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DEVELOPMENT AND VALIDATION OF UV-SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF FOLIC ACID IN BULK AND TABLET DOSAGE FORMS

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ABSTRACT
The purpose of this study was to create and verify a simple and accurate UV-Spectrophotometric technique for determining Folic acid in bulk and tablet dosage form. The approach was based on the measurement of folic acid solution absorbance at 255nm in 0.01 M NaOH. In the concentration range of 10-50 g/ml, the constructed calibration curve was found to be linear (R^2 = 0.9996). Limits of detection and quantification were 2.73 µg/ml, and 8.27 µg/ml, respectively. The precision of the developed method was confirmed by the obtained low RSD% values. Recoveries percentages were found 98.46%±1.42, 100.0%±0.20, 98.92%±1.12; n=3 for 50%, 100% and 150% levels, respectively indicating the method accuracy and freedom of interference from excipients. Folic acid content percent in the two analyzed brands were found to be equal 96.59%±0.005; n=3, and 97.28%±0.003; n=3 which complies with the official range (90-110%).

INTRODUCTION

Folic acid (FA) or Pteroyl-L-glutamic acid (Fig.1), is chemically known as N-[4-[[(2-amino-1,4-dihydro-4-oxo-6-pteriodinyl) methyl]amino]benzoyl]-L-glutamicacid [1]. It is important for the formation of red blood cells and is essential for growth and the prevention of anemia [2]. FA deficiency can lead to congenital deformations in the fetus (spina bifida, encephalocele, and hydrocephalus), as well as heart disease [3]. It is used for treatment of folate deficiency megaloblastic anemia, nutritional supplement, supplementation in patients with sickle cell anemia, and prevention of fetal neural tube defects.

Folic acid, a biochemically inactive compound, is precursor for tetrahydrofolic acid and methyltetrahydrofolate which are essential for the maintenance of normal` erythropoiesis and are also required cofactors for the synthesis of purine and

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thymidylate nucleic acids [4]. Folic acid is nontoxic, although there is some concern that high doses may mask pernicious anemia. This effect is only likely following taking amounts higher than 5 mg.

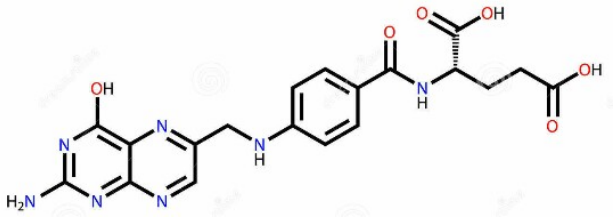

Figure 1: Chemical structure of folic acid

Literature review revealed number of methods for the determination of folic acid. These methods include: HPLC [5-10], electrochemical [11, 12], flow injection analysis [13] and derivative spectrophotometry [14-17]. Most of these methods are complicated, time-consuming using sophisticated expensive instrumentation and use organic solvents. The objective of the present work was therefore to develop and validate a simple direct UV spectrophotometric method for the determination of folic acid in bulk and tablet dosage forms.

MATERIALS AND METHODS

All materials and reagents used were of analytical grade. Folic acid Standard (99.08%) was kindly provided by City-Pharm Khartoum, Sudan. Folic acid tablets (two brands) were purchased from the local market. Sodium hydroxide, Scharlab S.L., Spain. Spectrophotometric studies were carried out on Shimadzu UV-1800, Kyoto, Japan

Preparation of stock solutions

Sodium Hydroxide solution

Sodium Hydroxide pellets (0.4g) were weighed, dissolved in 100ml distilled water and transferred into 1000ml volumetric flask. The volume was then completed to 1000ml with distilled water to obtain a solution of concentration 0.01M.

Preparation of FA standard solution

FA standard powder (100mg) was accurately weighed and transferred into 200 ml volumetric flask. The volume was completed to the mark with 0.01M NaOH solution to obtain solution with concentration 500µg/ml (solution A)

Preparation of FA sample solution

Twenty tablets of FA were weighed and powdered. An accurate weight equivalent to 100 mg FA was transferred to 200 ml volumetric flask. 0.01M NaOH was added, shaken, and then the volume was completed to the mark with 0.01M NaOH. The resultant solution was filtered to obtain solution with concentration 500µg/ml (solution B).

Estimation of optimum detection wavelength

Solution A (2 mL) was further diluted to 10 mL with distilled water. The resultant solution was then scanned between 200 – 400 nm against distilled water as blank in order to determine the optimum wavelength.

Method Validation

Linearity

Aliquot volumes of solutions A (1-5ml) were transferred each to 50 ml flask and completed with 0.01MNaOH to obtain concentration (10-50µglml). The absorbance of each solution was measured at 255 nm using sodium hydroxide as blank. The calibration curve was constructed by plotting concentration versus absorbance.

The regression analysis data were then calculated from the constructed curves according to the following equation [18]:

$$y = (b - ts_b)x + (a - ts_a)$$

where b is the slope, a is the intercept, s_b is the standard deviation of the slope, s_a is the standard deviation of intercept, t is the t-value at 95% confidence level for $(n - 2)$ degrees of freedom.

