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Article *in* Materials Today: Proceedings · April 2022

DOI: 10.1016/j.matpr.2021.09.453

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Spectrophotometric determination of folic acid using 1,10- phenanthroline materials with ninhydrin reagent

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ARTICLE INFO

Keywords:
Ferric chloride
Ninhydrine
Complexation processes
Folic acid
Pharmaceutical preparation

ABSTRACT

There have been 2 spectrophotometric techniques developed, validated to find out how much folic acid there is that is simple to use, sensitive to detect, and selective. One method reduces ferric chloride in a neutral medium while simultaneously adding folic acid, and the second method is to chelate iron (II) along with 1, 10-phenanthroline (method A) on one hand and Method B depends on the interaction of the primary amino group of folic acid with the amino group of Ninhydrin in an alkaline solution, On the other hand. In Method A, red chromogens with a wavelength of 505 nm are produced, whereas, in Method B, violet chromogens with a wavelength of 575 nm are produced, and so on. Beer's law holds under ideal conditions in molar absorptivity values of 2.331104 L.mol-1 cm-1 and 6.771104 L.mol-1 cm-1, respectively, for concentration ranges of 1.0–35.0 g ml-1 and 0.5–10.0 g ml-1. Sandell's sensitivity values of 1.944 × 10–6 μg cm-2 and 1.6728 × 10–6 μg cm-2, respectively, and Sandell Technically, technique A and method B had limits of detection (LODs) of 0.198 and 0.488, respectively, and limits of quantification (LOQs) of 0.600 and 1.479, respectively. The correlation coefficients (R2) for technique A and method B were both 0.9979 and 0.9988. A and B used stability constants of 5.6192 and 5.4633 kJ.mol-1, respectively, and Gibbs free energy change (ΔG) values of −25.6071 and −24.8963, respectively, in their calculations of the free energy change (ΔG). The proposed methodologies were successfully used to evaluate folic acid in both pure and tablet form, with no influence from common excipients found in pharmaceutical formulations, demonstrating their efficacy. According to the study, there were no statistically significant differences between the precision and accuracy of the suggested procedures and those produced using the reference approach.

1. Introduction

N-[4-[(2-amino-1,4-dihydro-4-oxo-6-pteridinyl)methyl]amino] benzoyl]-L-glutamic acid is a chemical compound that can be found in a variety of shapes and forms throughout nature. In addition to preventing birth abnormalities, anaemia, and glutamic acid toxicity, L-glutamic acid is an essential B-vitamin in preventing anaemia. The treatment of diabetes can also be made more effective with this supplement. A member of the B-vitamin family, which is made up of water-soluble vitamins, which include vitamin B12 [1], Fig. 1, depicts the document's overall structure and organization.

Plants (green leaves and algae) and microbes (bacteria and viruses) both contribute to its production (bacteria, yeast). Folic acid and its derivatives, the folates, play a role in the production of amino acids and nucleotides in animals and serving as carbon acceptors and donors in the environment. Folic acid and its derivatives [37–41], folates, are also crucial in producing amino acids and nucleotides in humans [2,3]. It is a tasteless and odorless yellowish-orange crystal member of the vitamin B family of water-soluble vitamins. It has no discernible flavor or odor and belongs to the vitamin B family of water-soluble vitamins. Long recognized as one of the essential vitamins for maintaining a healthy human metabolism [42–45] , folate is now an essential supplement. According to research, folic acid may be beneficial in treating neural tube defects (NTD), cardiovascular disease, colon cancer, and a variety of anemias in children, among other conditions [4,5].

Folate is a nutrient that the human body cannot produce. As a result, it must be obtained through the consumption of foods. Studies have revealed that the average folate consumption in various countries is significantly below the levels that are considered healthy or desirable [46–48] . Some governments have approved the fortification of staple

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https://doi.org/10.1016/j.matpr.2021.09.453
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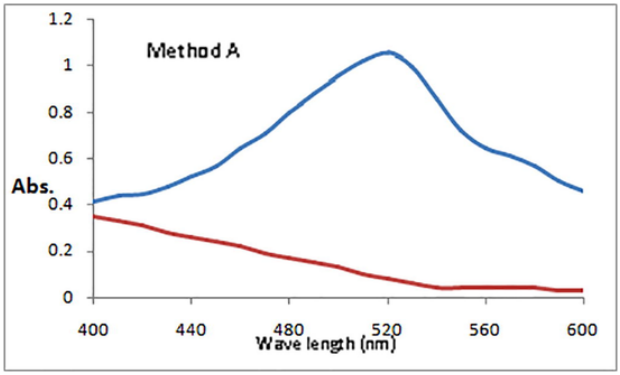


Fig. 1. method A.

