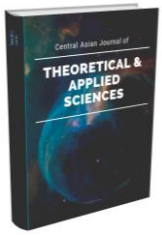

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Article

Development and Validation of Visible Spectrophotometry Method For Determination of Vitamin B9 Folic Acid In Pure And Pharmaceutical Formulations

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Abstract: Folate, forms of which are known as folic acid and vitamin B9, is one of the B vitamins 2 Folic acid helps your body produce and maintain new cells, and also helps prevent changes to DNA that may lead to cancer therefor our study describe sensitive, precise, and dependable spectrophotometric technique has been developed to accurately detect the concentration of vitamin B9 Folic acid in pure and pharmaceutical formulations. Depend on formation of ion-pair between vitamin B9 and methyl violet 10B dye. This reaction results in an orange-coloured solution that is soluble in water and shows absorption maxima at wavelengths of 446 and 367 nm when compared to a blank solution. The analytical technique was implemented and validated by thoroughly examining and optimizing various factors that could potentially disrupt the reaction. Significant linear relationships, characterized by correlation coefficients ranging from 0.9968 to 0.9974, were observed under optimal conditions. These associations remained consistent throughout concentration ranges of 0.625- 12.5µg/ml for 446 ,367 nm respectively. While the value of the molar absorption coefficient 48907.12 & 40873.64 $L.mol^{-1}.cm^{-1}$ at 446,367 nm respectively with the limits of qualitative and quantitative detection 0.1624,0.5415 and 0.1619 , 0.5399 µg / ml , respectively.The proposed method was successfully applied for the determination of folic acid in its pharmaceutical preparations, from Erbil Company – Iraq. Trade name (folic acide).

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1. Introduction

Vitamins mainly play as important and primary role in the prevention and Vitamins can be categorized into fat soluble vitamins and water soluble vitamins the various deficiency diseases. Various factors that affect the deficiency of vitamins include malabsorption, inadequate intake, increased excretion, genetic abnormalities. 1 folic acid is used to treat folic acid deficiency and certain types of anemia (lack of red blood cells) caused by folic acid deficiency. The recommended daily intake level of folate is 400 micrograms from foods or dietary supplements (2)

IUPAC name of Folic acid: (2S)-2-[[4-[(2-Amino-4- oxo-1H-pteridin-6- yl) methylamino] benzoyl] amino] pentanedioic acid.

Formula $C_{19}H_{19}N_7O_6$, M.wt : 441.40 $g{\cdot}mol^{-1}$ one of the water soluble B

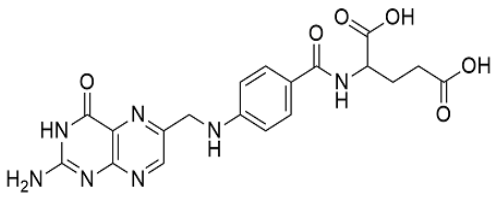

Figure 1. The structure of folic acid(3)

it's found as yellowish crystals .Folic acid is stable in the air, but by the ultraviolet light that the decomposition of losing its vitality. Thermally unstable in acidic solution, but in the neutral and alkaline environment is very stable and heated under 100 ° C for 1 hour will not be damaged (4). There are different analytical techniques for determination of Vitamin B9. Such Spectrophotometric(5- 9) spectroelectrochemistry (10) photoluminescence (11) HPLC(12) FIA (13) HPLC/DAD (14) LC-MS/MS (15) Given the importance of vitamin B9 in increasing the body's immunity, the aim of this study was to recommend a sensitive spectrophotometric estimation method that depend on ion-pair formation with methyl violet 10B dye [16].

Experimental Part

T80 UV-Visible Spectrophotometer PG Instrumental Ltd, UK with 10 mm quartz cell used for all spectrophotometric quantities, and Sartorius Balance 210S kern used to perform all weight measurements [17].

2. Materials and Methods

Totally the chemicals and solvents used from Aldrich and Fluka products and used without further purification vitamin B9 Folic acid typical material provided from State Company for Drug Industries and Medical Appliance (SDI) Samarra-Iraq. Distilled water used to formulate all solutions.

1) Standard solution (VitB9), 250μg/mL

In a volumetric flask, the volume was diluted to 100 ml using distilled water after dissolving 0.025g of vitB9, molecular weight 441.40 $g.mol^{-1}$, in a small amount of 0.1 M of sodium hydroxide solutio and added distilled water to the mark in volumetric flask 100mL, more dilute working solutions of the vitB9 prepared by serial dilutions with distilled water.

