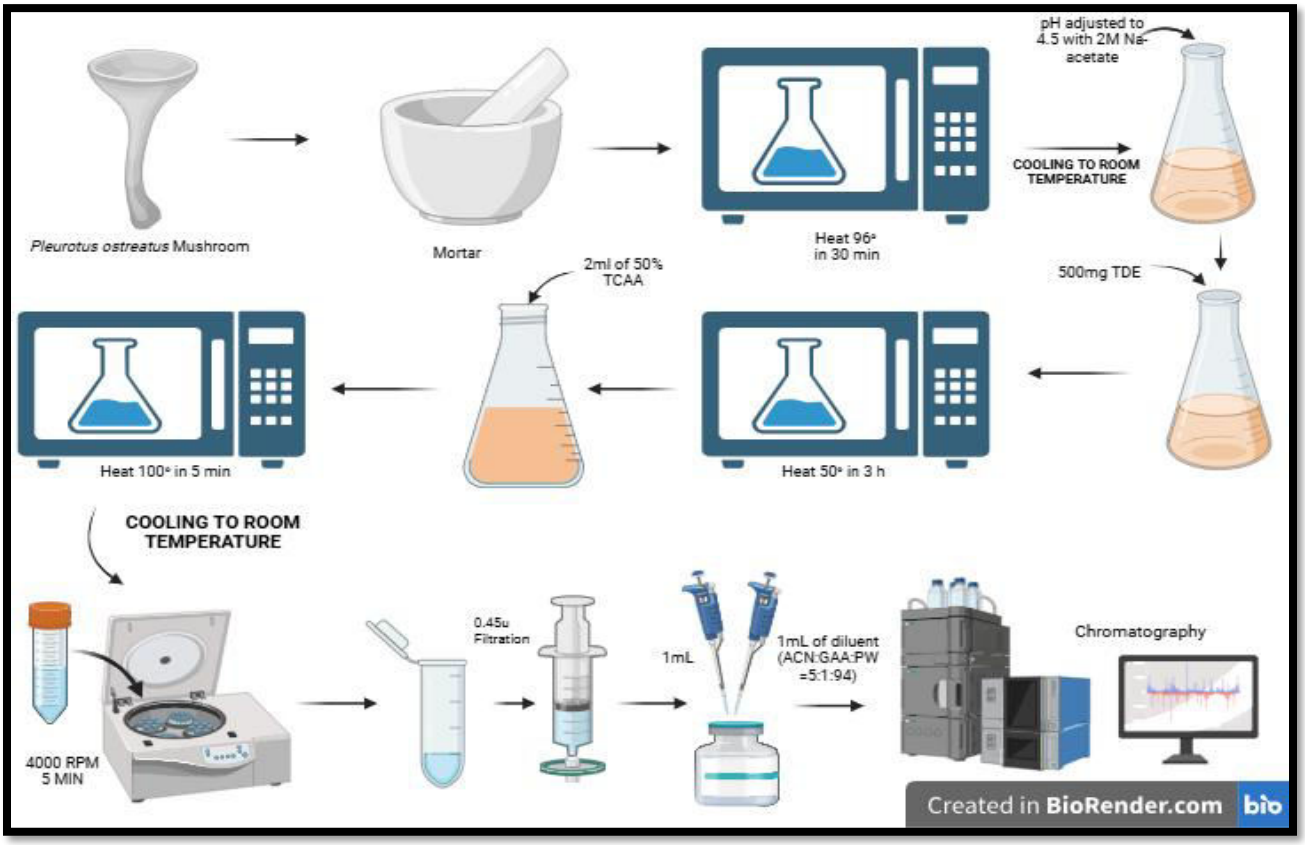

Figure 1: Schematic diagram of the mushrooms extraction method (created in BioRender software)

3. Results and Discussion:

3.1 Thiamine Concentration -

The quantification of thiamine in the samples (P1, A1 and S1) was performed using a 3-point calibration method. The results are summarized in the table below:

S.No.	RT	Concertation (µg/ml)	Peak area
1	0	0	0
2	8.2	50	57248464
3	8.19	25	24461813
4	8.2	12.5	10087231

Table 1: 3-pt calibration of standard Thiamine and calculation of concentration of thiamine in samples (P1, A1 and S1)

S.No.	Sample Code	RT	Concertation (µg/ml)	Peak area
1	P1	8.120	3.003	3000
2	A1	8.203	3.0187	18751
3	S1	8.127	3.0229	22977

Table 2: Concentration of Thiamine determined in samples (A1, P1 and R1) from calibration graph of standard.