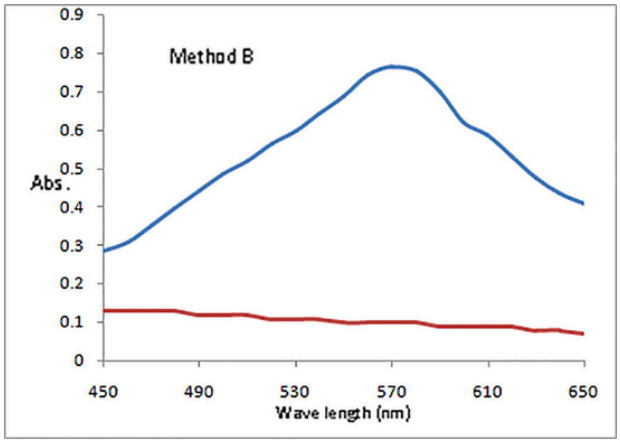


Fig. 2. method B.

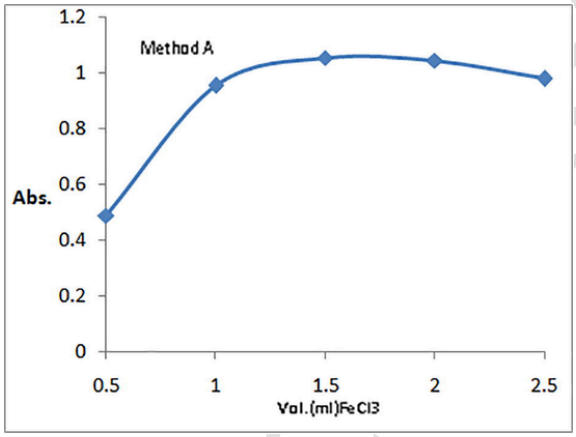


Fig. 3. Effect of Ferric chloride oxidation agents.

foods with folate to reduce the likelihood that women of reproductive age will give birth to a child born with a neural tube abnormality [6].“

Folate“ refers to a collection of naturally occurring compounds in food, such as some polyglutamates, that exhibit vitamin-like qualities similar to folic acid and are hence considered a nutritious supplement. The word ”folic acid“ comes from the Latin word ”folic acid molecule,“ which meaning ”folic acid molecule.“ While the terms folic acid and folates are sometimes interchanged, folic acid has almost double the bioavailability of folates [7].

In addition to high-performance liquid chromatography (HPLC) [8, 9], HPLC–MS [10], Phosphorimetry [25], inductively coupled plasma-attenuated mass spectrometry [18,19], bioassays [20], enzymatic methods [21], chromatography [22,23], Flow injection [24], chemiluminescence [11–13], colorimetry [14], flouorimetric [15,16], capillary electrophor are some of the techniques used to determine folic acid. Many previously described spectrophotometric procedures have significant drawbacks, including a limited range of determination, the necessity for heating or extraction, long reaction times, and product instability [49–50]. The goal of this project is to develop straightforward, fast, and sensitive spectrophotometric methods for identifying folic acid. The project's specifics are listed below. The methods' characteristics must be free of coercion when additives, such as specific vitamins, are prevalent.

As a result of these methodologies, researchers will be able to detect the presence of folic acid in pristine conditions and dose forms with greater accuracy and precision. None of the previously unknown folic acid determination methods have been used in the publication of folic acid determination methods. The most widely used analytical methods to date are the oxidation of folic acid with ferric chloride and determination of the iron(II) produced by complexing with 1,10-phenanthroline, in method A, or the reaction of the primary amino group of folic acid with Ninhydrin in an alkaline medium, in method B. The new methods were tested for linearity, range of determination, selectivity, accuracy, and precision using current ICH criteria [28].