2) 100 μg/ml of methyl violet 10B dye prepared by dissolving 0.01gm substance in 100 mL volumetric flask and diluted to the mark, then prepare a solution with a concentration of 10 μg/ml
3) Solutions 1M of each of sodium hydroxide , hydrochloric acid prepared and used.

4) Sample Preparation

A 10.0 tablets (folic acid) (each contain 5 mg folic acid), from Erbil Company – Iraq. Trade name (folic acide). are weighed (0.1081 g) and granulated to a fine articles then weight 1.081 g which dissolved in a small amount of 0.1 M of sodium hydroxide solutio and added distilled water then the solution filtered and the volume is complete to 100 ml to obtained 500 μg ml^{-1} solution other dilute working solutions were organized by serial dilutions with distilled water [18].

Preliminary study

In a 20 ml volumetric flask , 0.5 ml of a 250 μg/ml vitamin B9 solution was added to 2.5 ml of a 10 μg/ml methyl violet 10B dye solution. The mixture is then diluted by distilled water It was observed that a yellowish green solution was formed that gave two absorption peaks at two wave lengths of 367 and 446 nm , while, the blank has not any significant absorbance in this region (Figure 1).

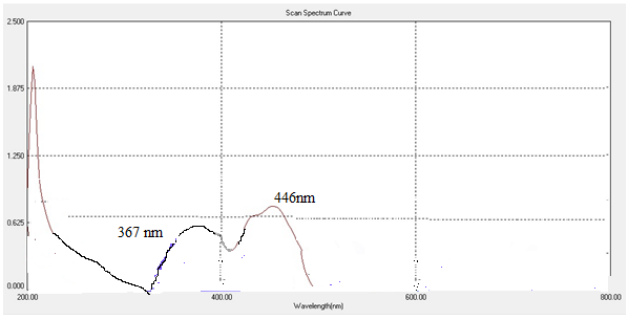

Figure 1. spectrum of the sample

3. Result and Discussion

Analytical parameters optimization The optimum conditions for the assay procedures and color development for method have been established by varying the parameters one at a time, keeping the others stable and observing the effect The effect that occurs absorbance of the colored solution [19]

Effect of methyl violet 10B dye volume

To ascertain the optimal concentration of methyl violet 10B dye, several volumes of dye solution (10 µg/ml) in the range of 0.5-4.5 ml were treated with a consistent quantity The study found that the greatest absorbance value was achieved by utilizing 2.5 ml of dye (Figure 2).

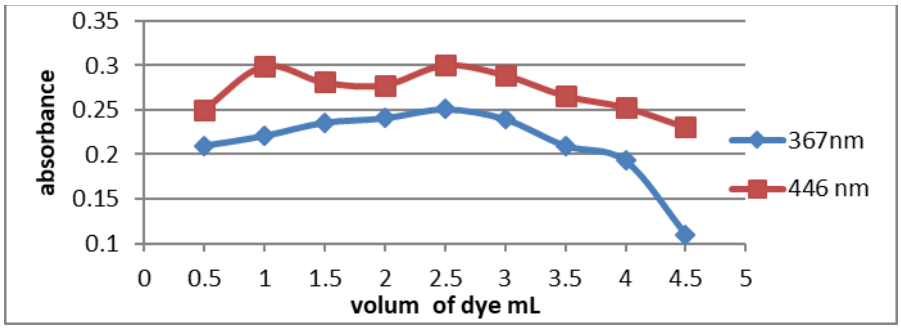

Figure 2. Effect of Volume of dye in Absorbance intensity

Effect of pH

The proposed reaction was carried out by changing the pH using solutions of hydrochloric acid and sodium hydroxide with different volumes of 0.1 molar solutions, and observing the change in absorption of the solutions at 367 and 446 nm. Note that the value of the pH before adding = 6 (Figure 3).

