IASJ المجلات الاكاديمية العراقية

Tikrit Journal of Pure Science

ISSN: 1813 – 1662 (Print) --- E-ISSN: 2415 – 1726 (Online)

Journal Homepage: http://tjps.tu.edu.iq/index.php/tjps

Spectrophotometric determination of Folic acid (B9) by oxidative Coupling Reaction with 4-nitro-aniline

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https://doi.org/10.25130/tjps.v27i6.755

ARTICLE INFO.

Article history:

-Received: 3 / 9 / 2022

-Accepted: 28 / 9 / 2022

-Available online: 25 / 12 / 2022

Keywords: Coupling Reaction; spectrophotometric; 4-nitro aniline; folic acid

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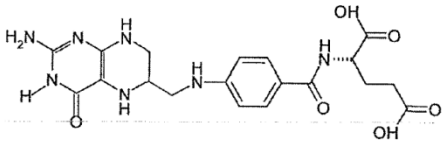
ABSTRACT

Development of an easy, inexpensive, fast and selective spectrophotometric method for the determination of Folic Acid (FA) by its reaction with 4-nitro aniline reagent, with good sensitivity, as an oxidative coupling method in the presence of Potassium permanganate solution, and it was found that the concentrations that were agreement with Beer-Lambert's law at the wavelength of 374 nm ranged between 1- 21 µg/ml of folic acid solution, and a deviation from Beer's law occurs at a concentration higher than 21 µg/mL, gave a linear coefficient and correlation coefficient its value is 0.9994, 0.9996, respectively, While the value of the molar absorption coefficient 12138.5 L/mol.cm and Sandel's sensitivity 0.0363 gm/cm^2 with the limits of qualitative and quantitative detection 0.103 and 0.363 µg / ml^{-1}, 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).

1. Introduction

Folic acid (FA) it is one of the types of B vitamins, which is vitamin B9 and is considered one of the most important vitamins beneficial to the human body in general. Its chemical formula is $C_{19}H_{19}N_7O_6$



and its molar mass is 441.4 g/mol. Its solubility is low in water and it is more soluble in alkaline, Malnutrition is one of the main causes of folic acid deficiency or those who eat a restricted diet. Symptoms of folate deficiency include weakness, fatigue, difficulty concentrating, irritability, headache, palpitations, and shortness of breath. Anemia and deterioration of the gastrointestinal mucosa due to the lack
of red blood replacement and mucosal cells [1]. The food carbon for one carbon especially as a donor of a methyl group during DNA synthesis [2].

Pregnant women are at risk for folate deficiency because pregnancy greatly increases folate requirements, especially during periods of rapid fetal growth [3]. Deficiency can occur anywhere where cell proliferation should increase, such as in pregnancies especially twins, during cancer, devastating skin diseases, burns, heavy blood loss during an accident, and damage to the digestive system. [4,5] Folic acid is found in natural sources. It can be obtained in large quantities, such as dried legumes and whole grains, and can also be obtained through a
dietary supplement in addition to folic grains [6-8]. It is also found in green leafy vegetables such as spinach, turnip greens, and lettuce. Folic acid is also found in abundance in cabbage, broccoli, strawberries, green peppers, red and yellow, and artichokes. and sunflower seeds and other fruits and vegetables at the same time [9]. A number of analytical techniques have been described in literature for the estimation of folic acid such as Spectrophotometric [10-13] electrochemical sensor [14].

2. Experimental

2.1. Apparatus

UV-Visible Spectrophotometer PG Instrumental T90 Ltd, UK, 10mm quartz cell used for wholly spectrophotometric quantities, sensitive Type of balance ,water bath.

2.2. Materials and Reagents

Absolutely the chemicals and solvents from Aldrich and Fluka products without further purification, folic acid typical material from State Company for Drug Industries and Medical Appliance Samarra-Iraq SDI. Distilled water used to formulate all solutions. were prepare by the following :-

Standard stock solution of folic acid 250 µg/ ml is prepared via dissolving 0.025 g in 10 ml of NaOH 1 M in a volumetric bottle of 100 ml and fill the volume with distilled water to the mark. 4-nitro aniline solution 0.01 M via dissolving 0.27g of pure in 200 ml, $KMnO_4$ 0.003M solution via dissolving 0.047 g in 100 ml distilled water

A 10.0 tablets (folic acid) (each contain 5 mg folic acid), from Erbil Company – Iraq. Trade name (folic acide). are weighed (0.1081g) and granulated to a fine articles then weight 1.081 g which dissolved in a small volume of 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.

3. Results and Discussion

3.1. Preliminary study

When adding 0.5 ml of folic acid (250 µg/ml) to a volumetric flask of 10 ml capacity, then adding 0.3 ml of reagent solution 10^{-2} M, then adding 0.3 ml of oxidizing agent solution of potassium permanganate 3 x 10^{-3} M, and completing the volume with distilled water to the mark measured the absorbance of the solution versus the blank solution and it was found that it gives the highest absorption at the wavelength of 374 nm.