2. Experimental

Mechanism Experimental:

Apparatus: Shimadzu UV–Vis Double Girder Spectrophotometer (model 2450) was used for all the spectral data and was calibrated using optical quartz matched cells.

Materials and Reagents: The whole investigation was done with pure reagent-grade chemicals, and deionized water was utilized the entire time.

Folic acid was procured since Sigma in (St. Louis, MO), (pharmaceutical preparation). The countries provided by kanawati are Syria, and Folded Reagents and solvent-based analytical reagent grade were supplied by the lab in Syria and the rest of the world. Locally sourced folic acid tablets were purchased. The only reagents that were used were of the highest analytical grade. 4 was where the answers were stored “C, kept refrigerated for not and over seven days and not exposed to light in dark bottles.

A stock standard solution of folic acid

(1000 mg mL-1) was made by dissolving 102 mg of folic acid in 0.2 ml of 2.0 M NaOH solution and diluting it to 100 ml with purified water. A standard working solution was obtained by diluting the stock solution to a sufficient volume (100 g ml-1).Ninhydrin Solution (0.5%):

To obtain 100 ml of solution, 500 g of Ninhydrin powder was dissolved and then mixed with distilled water.

Sodium Bicarbonate Preparation (Saturated Solution):

A magnetic stirrer was used to stir 100 ml of distilled water and 25 gm of sodium bicarbonate in a beaker for 20 min. The quantification filter paper was used to separate the product after the solution was filtered.

Ferric chloride stock solution (0.1 M):

2.7 g of the chemical was suspended in 100 ml of water and maintained in a glass vial in this solution. The stock solution was diluted to the required concentration with water to make a 0.05 M effective solution. The solution was ready to work again before the experiment was carried out.

1,10-phenanthroline reagent (0.01 M):

In solution, phenanthroline (1, 10-phenanthroline) is obtained by dispersing 198 mg of the substance in water and diluting the solution to 100 ml with water.

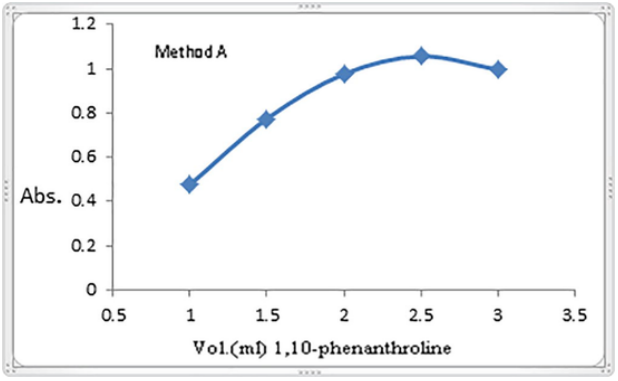


Fig. 4. Effect of volume 1,10-phenanthroline reagent.

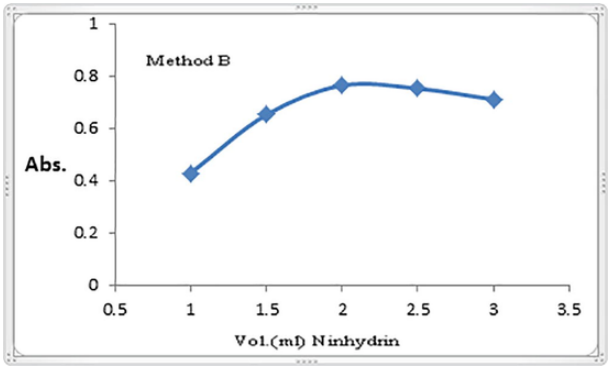


Fig. 5. Effect of volume Ninyidrin reagent.

