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COMPARATIVE EVALUATION OF WATER-SOLUBLE VITAMIN CONTENT IN CHICKEN MEAT FROM LAHORE MARKETS AGAINST WHO STANDARDS

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Abstract

Water-soluble vitamins (WSVs) are vital micronutrients that play important roles in metabolism and general health. These include vitamin C and the B-complex vitamins (thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, biotin, folic acid, and cobalamins). Through the use of high-performance liquid chromatography (HPLC), the existence of seven important WSVs in chicken meat was to be quantified. Samples of chicken were bought from Lahore's regional markets, and their vitamin content was examined. For HPLC calibration, standard solutions for each vitamin were made. The findings showed that the chicken samples had considerably lower quantities than the reference levels. Numerous elements of Pakistani poultry production are blamed for this shortfall, including the use of growth-promoting chemicals, depleted soil, low feed effectiveness, intensive farming methods, and contaminated water supplies. The results emphasize the necessity of bettering nutritional guidelines and farming methods in order to increase the amount of vitamins in chicken meat and provide consumers with higher-quality nutrition.

INTRODUCTION

In accordance with their ability to dissolve, vitamins—a vast set of vital micronutrients—can be divided into two categories: fat-soluble vitamins (FSVs) and water-soluble vitamins (WSVs). Vitamin C and vitamins B complex, including thiamine (B1,VB1), riboflavin (B2,VB2), niacin (B3,VB3), pantothenic acid (B5,VB5), pyridoxine (B6,VB6), biotin (B7,VB7), folic acid (B9,VB9), and cobalamins (B12,VB12), are included in WSVs [1]. Previously known as vitamin H/coenzyme R, biotin is also known as vitamin B8 (VB8). A number of vitamins are included in some B vitamins, including VB1, VB2, VB6, and VB9. The chemical makeup, structural intricacy, stability, and physical characteristics of these WSVs vary. While they are all hydrophilic, there is a significant difference in how soluble they are in water, ranging from exceptionally soluble vitamin C to less soluble Vitamin B2 [2].

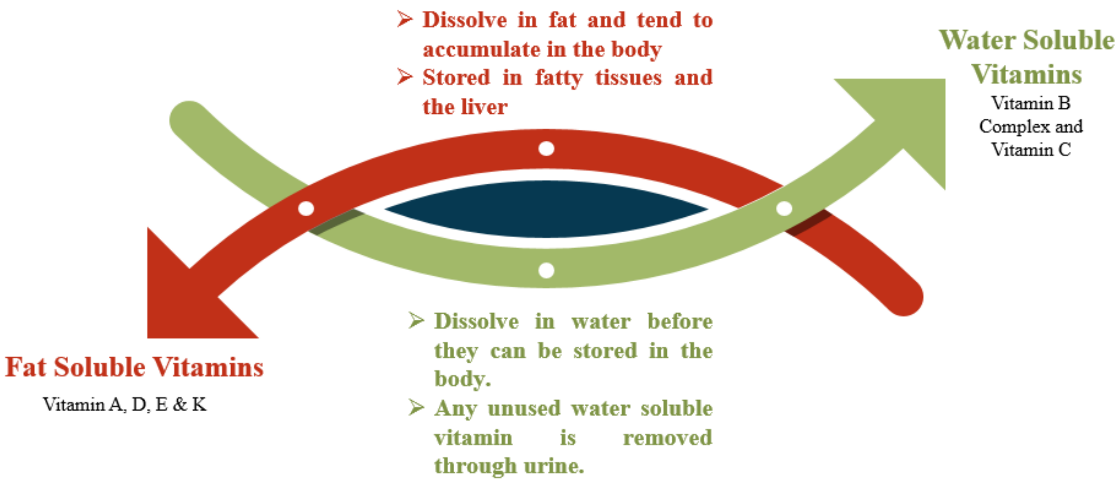

Figure 1. Classification of Vitamins.

WSVs are widely recognized for their unique function in liver, muscle, skin, eyes, and hair health as well as in energy metabolism. They function as precursors of coenzymes and cofactors of enzymes in various metabolic processes that take place in the body, such as the cellular breakdown of lipids, carbohydrates, and proteins. Nearly a century ago, it became clear that vitamins were essential for human health. Conditions including rickets, pellagra, scurvy, and beriberi were once brought on by a lack of water-soluble vitamins [3].

Clinical investigations led to the establishment of a connection between the physiological and biological roles of certain vitamins. For instance, proline and lysine hydroxylase enzymes, which fix the tertiary structure of the collagen molecule and stimulate collagen gene expression, require vitamin C as a coenzyme [4].

The present scientific justification for assessing the health impacts of vitamins frequently rests on fundamental metabolic concepts and clinical symptoms observed during evident clinical deficits. While vitamin shortages are frequent and vary by location, gender, and age, clinical observation of vitamin deficiencies is uncommon internationally, particularly in developed nations [5].

1.1. Desired Water Soluble Vitamins for this Research

Vitamins of this kind are easily absorbed by our body due to their fluid composition, and the renal system have the ability to eliminate them. Vitamin C and the B-complex vitamins are examples of water-soluble vitamins.