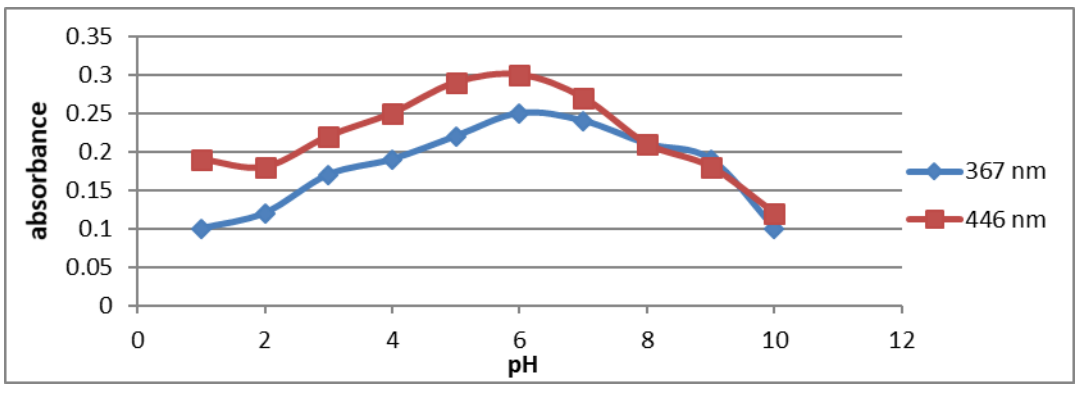

Figure 3. Effect of pH in Absorbance intensity

Effect of reaction time

The reaction time between the vitamin B9 solution and dye solution was studied before completing the volume with distilled water to the mark in sample flask for periods of time that ranged from the beginning of mixing to 30 minutes. It was found that 15 minutes is sufficient time to give the best absorption value (Figure 4).

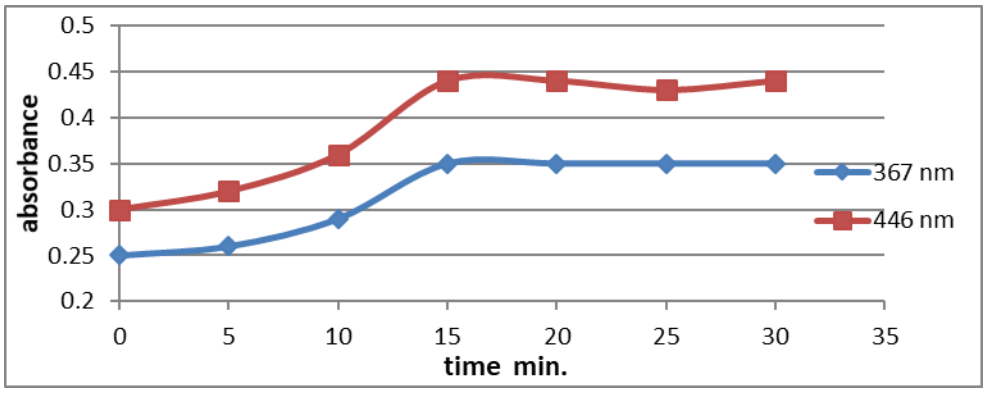

Figure 4. Effect of reaction time in Absorbance intensity

Effects of temperature

A study was carried out to analyze the influence of temperature on a series of sample and blank solutions. The solutions underwent temperature variations, ranging from 20-100 °C, through immersion in a water bath. The most elevated degree of color intensity was attained at boiling degree (Figure 5).

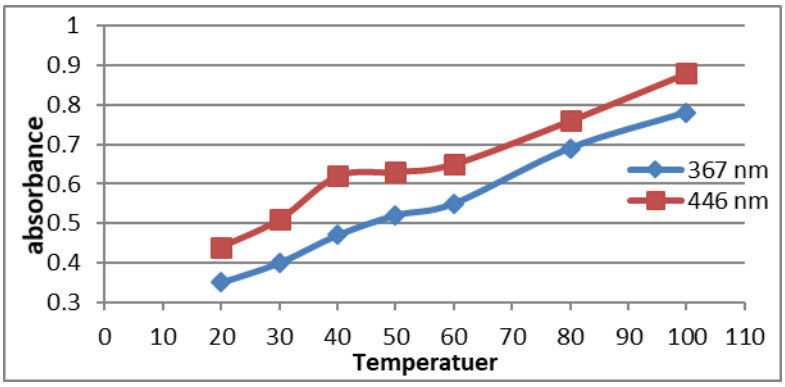

Figure 5. Effect of Temperature in Absorbance intensity

Procedure for construction of calibration curve

An evident correlation was noted between the absorbance at the maximum tow wavelengths 367,446 nm respectively and the vit.B9 concentration. This association was determined under optimum conditions . The vit.B9 concentration ranges were 0.625 - 12.5µg/ml using methyl violet 10B dye 10 µg/ml In a series of volumetric flasks 20mL . To get accurate results gave a correlation coefficient of 0.9968 & 0.9974. The value of the molar absorbance was calculated at 48907.12 & 40873.64 $L.mol^{-1}.cm^{-1}$ at 446,367 nm respectively (Figure 5,6) [20].