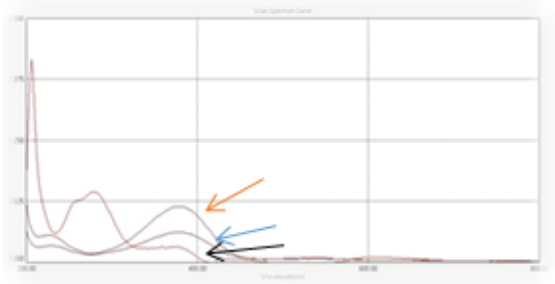


Fig.: SB: Spectrum of absorption of folic acid in coupling reaction solution against blank

SW: Spectrum of absorption of folic acid in coupling reaction solution against Water

BW: Absorption of blank against distilled water

Optimization of reaction conditions

Several conditions studied which are affecting the absorbance so as to in order to improve it. The effect of type of base and oxidation reagent, amount of NaOH, 4-nitro aniline, and potassium permanganate, temperature, stability) was investigated. The results show that use 15 ml of 1 M NaOH with 0.5 ml of 0.003M of $KMnO_4$, and 0.4 ml of 0.01 M of reagent,) (give best results (table 1- 6 respectively).

Table 1: Effect of type of base

type of base solution	Absorbance
KOH	0.260
NaOH	**0.407**
NH4OH	0.173

Table 2: Effect volume of base

Volume of base (ml)	Absorbance
0.05	0.387
0.1	0.398
0.3	0.422
0.5	**0.425**
0.8	0.420

Table 3: Effect of type of oxidation reagent

oxidition agent	Absorbance
K_2Cr_2O7	0.374
K_2CrO_4	0.367
KIO_4	0.213
$NaIO_4$	0.201
$KMnO_4$	**0.422**

Table 4: Effect volume of 4- nitro aniline

4-nitro aniline solution 0.01 M volume/ml	Absorbance
0.1	0.328
0.3	0.425
0.4	**0.429**
0.5	0.425
0.6	0.408
0.8	0.401

Should be followed the order of addition of the reactants as cited in the suggested procedure. Table 5.

Table 5: Effect of addition order

add sequence	Absorbance
A+B+C	0.429
A+C+B	0.369
C+A+B	**0.442**

C= Vit.B9 A= $KMnO_4$ B=4-nitro aniline

Table 6:.Effect stability

Absorbance	Stability time
0.446	0
0.446	5
0.445	10
0.445	20
0.446	30
0.445	45
0.446	60

4. Calibration curve and the statistical data

After studying and adjusting the optimum conditions for the reaction and fixing them, the standard calibration curve was prepared by taking increasing volumes (0.05-0.84 ml) of folic acid solution 250 µg/mL to a series of volumetric flasks of (10 ml) capacity containing 0.5ml of potassium permanganate solution at a concentration of 3x10^{-3} M, then adding 0.4 ml of 4-nitro-aniliene solution after five minutes and completing the volume to the mark with distilled water. The results were obtained as in Figure (2), and a deviation from Beer's law occurs at a concentration higher than 21 µg/ml and it gives a linear coefficient

and a correlation coefficient of 0.9994 and 0.9996, respectively, while the value of the molar absorption coefficient (12138.5) and Sandel's sensitivity (0.0363).

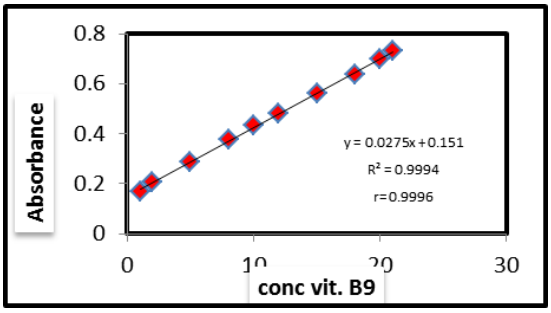


Fig. 2: Calibration curve for the determination of folic acid corresponding optimal conditions.

Table 7: Accuracy and precision

Conc of FA µg/ml Taken	found	Erel %	*RSD %
10	10.25	2.5	0.184
15	14.94	3-	0.258
20	19.89	0.5-	0.116

*n=6

4. Accuracy and precision

The capability of the method was statistically evaluated by measuring accuracy as relative error percentage (Erel % and precision as relative standard deviation percent of the proposed methods. Table 6 illustrates that the results obtained for 6 replicates at 3 concentration levels of FA were satisfactory which indicate that the proposed methods have a good accuracy and precision.

Table 8: Statistical data of the calibration curve

Parameter	Value
λmax	374 nm
Linear range µg/ml	1-21
Molar absorptivity (L/mol.cm)	12138.5
Sandell's sensitivity (µg/cm^2)	0.0363
Correlation coefficient	0.9996
Slope	0.0275
LOD (µg/mL)[15-16]	0.103
LOQ (µg/mL)[15-16]	0.363

5. Stoichiometry of reaction

The consequences obtained between FA and the mixture was investigated using Job's method and mole- ratio indicate that the stoichiometry of the water-soluble coupling product between FA and the reagent is 1:1 Fig. 3 and Fig4.