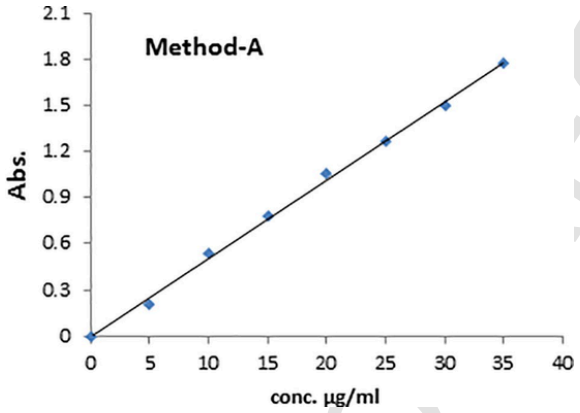


Fig. 6. Calibration curve of Folic acid with method A.

3. Procedure for pharmaceutcal formulations:

Twenty tablets were thoroughly mixed again and ground into powder form. Approximately 10 mg of folic acid were dissolved with 2 ml of 2 M sodium hydroxide (NaOH) solution, and the solution was then diluted with two liters of deionized water. It took 30 min to agitate the volumetric flask thoroughly; It was necessary to filter the solution through a Whatman filter paper (No. 1) to obtain a pure solution.

4. Method a

Two milliliters of average work solution (100 µg/ml) were accurately measured in a micro burette. Then, 5 ml of the solution was added to the total volume to make it 10 ml. A standard solution of ferric chloride (0.05 M) and 1, 10-phenanthroline (0.01 M) was first added to each flask to give a total volume of 10 ml, and these solutions were then successively added to give the required 1.5 ml in each flask. Stoppard flasks mixed well and left to exist for 15 min with infrequent shaking produced excellent results. After which, the solution absorbance was read at 505 nm in the presence of the reagent blank.

5. Method B

Let 0.5 ml of the 10 ml normal working solution (100 µg/ml) of the folic acid solution, 1 ml of Ninhydrin, and 1 ml of saturated sodium bicarbonate solution be added to a 10 ml volumetric flask. Mix it well and fill the rest of the flask with distilled water to the threshold. This process was conducted in a boiling water bath where the temperature was held at 97 ± 1 °C for 15 min and then allowed to return to ambient temperature. The spectrophotometer registered the absorption maxima at 575 nm in the spectra and found that the spectrum matched the reagent blank.

6. Results and discussion

This research sought to create a simple spectrophotometric approach to the assessment of Folic acid in its therapeutic dosage forms and in its pure form. The medication containing folic acid was discovered to become oxidized with $FeCl_3$ in a neutral solution. Fe^{2+} has been observed to complex with phenanthroline (a derivative of 10-phenanthroline) to form a complex called Fe^{2+}: 1,10-phenanthroline complex (also called the 1,10 phenanthroline complex). Folic acid remains unmeasured in the market at this time, though preliminary studies have suggested that this new approach is appropriate for Folic acid analysis. Oxidation of Folic acid with excess $FeCl_3$ in neutral media yielded a Fe^{2+} which was measured after being absorbed in the various wavelengths with 1, 10-phenanthroline (Fig. 1).

In an alkaline medium, 2-hydroxyindan-1,3-dione is formed in the conversion of Ninhydrin to ocarboxyphenyl glyoxal. In the presence of saturated $NaHCO_3$, the primary amino group of Folic acid reacted with Ninhydrin to give a violet-colored solution. Folic acid and Ninhydrin are oxidized, deaminated, and condensated (using water) to form the colored Ruhernann purple, as demonstrated in Fig. 2.

7. Optimization of the reaction circumstances

In order to get the best enzymatic browning in the detection and quantification of Folic acid, various tests were conducted. Each of the dependent variables was examined to see how they influence the reaction.

A variable volume of oxidizing agent (0.5–2.5 ml) was put to 10 ml volumetric flasks to determine the appropriate volume of oxidizing agent (Fe3 +). When the Fe3 + concentration was raised, the reagent blank absorbance increased. The reaction's “feasibility,” as determined by measuring the minimum blank absorbance with a fixed amount of 1.5 ml of 0.1 M ferric chloride in a total volume of 10 ml, led to the conclusion that 1.5 ml of 0.1 M ferric chloride in a total volume of 10 ml was the best solution for method A, as shown in Fig. 3.