3.2 Riboflavin Concentration -

The quantification of riboflavin in the samples (P1, A1 and S1) was also performed using a 3-point calibration method. The results are summarized in the table below:

S.No.	RT	Concertation (µg/ml)	Peak area
1	0	0	0
2	7.61	12.5	804761
3	7.612	25	2321043
4	7.612	50	4913141

Table 3: 3-pt calibration of standard Riboflavin and calculation of concentration of riboflavin in samples (P1, A1 and S1)

S.No.	Sample Code	RT	Concertation (µg/ml)	Peak area
1	P1	7.617	7.22	532124
2	A1	7.633	11.050	917951
3	S1	7.627	8.753	686273

Table 4: Concentration of Riboflavin determined in samples (A1, P1 and R1) from calibration graph of standard.

3.3 Discussion -

The study's goal was to quantify the levels of thiamine and riboflavin in three samples (P1, A1, and S1) using gradient High-Performance Liquid Chromatography. The procedure involves developing standard calibration curves for thiamine and riboflavin, followed by sample analysis.

3.4 Thiamine Analysis -

The retention time for thiamine was consistent across all samples, indicating reliable detection and measurement conditions. The concentrations of thiamine in the samples were found to be quite similar, ranging from 3.003 µg/ml to 3.0229 µg/ml. This close range suggests that the thiamine content is evenly distributed among the samples, demonstrating the precision of the HPLC method used.

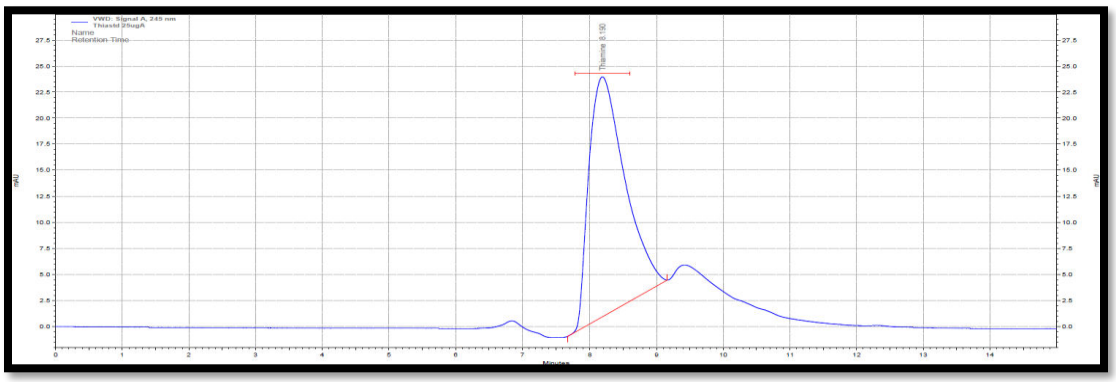

Figure2: Chromatogram of standard-25µg/ml (Thiamine) at 245 nm andrun time of 15 minutes (RT: 8.190min)

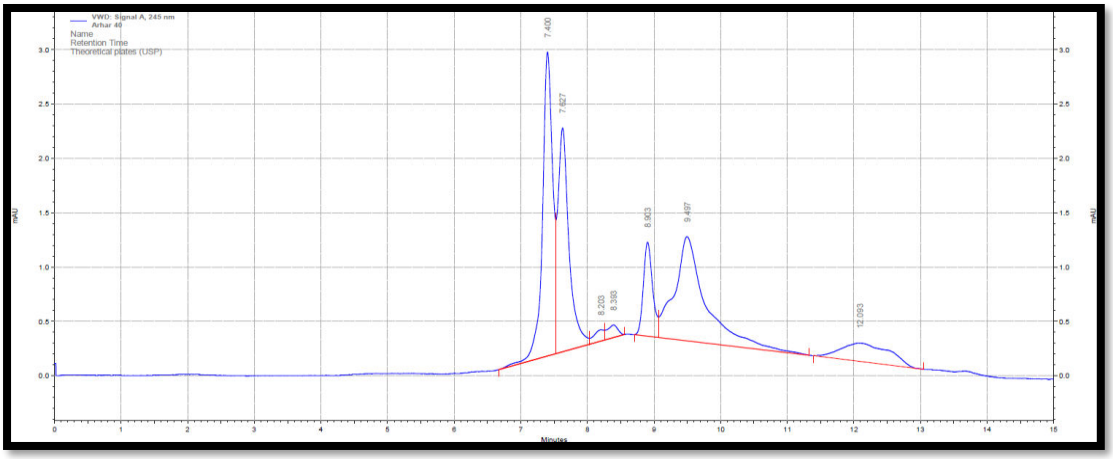

Figure3: Chromatogram of sample (A1) for estimation of thiamine at 245 nm and runtime of 15 minutes.