1.1.1. Vitamin C

Ascorbic acid, or vitamin C, is a crystalline substance that dissolves in water. It is synthesized by most animals and plants; however, human beings do not have gluconolactone oxidase, an essential enzyme in the last stage of ascorbate formation. Therefore, the diet must supply these species with the necessary amount of vitamin C. The best foods to receive vitamin C from include oranges, grapefruits, green vegetables, and beef liver [6]. Vitamin C is necessary for wound recovery, a healthy immune system, and the formation of collagen, which strengthens connective tissues. A lack of vitamin C alters the connective tissue, which can result in scurvy, a health problem involving the synthesis of collagen [7].

1.1.2. Vitamin B1

Thiamine, frequently referred to as vitamin B1, functions as thiamine Pyrophosphate, a kind of coenzyme engaged in the breakdown of carbohydrates. The hexose monophosphate shunt also involves thiamine pyrophosphate. It's an agent that protects neurons [8]. Nuts, potatoes, pork, legumes, and cereals all contain thiamine. Beriberi is an illness caused by its lack. Because the vitamin is lost from the seed coat of refined rice, it is frequently seen in cultures that consume processed rice. Beriberi comes in three varieties: dry, moist, and infantile [9]. It is essential for the cardiac system, neurons, and muscles to work properly and is a co-catalyst in the digestion of sugar. Unmilled oats, wheat germ, beets, almonds, animal products, lentils, yolks, chicken, green peas, and lush green vegetables are some of the foods high in vitamin B1. Deficiency of this vitamin may result in alcoholics developing polyneuritis, mental disorder, ataxia, or Wernicke-Korsakoff syndrome. Overconsumption can result in headaches, tachycardia, or peevishness, a sleeping disturbance [10].

1.1.3. Vitamin B2

Yellow-crystalline riboflavin is commonly known as vitamin B2. Grain, eggs, dairy products, and oats are good sources of riboflavin. It contributes to the respiration of tissue. Ariboflavinosis is the disorder caused by its insufficiency. The symptoms of ariboflavinosis include cheilosis, which is a patterned degeneration of the skin that surrounds the mouth; glossitis, which is a sparkling red and sensitive tongue; sore lips; eye problems; light sensitivity; an oily complexion surrounding the nose; and scrotal dermatitis [11].

1.1.4. Vitamin B3

Reduces the risk of heart disease, helps reduce bad cholesterol levels, and relieves arthritic pain. It was discovered in animal products like liver, chicken, and lean red meat. An outstanding way to obtain niacin is pea butter. Coffee, tea, whole grain cereals, etc. are other helpful resources. Numerous metabolic processes, such as those involving the skin, neurons, and digestive system, are either directly or indirectly impacted by niacin. It's crucial for turning food into energy as well. Extreme niacin deficiency results in the disease pellagra, which is characterized by diarrhea, dermatitis and other skin problems, dementia, inflammation of the mouth and tongue, and additional signs that, if neglected, can be fatal. Minor shortages may also result in slow metabolism and migraine [12].

1.1.5. Vitamin B5

Pure pantothenic acid has a thick liquid consistency. Its pKa value is 4.41. It will hydrolyze in acidic condition, where it is less persistent; pH 5-7 is the optimal range for stability. Typically, pantothenic acid is given as calcium pantothenate, which is soluble and more resistant to light, heat, and oxygen than pantothenic acid, but unstable in both basic and acidic environments [13]. The majority of plant and microbial life produce pantothenic acid through biosynthesis. Because human beings, animal, and some microscopic bodies are unable to produce this vitamin, they must obtain it from outside sources [14]. Meat, eggs, dairy products, almonds, mushrooms, yeast, and offal (liver and kidney) are important sources [15]. The synthesis and breakdown of fatty acids depend on vitamin B5. It participates in both the production of fatty acids and the breakdown of fatty acids to provide energy, a process known as beta-oxidation. In yeast and cells from mammals, the production of coenzyme A (CoA) and acyl-carrier protein (ACP) depends on Pantothenic acid [16].

1.1.6. Vitamin B6

Being a coenzyme engaged in more than 150 metabolic events, vitamin B6 is an intriguing substance implicated in most changes that occur in the human body. Pyridoxal phosphate (PLP), the active form, is a cofactor in over 160 bodily reactions. Thus, the terms "PLP" and "vitamin B6" are synonymous [17]. While bacteria and yeasts can make this chemical in a different fashion, neither humans nor other higher species are able to manufacture it. Because a particular pyridoxal kinase (PDXK) is present, humans can transform dietary pyridoxal, pyridoxine, and pyridoxamine into active phosphates [18]. PLP regulates various physiological processes that affect human well-being and illnesses, including blood pressure management (which has an effect on the renin-angiotensin pathway), coagulation of blood, endothelial integrity, and the accumulation of platelets [19].

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1.1.7. Vitamin B9

The synthetic form of folate, a water-soluble vitamin present in fruits, vegetables with green leaves, and liver is called vitamin B9, or folic acid. Folate, or vitamin B9, is converted to its active form, tetrahydrofolate. It is a necessary chemical for the creation of DNA and RNA. Pregnant women should take folate supplements as a prophylactic measure since folate deficiency can result in neural tube abnormalities. Megaloblastic anemia is a form of macrocytic anemia that can also be caused by a folate shortage. This differential diagnosis is made in conjunction with a lack of vitamin B12, which can also induce megaloblastic anemia [20].