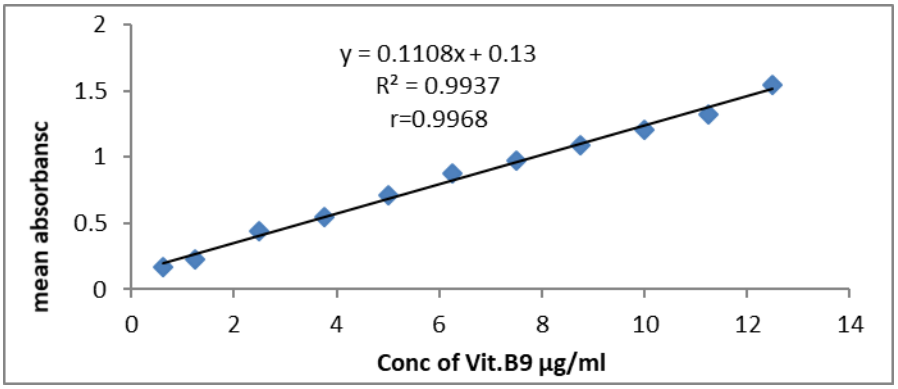

Figure 6. Calibration curve for determination vit.B9 at λ=446nm

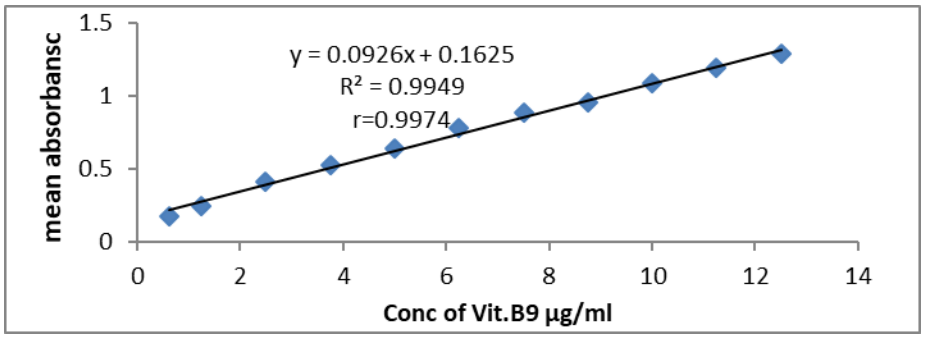

Figure 7. Calibration curve for determination vit.B9 at λ=367nm

Accuracy and precision

Accuracy and precision studied by measuring absorption (n=3) at 446 and 367nm for three different concentrations of the drug within the limits of Beer's law, the average recovery (101.6% & 99.65) at 446 and 367nm respectively indicate that the method has high accuracy and precision . The results in Table (1,2).

Table 1. Results of accuracy and precision at 446nm

Conc.of vit.B9µg/ml	Conc.of vit.B9µg/ml observed*	Rec%	E_{rel}%
3.75	3.79	101.08	1.08
5	5.23	104.69	4.69
8.75	8.66	99.03	-0.97

Table 2. Results of accuracy and precision at 367nm

Conc.of vit.B9µg/ml	Conc.of vit.B9µg/ml observed*	Rec%	E_{rel}%
5	5.15	103.13	3.13
8.75	8.61	98.43	-1.57
12.5	12.17	97.41	-2.59

*n=3

Detection limits

The detection (LOD = 3s /p) and (LOQ=10 s /p(quantitation limits for the suggested procedures were determined. The variable "s" represents the standard deviation of 10 repeated measurements of the reagent blank, where as "p" which is defined as the slope of the calibration curve Table (3).

Applications

Direct Method

Different concentrations (3.75,5,8.75,12.5 µg mL^{-1}) of a pharmaceutical formulation treated such as in construction on of calibration curve. The absorbance measured at 367, 446 nm for 3 times. Erel% calculated the results in Table 4&5

Conc.of vit.B9µg/ml	Conc.of vit.B9µg/ml observed*	E_{rel}%
3.75	3.819	1.84
5	5.22	4.4
8.75	8.69	-0.68

Table 4. Determination of vit.B9 in pharmaceutical formulation at 446nm

Conc.of vit.B9µg/ml	Conc.of vit.B9µg/ml observed*	E_{rel}%
5	5.215	4.3
8.75	8.421	-3.76
12.5	12.191	-2.472

*n=3

Table 5. Determination of vit.B9 in pharmaceutical formulation at 367nm

4. Conclusion

A rapid, simple and precise spectrophotometric method has been suggested for the determination of Folic acid (Vit.B9) in aqueous solution based on reaction with methyl violet 10B dye. The suggested method does not require the solvent extraction step, the method was applied, successfully for the determined of amounts commercial Vit.B9 product.

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