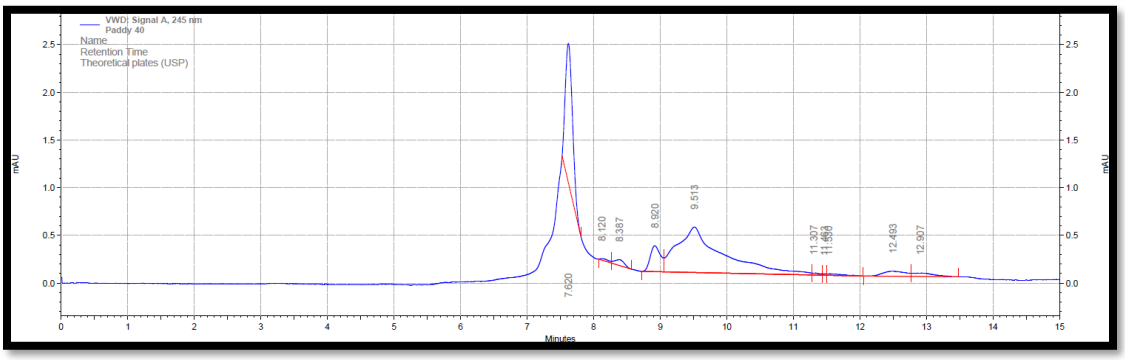

Figure 4: Chromatogram of sample (P1) for estimation of thiamine at 245 nm and runtime of 15 minutes

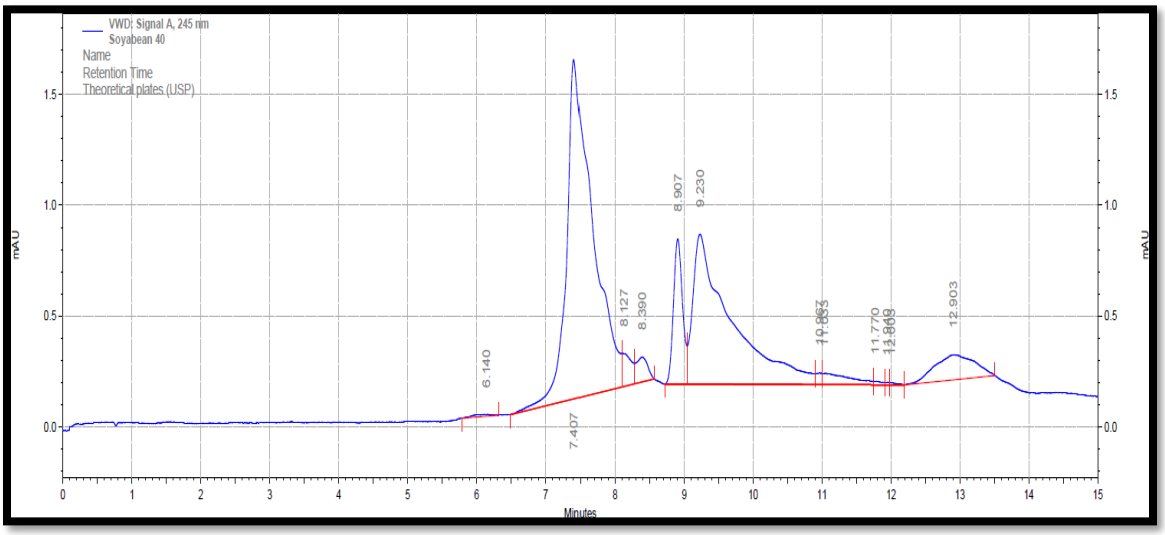

Figure 5: Chromatogram of sample (S1) for estimation of thiamine at 245 nm and runtime of 15 minutes

3.5 **Riboflavin Analysis -**

The retention period for riboflavin was likewise constant, albeit compared to thiamine, the concentrations varied a little more. Compared to samples P1 (7.224 µg/ml) and S1 (8.753 µg/ml), sample A1 had a concentration that was significantly higher (11.050 µg/ml). This variance may be the result of minor variations in the preparation or measurement procedure, or it may be the result of alterations in the original sample composition.