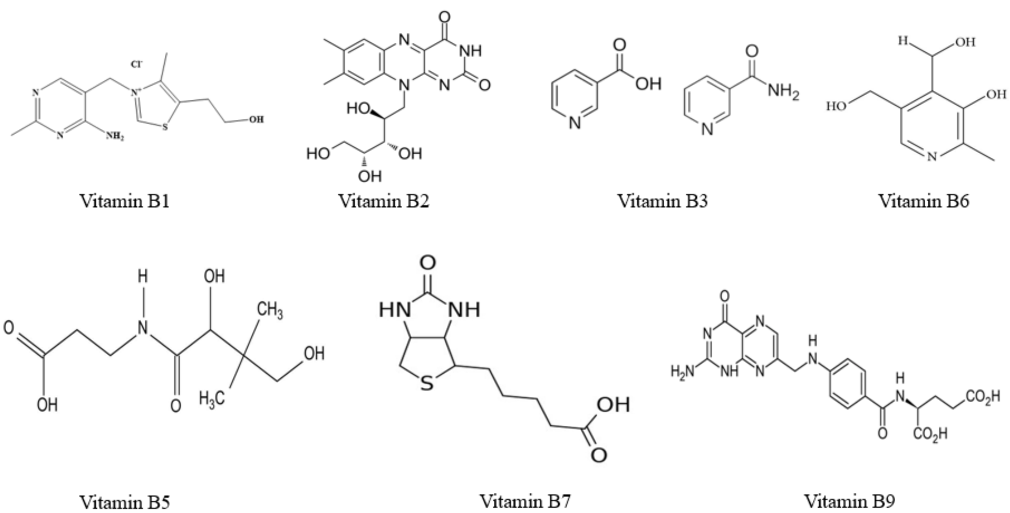


Figure 2. Molecular Structures of water soluble vitamins.

Hong et al. (2021) looked into the B1, B2, B3, and B7 water-soluble vitamins that are present in Korean meat, cereal, and noodle products. Regarding meat items, B1's values varied between 0.039 and 0.295 mg/100 g, whilst B2's contents varied between 0.029 and 0.327 mg/100 g [21].

Alugwu et al. (2023) carried out their research to determine the impact of heat treatments on the water soluble vitamins and selected mineral (Ca, K, Mg, Na, P, Fe, and Zn) concentrations of chicken breast meat. Samples of industrially free of skin chicken breast meat were bought, shipped in a cool environment to the Bioprocess labs, chilled, cut into the appropriate sizes, and then melted. The samples were cooked at 170, 180, and 1900 degrees Celsius for 0, 4, 8, and 12 minutes for minerals and 0, 8 and 16 minutes for vitamins using air frying (AF), baking (BK), deep fat frying (DF), and grilling (GR). Prepared and uncooked items were incubated in wet acid for one night, followed by five hours of digestion in a block digester at a gradually raised temperature of 1200 degrees Celsius, cooling, and deionization. Optima 4300DV inductivity coupled plasma mass spectrometry (ICP-MS) and inductivity coupled plasma optical emission spectrometry (ICP-OES) were used to analyze the mineral elements. In the meantime, the vitamins were determined by measuring the absorbance of the filtrates of the samples soaked in the corresponding solvents in the Spectrophotometer at various wavelengths in comparison to the blank samples. Vitamins B1, C, B2, B9, and B6 showed greater percentage declines in samples cooked by DF, at 55.10%, 37.93%, 37.11%, 34.44%, and 30.99%. Conversely, samples subjected to baking (Bk) and grilling (GR) showed greater percentage losses in vitamins B12 and B3, of 41.67% and 31.84%, respectively [22].

Fatima et al. documented the recent developments in the investigation of water-soluble vitamins (WSVs), taking into account the benefits and drawbacks of several methods for extraction, differentiation, and identification that are frequently employed to quantify these vitamins. It has been suggested to use acid hydrolysis, enzyme treatment, SPE based approaches, and various other extraction techniques. Analytical methods based on liquid chromatography and electrophoresis have received special attention. Additionally, a thorough discussion of the applicability and specificity of hydrophilic interaction liquid chromatography (HILIC) for WSVs has been held. Issues pertaining to these methods and potential fixes have also been taken into account. From 2014 to 2019, particular attention has been paid to the use of liquid chromatography in the simultaneous study of WSVs and their homologue in complicated dietary samples [2].

2. Materials and Methods

2.1. Chemical Reagents

The following materials were used to analyze the vitamins that are soluble in water:

- Acetonitrile (HPLC grade)
- Trichloroacetic Acid (HPLC grade)
- Oxalic Acid Solution
- Sodium hydroxide (ACS-grade)
- Thiamine hydrochloride standard (B1)
- Riboflavin standard (B2)
- Nicotinamide-niacin amide standard (B3)
- Pyridoxine hydrochloride standard (B6)
- Calcium D-Pantothenate standard (B5)
- L- Ascorbic Acid (Vitamin C) and folic acid (B9)

Riboflavin was dissolved in water using a 0.1 N NaOH solution to create a basic medium.

2.2. Standard Preparation

In order to get our B Vitamins Standards solutions at 0.2 mg/ml concentration 0.01g of each: thiamine hydrochloride for B1, nicotinamide for B3, Calcium D-pantothenate for B5, pyridoxine hydrochloride for B6, folic acid for B9 and ascorbic acid for vitamin C were diluted in a 50 ml of double-distilled water in a measuring flask. To make the vitamin B2 standard solution 0.01g of Riboflavin was dissolved in 50ml of 0.1N NaOH solution. The reference solution was ultra sonicated for 20 minutes at room temperature. Preparation processes were carried out under reduced lighting conditions. Using foil-covered glasses can prevent vitamin breakdown, particularly vitamin B6, B2 and VC.