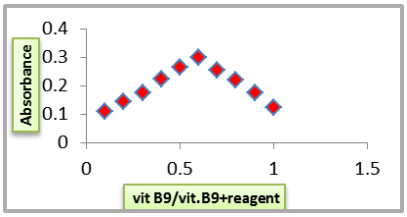


Fig. 3: Job's method.

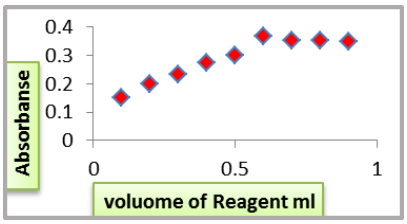
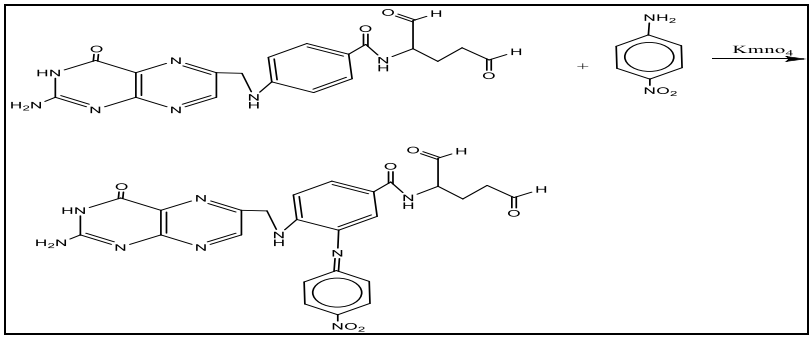


Fig. 4: Mole ratio for reaction.



6. Applications

1- Direct Method

Different concentrations (2,10,15,20 µg mL^{-1}) of a pharmaceutical formulation treated such as in construction on of calibration curve. The absorbance measured at 374 nm for 3 times. Erel% calculated the results in Table 8 .

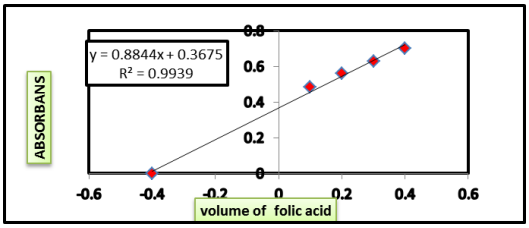
Table 9: Determination of FA in pharmaceutical formulation

Conc of FA µg/mL	Observed µg/mL *Conc of FA	Erel %
2	2.072	3.63
10	10.18	1.81
15	15.01	0.066
20	19.818	0.909-

* n=3

Table (8) illustrations the efficiency and success of the developed method for the determination of FA in its pharmaceutical formulation

2- Standared addition method



Rec	Con measured µg/ml	Con present Of FA µg/ml	Type of Drug
100.03	10.388	10	حبوب من حامض الفوليك 5 mg

Conclusion

A rapid, simple and precise spectrophotometric method has been suggested for the determination of Folic acid (Vit.B9) in aqueous solution based on oxidation with 4-nitro-aniline.The suggested method does not require temperature control or the solvent extraction step, the method was applied, successfully for the determined of amounts commercial Vit.B9 drug.

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التقدير الطيفي لحامض الفوليك (B9) عن طريق تفاعل الاقتران التاكسدي مع 4–نيترو–أنيلين

رشا سعد صابر التكريتي ، أسماء أحمد محمد الراشدي

قسم الكيمياء ، كلية التربية للعلوم الصرفة ، جامعة تكريت ، تكريت ، العراق

الملخص

تطوير طريقة طيفية سهلة وغير مكلفة وسريعة وانتقائية لتقدير حمض الفوليك (FA) من خلال تفاعله مع كاشف 4–nitro aniline ، بحساسية جيدة ، كطريقة اقتران تاكسدي بوجود محلول برمنجنات البوتاسيوم، وجد أن التراكيز التي كانت مطاوعة لقانون بير لامبرت عند الطول الموجي 374 نانومتر تراوحت بين 1–21 ميكروغرام / مل من محلول حمض الفوليك، ويحدث انحرافًا عن قانون بير عند تركيز أعلى من 21 ميكروغرام / مل ، أعطى معامل خطية ومعامل ارتباط قيمته 0.9994 ، 0.9996 على التوالي ، بينما قيمة معامل الامتصاص المولاري (12138.5) وحساسية ساندل (0.0363) بحدود الكشف النوعي والكمي 0.103 و 0.363 ميكروغرام / مل –1 على التوالي. تم تطبيق الطريقة المقترحة بنجاح لتقدير حمض الفوليك في مستحضراته الصيدلانية من شركة اربيل – العراق