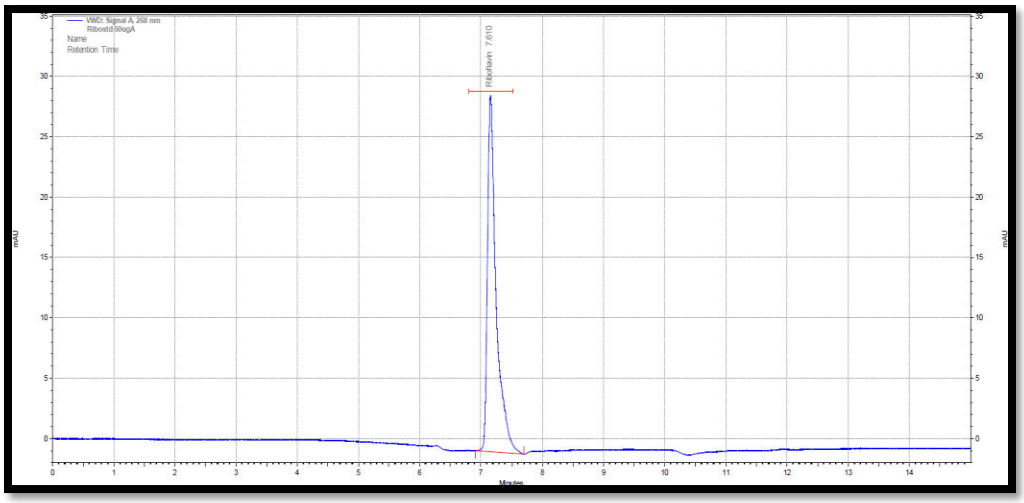

Figure 6: Chromatogram of standard-50µg/ml (Riboflavin) at 268nm and runtime of 15 minutes (RT: 7.61min)

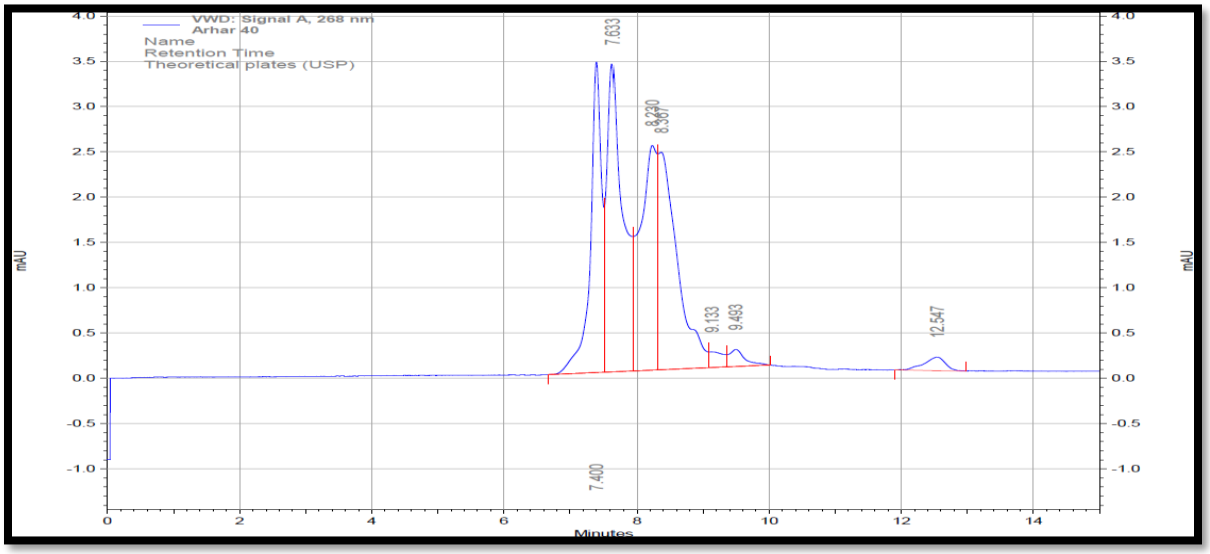

Figure 7: Chromatogram of sample (A1) for estimation of Riboflavin at 268 nm and runtime of 15 minutes