2.3. Sample Preparation

Chicken meat was bought from local market of Lahore. It was thoroughly washed, taken from different parts of chicken and was crushed using pestle mortar. This was done to have a homogenized and evenly distributed sample. Crushed chicken sample was then used in order to extract its vitamins that are specifically soluble in water.

2.3.1. Preparation of Extracting Solution

Oxalic Acid solution was used to extract the water soluble vitamins from the chicken meat sample. 1 g of oxalic acid was added drop wise in 99 ml distilled water and was stirred for almost half an hour to ensure thorough mixing.

2.3.2. Extraction of Vitamins from sample

Almost 11 grams of sample was taken in petri dish and 25 ml of extracting solution was added to it. The petri dish was covered and allowed to stay for almost 2 hours. After almost 2 hours petri dish was uncovered and color of chicken sample turned whitish from its original appearance.

2.3.3. Centrifugation

The centrifugation of sample was then done to separate the liquid obtained after the sample was allowed to stay in oxalic acid solution in order to extract desired vitamins from that sample. The centrifugation was done at 7000 revolutions per minute (rpm) for 10 minutes. This resulted in the separation of liquid from solid portion. The solid sat down and liquid above was taken for further processing.

2.3.4. Filtration

Filtration was done in two steps:

1) Filtration with ordinary Filter Paper.
2) Syringe Filtration.

Firstly, the liquid obtained after centrifugation was filtered using ordinary filter paper. Setup was arranged to perform this filtration to get rid of the presence of solid inclusions in the liquid obtained as a result of centrifugation. After this, to ensure the complete absence of any type of solid inclusions in the liquid sample, syringe filtration was performed. The filter used for this purpose was of 0.45 micrometer.

2.3.5. Analysis by HPLC

The equipment used for this study was a High-Performance Liquid Chromatography (HPLC) device with a four solvent delivery system quaternary pump and an Ultra Violet/Visible (UV/Vis) detector. Chromatographic separation was performed using C18 column.

The mobile phases used for this analysis were:

- Acetonitrile
- Trichloroacetic Acid

Acetonitrile	Trichloroacetic Acid
40%	60%

The analysis was performed for 40 minutes with the flow rate of 0.8 ml/min.

The whole methodology is shown below:

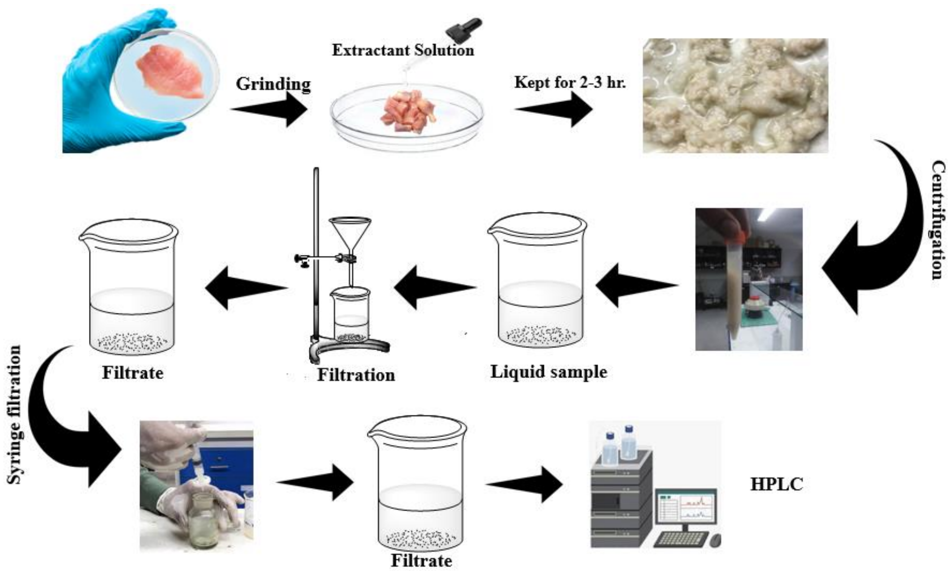


Figure 3. Working Methodology

3. Results and Discussions

The amount of water-soluble vitamins in chicken meat that was readily available was studied. Seven different water-soluble vitamins were examined using the HPLC method in conjunction with a UV detector.

Followings are the vitamins investigated for this study:

- Vitamin B1
- Vitamin B2
- Vitamin B3
- Vitamin B5
- Vitamin B6
- Vitamin B9
- Vitamin C

4.1 Vitamin Standards

Chromatograms of vitamin standards were obtained from HPLC showing their retention times (RT) in the table below:

Table 1. Retention Time and Concentration of Vitamins.