Limit of Detection and Limit of Quantification

LOD and LOQ values were calculated using the following formulas [19]:

$$\text{LOD} = \frac{3.3\ \text{Sy/x}}{\text{b}}\ ;\ \ \text{LOQ} = \frac{10\ \text{Sy/x}}{\text{b}}$$

Where b is the slope and Sy/x is the standard deviation of y to x

Precision

Intra-day precision and inter-day precision were evaluated by measuring the absorbance values of six replicates from solution A. Results were then expressed as RSD% values.

Accuracy

The accuracy of the method was determined by calculating the recoveries of folic acid by standard addition method .Study was carried out by addition of known amount of standard drug in the pre-analyzed tablet formulation, in 50%, 100% and 150% of label claim.

The amount of folic acid recovered was estimated using the following formula [20]:

% $recovery$= (Cs - Cu)/CA × 100

Where

Cs =concentration of spiked samples.

Cu = concentration of unspiked samples.

CA = concentration of analyte added to the test sample

Determination of Drug content in Tablets form

3ml of solution A and B were treated as under linearity. The absorbance value of the resultant solution was then measured at 255 nm. The content was calculated by the direct sample/ standard comparison.

RESULTS AND DISCUSSION

The main objective of pharmaceutical analysis is to get consistent, reliable and accurate data for the analysis of a drug and the main way to get this aim is to develop new methods of analysis which can fulfill these criteria's. In developing countries where the cost and availability of the resources are limited, the need for sensitive, selective and accurate analytical method is crucial. UV –Vis spectrophotometry is one of the most frequently employed techniques in pharmaceutical analysis which include many methods for assay of substances. Folic acid UV-Vis absorbance is mainly attributed to the presence of pterine nucleus, para-amino benzoic acid part, and glutamic acid part in it its structure. The UV spectrum of FA solution exhibited maximum absorption at 255 nm.

Method validation

The linearity of the method was checked in pure solution of FA. Regression analysis of Beer's plots showed good correlation in concentration range of 10-50 µg/ml with good correlation coefficient (Figure 2). The obtained regression analysis data was summarized in Table 1.

Precision

The precision of the developed method was assessed in terms of repeatability and intermediate precision by analyzing six replicates. RSD% values were 0.158%and 0.680%, respectively reflecting the precision of the developed method (%RSD > 2).

Accuracy

The accuracy of the developed method was determined by calculating the percent recoveries of folic acid after addition of known amount of standard drug in the pre-analyzed tablet formulation, in 50%, 100% & 150% of label claim. % recoveries were found 98.46%±1.42, 100.0%±0.20, 98.92%±1.12; n=3 for 50%, 100% and 150% levels, respectively table2. These results reflect the accuracy of the developed method and its freedom from interference of excipients.

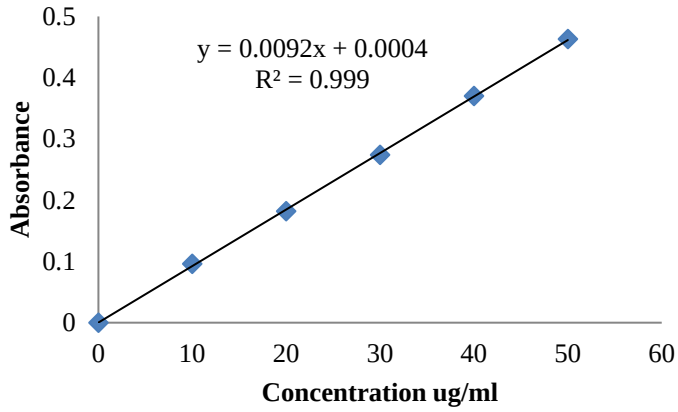

Figure 2: Calibration curve of FA

Table 1. Regression Analysis Results

Parameter	Value
R^2	0.999
Regression equation	y =0.009211x+0.0004
Slope ± ts_b*	0.0092±0.0005
Intercept ± ts_a*	0.0004±0.022
Range	(10-50)µg/ml
LOD	2.73µg/ml
LOQ	8.27 µg/ml /ml

*Standard error of slope and intercept calculated at 95% confidence limit for n- 2 degrees of freedom.

FA content

The developed method was then applied to determine the content of FA in two tablet brands collected from the local market, each containing 5mg folic acid. The content % was found to be 96.59%±0.005; n=3, and 97.28%±0.003; n=3 which complied with the official range.

CONCLUSION

The method developed for the determination of folic acid in bulk and in tablet formulations was proved to be simple and selective. Good linearity was achieved in the concentration range studied, together with satisfactory accuracy and precision. The proposed method offers an alternative use in the quality control

laboratories for being simple and requiring environment friendly reagents compared to chromatographic methods

FINANCIAL ASSISTANCE
Nil

CONFLICT OF INTEREST
The authors declare no conflict of interest

AUTHOR CONTRIBUTION
Takreem Elkhazein, Tahani Abdeljabar, and Amal Abdelrahman conducted the practical work. Mohamd Adam wrote the manuscript draft. Shaza Shantier wrote the abstract and revised the draft. All the authors approved the final draft.

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