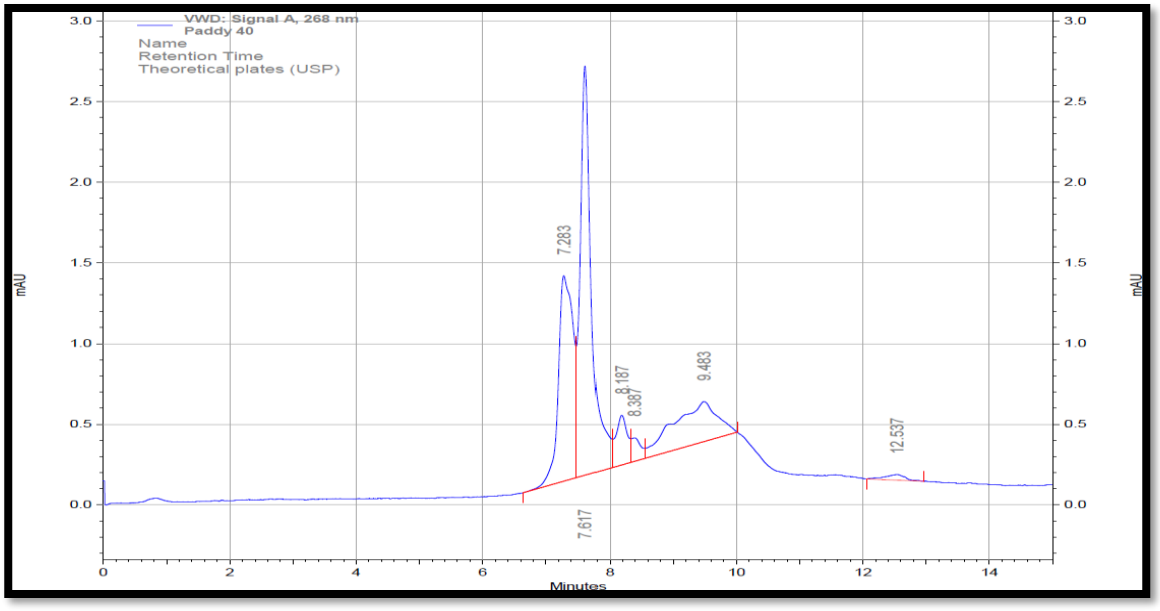

Figure 8: Chromatogram of sample (P1) for estimation of Riboflavin at 268 nm and runtime of 15 minutes

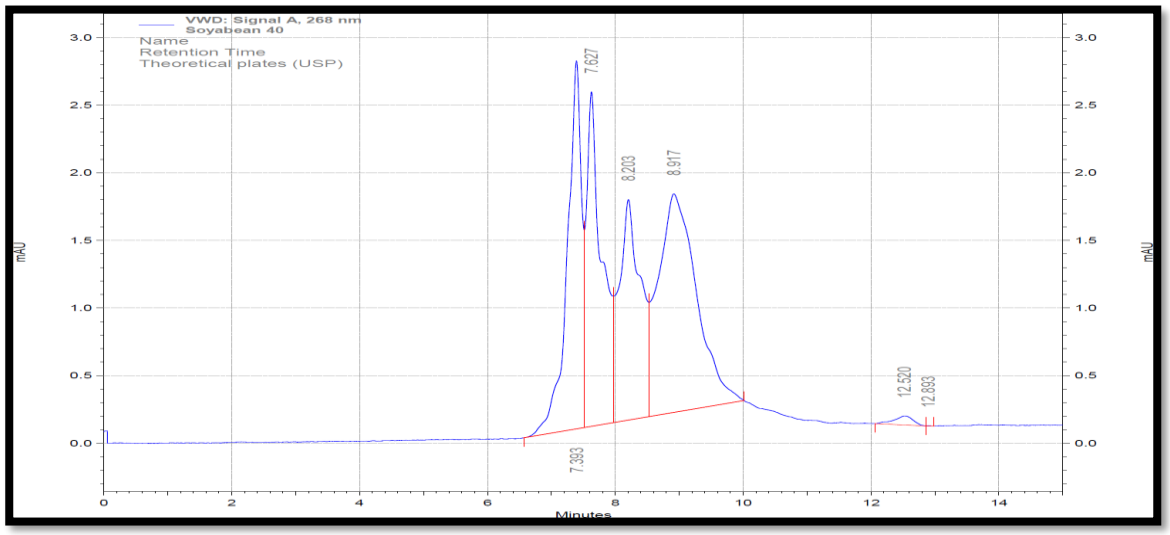

Figure 9: Chromatogram of sample (S1) for estimation of Riboflavin at 268 nm and runtime of 15 minutes

3.6 **Method Reliability** -

The separation and measurement of these vitamins were successfully accomplished by using HPLC under particular chromatographic circumstances (e.g., Agilent TC-C18 column, 90:10 Acetonitrile: Water mobile phase ratio, and detection

wavelengths of 245 nm for thiamine and 268 nm for riboflavin). Accurate and repeatable findings were guaranteed by the meticulous preparation procedures and calibration techniques.

4. Conclusion:

Gradient HPLC was used to successfully quantify the thiamine and riboflavin in the samples, yielding accurate concentration readings and consistent retention times. The findings show that the approach is reliable and appropriate for analyzing these vitamins in sample matrices that are comparable. Subsequent investigations may delve into the causes of the noted fluctuations in riboflavin concentrations and evaluate the method's suitability for use with different kinds of samples.

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