Sr.#	Vitamins	Retention Time	Concentration (mAU)
1	B1	39.687	92
2	B2	17.343	150
3	B3	5.373	117
4	B5	6.676	110
5	B6	11.245	74
6	B9	22.797	128
7	VC	3.416	502

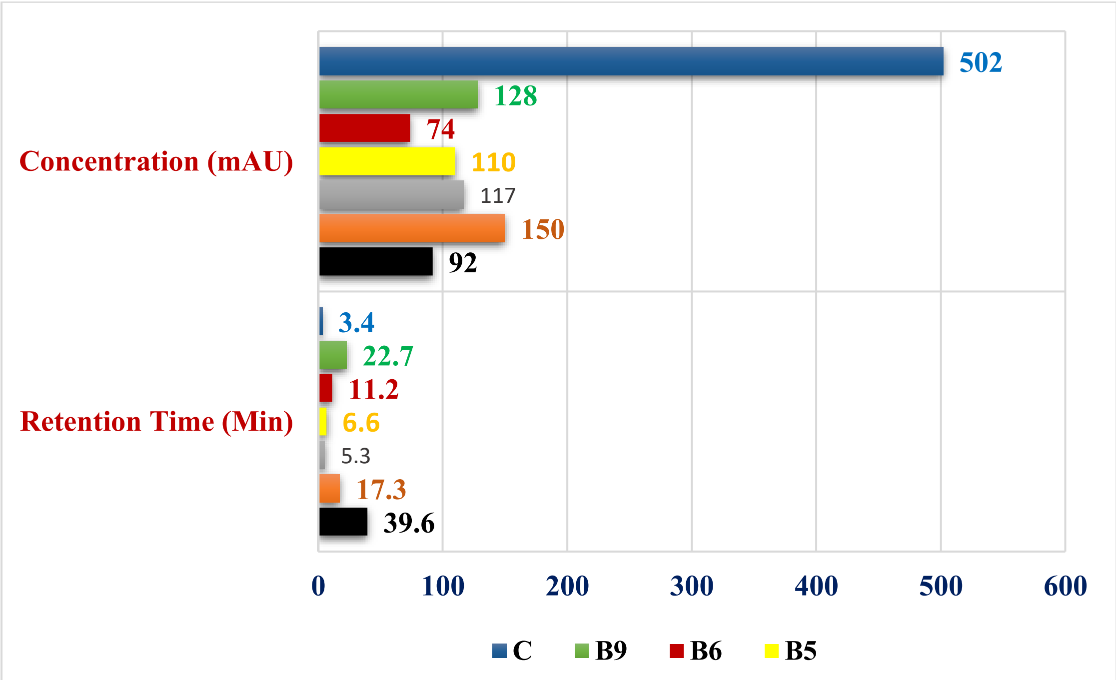


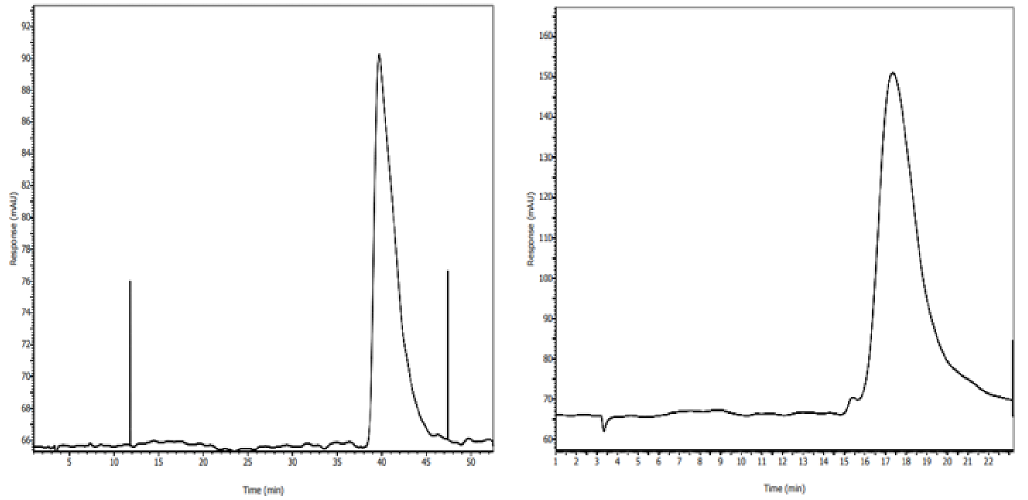
Figure 4. Concentration of Vitamin Standards.

In HPLC chromatogram, the x-axis typically represents time or elution volume, while the y-axis represents the detector response. The concentration of the substance under study can be determined by the detector response.

A peak for vitamins would normally be visible at a particular retention time on the chromatogram. The amount of time the compound needs to pass through the column of chromatography and arrive at the detector is known as the retention time. The area beneath the peak corresponds to the sample's vitamin content.