Fig. 4 shows that the volume of 2.5 ml of 1,10-phenanthroline reagent solution (0.01 M) is the ideal amount, according to the study done with varied volumes of 1,10-phenanthroline reagent solution (0.01 M) with procedure A. It was determined to be meaningful since it was picked in subsequent studies.

In study Method B, 2.0 ml of ninhydrin reagent was used to replace 2.0 ml of the folic acid drug in a conical flask, and after the addition of 0.01 M of Ninhydrine reagent, the resulting solution volume was completed to 10 ml with distilled water. The results can be seen in Fig. 5, which shows that using 2.0 ml of ninhydrin reagent yielded the maximum absorption. as a result, the new discovery was realized in further research.

The research revealed that standing periods for full-colour development were 15 min for meth‹xt A and 30 min for meth‹xt B, and the

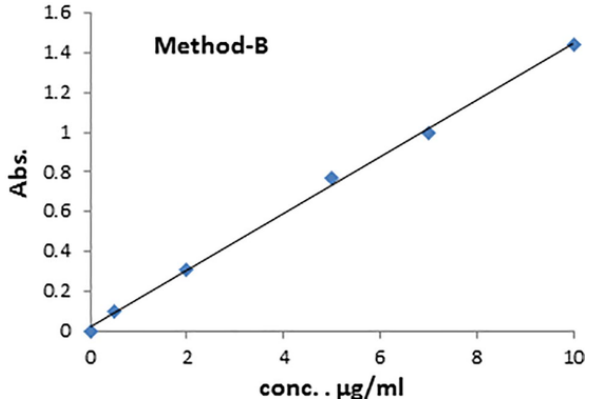


Fig. 7. Folic acid calibration curve using technique B.

Table 1
Folic acid optical data and validation parameters.

Method B	Method A	Parameters
575	505	λ_{max}. (nm)
0.1–10	1.0–35	Beer's law limits (µg/ml)
0.9988	0.9979	Correlation coefficient (R^2)
0.021	0.003	Intercept
0.142	0.050	Slope (b)
0.488	0.198	Limits Of Detection LOD(µg/ml)
1.479	0.600	Limits Of Quantitation LOQ(µg/ml)
6.771×10^4	2.331×104	Molar Absorptivity (l mol^{-1} cm^{-1})
16.728×10^{-5}	$1.944 \times 10–6$	Sandell's sensitivity (µg/cm2)

Table 2
shows the accuracy of techniques A and B.

RSD %	RE %	Recovery%*	Conc. of DCL (µg.ml^{-1})		Method
			Found*	Taken	
1.275	0.60	100.60	5.03	5.0	**Method A**
0.967	0.50	100.50	10.05	10.0	
1.088	2.25	102.25	20.45	20.0	
0.989	1.66	98.33	2.95	3.0	**Method B**
1.065	1.5	101.50	6.09	6.0	
1.329	0.22	99.88	8.98	9.0	

colour was stable for 50 min after completion of the standing time in both methods.

An experiment was conducted on the effect of temperature (5–60 °C) on the coloured product's absorption. I discovered that the best absorption occurred at 25 °C for method A, which is why the results are shown. The findings of the previous experiments allow being used in the following experiments.

In method B, when Ninhydrin was added to saturated sodium bicarbonate, it was found that the solution required 97 ± 1 °C for attaining optimal and steady absorbance, which was stable for about 5 h after that.

To the solution with 0.5–2.0 ml of 1.0 M sodium carbonate, 0.5–2.0 ml of a saturated sodium carbonate solution was added. In the studies that followed, 1.0 ml of this sodium carbonate solution was used, as shown in Fig. 5.

8. Method validation

Research methodology for linearity, responsiveness, detection limits, quantification, precision, accuracy, specificity, and recovering was validated using ICH guidelines [29,30].

To a series of volumetric flasks, 1.5 ml ferric chloride (0.1 M) and 2.5 ml 1, 10-phenanthroline reagent solution (0.01 M) were added (10 ml). At that point, the problem became somewhat more complicated; hence, all of the solutions had to be finished within 15 min, and the volumes had to be filled to the mark with distilled water. The absorption spectrum was found to be 505 nm concerning the blank. When using a calibration curve, as in Fig. 6, the calibration curve is linear for a range of concentrations of 1.0–35.0 µg/mL for method A.