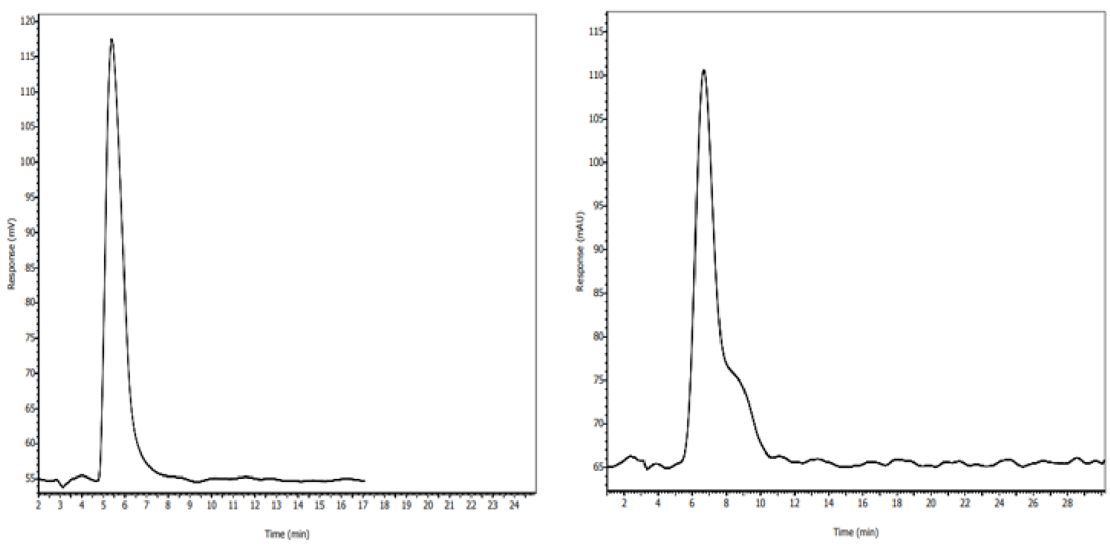
An ideal HPLC chromatogram would have a symmetrical peak that is well-defined, signifying good separation from other compounds in the sample. The lack of other peaks indicates that the analysis for vitamins was highly specific and pure.

Remember that the particulars of the chromatogram, like the retention period and peak form, can change based on the HPLC parameters that are employed, like the kind of column, the makeup of the mobile phase, and the wavelength of the detector. The chromatograms for vitamin standards obtained from HPLC are shown below:



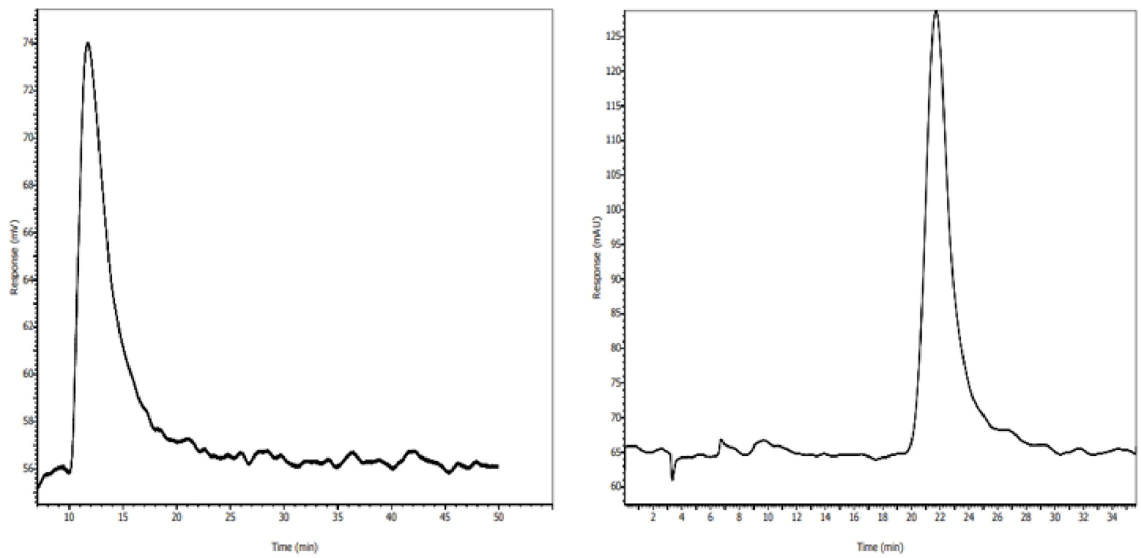
Vitamin B1 Vitamin B2

Figure 5. Chromatograms of Vitamin B1 and B2.



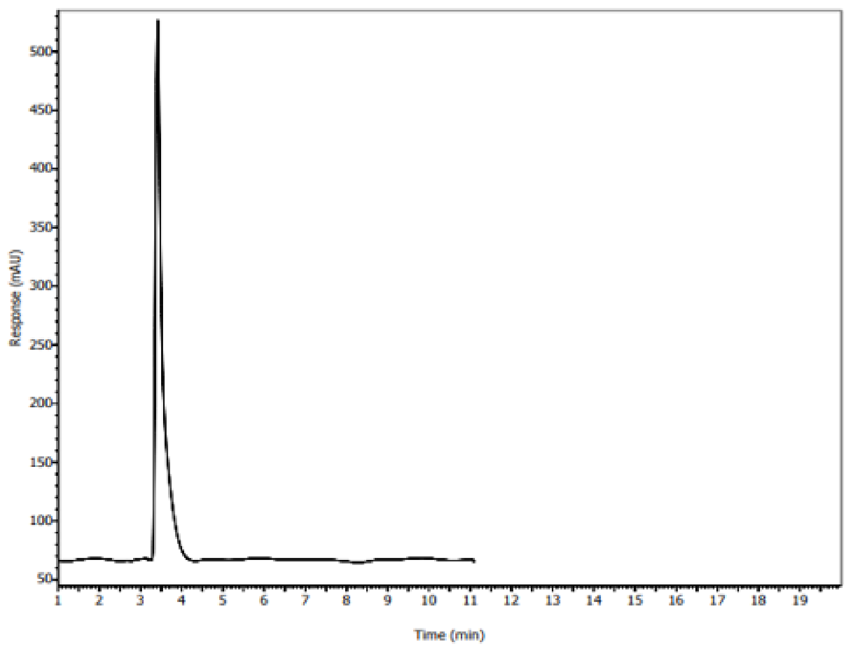
Vitamin B3 Vitamin B5

Figure 6. Chromatograms of Vitamin B3 and B5.



Vitamin B6 Vitamin B9

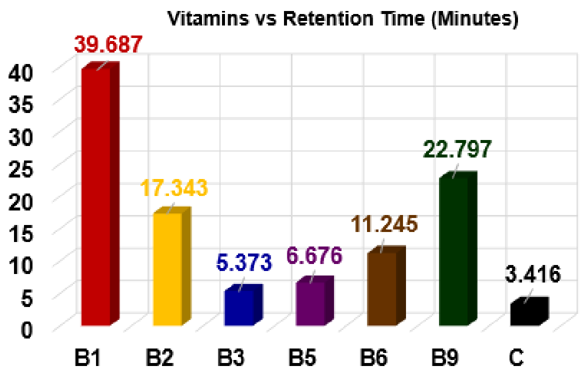
Figure 7. Chromatograms of Vitamin B6 and B9.



Vitamin C

Figure 8. Chromatogram of Vitamin C.

Figures 5-8 show the concentration of all the vitamins at different times. Remember this time is called Retention Time when vitamin samples were eluted from the HPLC column. This time varies for different vitamins. The retention time for all the vitamins under study is shown by the figure 8 below:



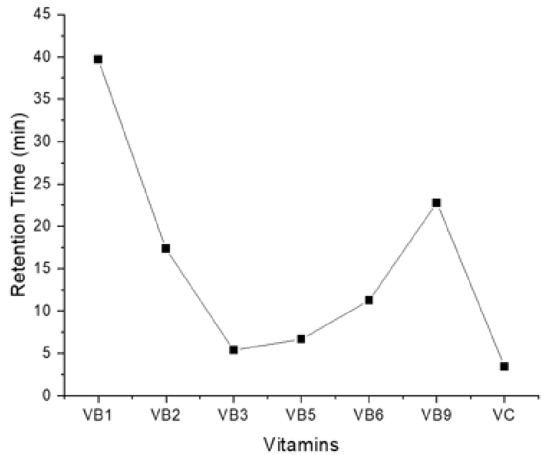


Figure 9. Retention Times of Vitamins under study.

The height of peak of chromatogram at these retention times shows the specific concentration of that vitamin. The concentrations of vitamins were found far away from the standard values of the vitamins. The concentration of the samples is given by the tables below:

Sample 1:
Table 2. HPLC Results of Sample 1.

Vitamin	Retention Time (Min)	Width (Min)	Area (mAU*s)	Concentration (mAU)	Area (%)
VB1	39.6	0.0312	5.91104	2.73343	0.0653
VB2	17.3	0.0354	5.6454	2.63048	0.0623
VB3	5.3	0.0395	11.23926	4.15854	0.1241
VB5	6.6	0.0734	19.37317	4.77055	0.2139
VB6	11.2	0.0444	9.24264	3.33064	0.1021
VB9	22.7	0.0514	20.21051	6.30468	0.2232
VC	3.4	0.0468	10.85235	3.26402	0.1198

Sample
HPLC Results of Sample 2.

Vitamin	Retention Time (Min)	Width (Min)	Area (mAU*s)	Concentration (mAU)	Area (%)
VB1	39.6	0.0631	10.48089	2.14184	0.0364
VB2	17.3	0.0882	23.86441	3.38676	0.0829
VB3	5.3	0.0717	11.15209	2.17956	0.0387
VB5	6.6	0.5277	124.8623	2.86903	0.4337
VB6	11.2	0.0431	8.30522	3.11838	0.0288
VB9	22.7	0.0476	6.75941	1.89583	0.0235
VC	3.4	0.163	1129.369	116.6298	3.9224

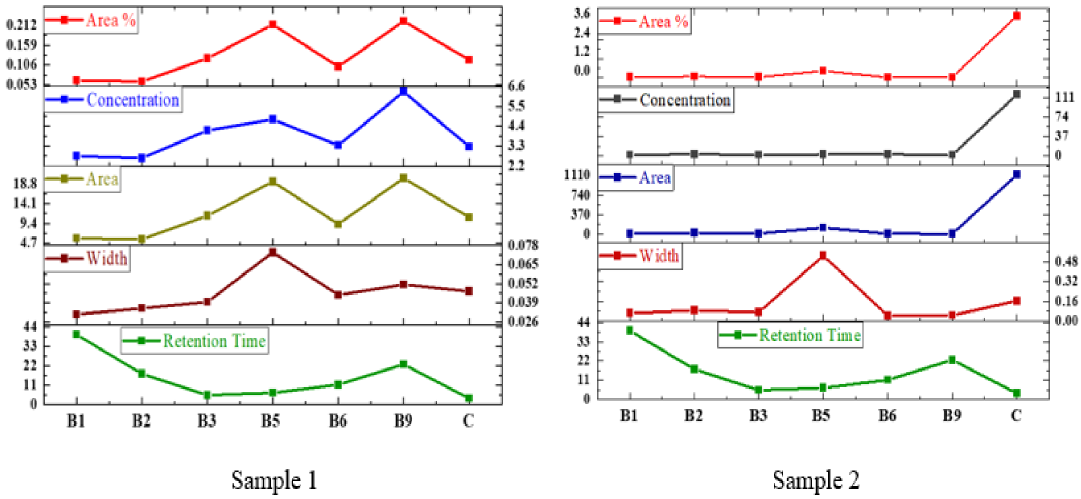


Figure 10. HPLC results of chicken samples.

Comparison with Standards:

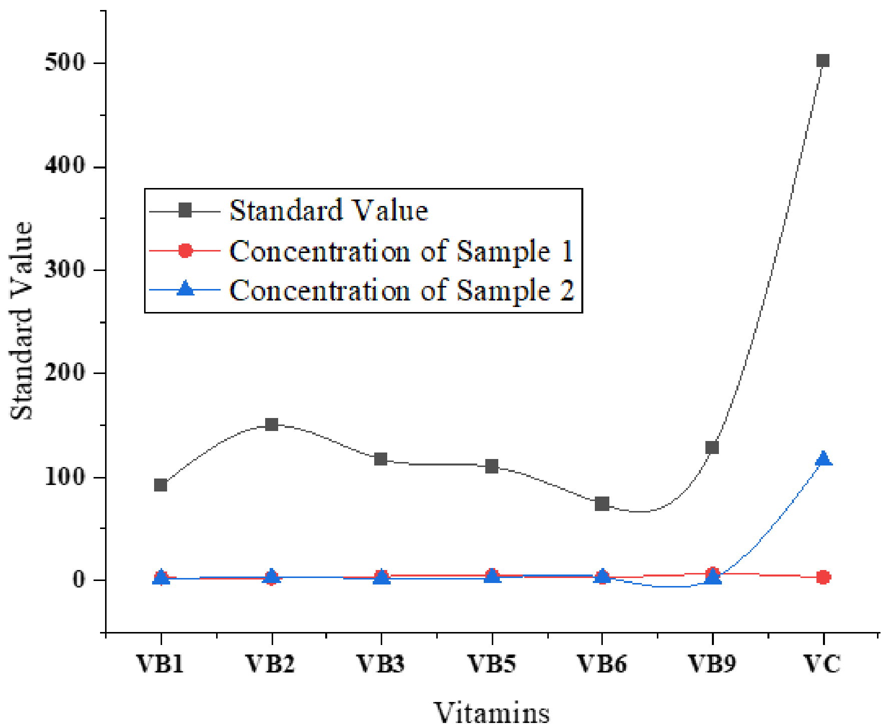


Figure 11. Comparison of results with standard values.

Vitamins were detected in the samples but were in lower amounts. Not even a single vitamin's concentration was closer to the standard values of vitamins. This shows how poor our diets are as these samples were taken from the local markets that are providing this chicken meat to the masses. Concerns have been raised about the discrepancy between the water-soluble vitamin found in chicken meat and the World Health Organization's (WHO) guidelines in Pakistani poultry farming. This discrepancy can be linked to a multitude of factors that work together to give broiler chickens subpar amounts of vital vitamins. Examining the many facets of poultry farming methods, environmental variables, feed quality, and Pakistan's overall farming nature is necessary to comprehend this complicated problem.

First and foremost, Pakistan's current agricultural practices are largely to blame for the low vitamin content of chicken meat. In order to maximize productivity, broiler chickens—chickens raised for meat production—are frequently subjected to intensive farming practices. In these kinds of settings, quick growth and high yield are usually prioritized over nutritional considerations. Because they are frequently raised in cramped quarters, hens have less access to sunlight, which is their main source of vitamin D. This shortfall can then affect how other vitamins are absorbed, which adds to the meat's overall low vitamin content [23].

The nutritional value of the feed used also has a significant impact on the vitamin content of chicken meat. Vital nutrients, such as water-soluble vitamins like the B complex vitamins (B1, B2, B3, B5, B6, B7, B9, and B12) and vitamin C, may be missing from the feed provided to broiler chickens in Pakistan. The feed's nutrient content is contingent upon the accessibility and cost of premium components. Farmers frequently choose less expensive feed mixes over more nutritious options for their birds due to financial constraints [24, 25].

The amount of nutrients in crops used as fodder for poultry is directly impacted by the quality of the soil. Depletion of the soil, a problem that many farming areas face, can lead to crops that are lower in vitamin content. Lower vitamin levels in the chickens' meat result from the feed's nutritional deficiencies being passed on to them [26].

Another important factor that appears to have an impact on the vitamin content of broiler chicken meat is water quality. A healthy, unspoiled water source is crucial for the general well-being of chickens. Poor water quality can make it more difficult for vital nutrients to be absorbed, which makes a hen's vitamin deficiency worse. Water supplies in many parts of Pakistan are tainted with pathogens and contaminants, endangering the health of the animals and thereby altering the nutritional value of their meat [27].

The consumption of growth-promoting ingredients and antimicrobial agents in chicken farming may have unforeseen effects on the amount of vitamins of the meat. These compounds might speed up growth and stave off illness, but they can also make it difficult for the birds to absorb and use vital vitamins. The excessive dependence on additives without appropriate oversight and control may be a factor in the meat's below-average vitamin content [28]. The birds' general health and metabolism may be impacted by stress caused by extreme temperatures and variations in the climate. Stress can also result in decreased appetite and absorption of nutrients, which can explain the meat's reported deficiencies in water-soluble vitamins [29].

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