Folic acid (100 µg/ml), a series of ten-milliliter volumetric flasks, 1.0 ml Ninhydrin, and 1.0 ml saturated sodium bicarbonate solution were aggregated into a single solution, with the addition of a tenth of a milliliter of distilled water. Then the volumetric flasks were finished to the mark. Flasks have been heated for 15 min in a water bath at 97 ± 1°Celsius, then brought back to room temperature and measured the absorbance of the solution at 575 nm, without the background reagent, against the blank reagent. The calibration curve in method B, shown in Fig. 7, is linear between concentrations of 0.5 µg/ml and ten µg/ml. Absorptivity values are 2.331 × 104 and 6.771 × 104 L.mol-1.cm-1, with the Sandell sensitivity index of 1.944 × 10–6 and 16.728 × 10–5 µg/cm2.

The regression analysis:

The a-intercept, b-slope, R-regression coefficient, b-slope, and a-intercept standard deviations are symbols (Table 1). The low Sandell specificity and sensitivity molar absorptivity numbers 6 and 7 [31] revealed that the approach had a relatively high sensitivity.

The detection limits (LOD) and quantitative limits (LOQ) were calculated using the identical formulas equations as the detection limits (LOD):

$LOD = 3.3\sigma/s$

$LOQ = 10\sigma/s$

Where σ is the slope of the calibration curve and is the standard deviation of five blank reagent results, table 1 [32] summarizes the LOD and LOQ values.

9. Accuracy

A healing study was conducted to assess the efficacy of the proposed sample, and the findings are presented in Table 2. Both approaches had good precision in their outcomes.**determinations on average**

The outcomes of proportion recovering (limit 98.33/r to 102.25/e) and the repeatability of repeatability were within the prescribed limit for tablets (Table 2). The results showed that the method was successful in estimating the folic acid content in the formulation.

10. The precision

The method's precision was tested across three days for samples exhibiting intra-day and inter-day variability (n = 5). Method A used concentrations of 10.0 to 0.0 and 30 g/ml, while method B used concentrations of 2.0, 4.0, and 8.0 g/ml. Method A used concentrations of 10.0 to 0.0 and 30 g/ml, while method B used concentrations of 2.0, 4.0, and 8.0 g/ml. The inter-day and intra-day calibration samples and the calibration solution were all prepared on the same day as the analysis.

Three samples of the same batch (same concentration) obtained at the start of the test period, 24 h into the test period, and 48 h into the test period demonstrate the precision of the test procedure (Table 3). Three similar specimens from the same batch (all of the same concentration) must be collected on three separate days, indicating good inter-day precision in the testing methodologies (Table 3).

The premeditated relative standard (RSD%) and relative error (RE%) of the found absorption were used as the primary basis for sensitivity and efficiency. The tables on the following page summarize the intra-day exact and accurate data for the suggested methods for determining folic acid, which is < 2.

Table 3
Assessment of intra-day and inter-day precision and accuracy

Inter-day Accuracy and precision			Intra-day Accuracy and precision			Folic acid taken (µg/ml)	Method
RSD%	RE%	Folic acid found (µg/ml)	RSD%	RE%	Folic acid found (µg/ml)		
1.55	0.50	9.95	0.99	0.30	10.03	10	**Method**
1.46	0.20	19.96	1.05	0.50	20.10	20	**A**
1.09	0.07	29.98	1.32	0.77	30.23	30	
0.98	0.53	1.99	0.89	0.50	2.01	2	**Method**
1.11	1.09	3.96	1.44	1.25	4.05	4	**B**
1.70	0.63	7.95	1.06	1.38	8.11	8	

11. Concern of interventions:

A volumetric flask (10 ml) containing 2.0 ml of folic acid (100 kg/ml) was used to test the efficacy and specificity of the suggested protocol. The flask also contained foreign materials such as those found in dosage forms (such as folic acid, found in most dosage forms). To obtain the best possible uptake and recovery, ideal conditions were applied, and the absorbance at 505 nm and 575 nm for methxt A and B was measured, and the results were compared to those obtained with a blank reagent and recovering. According to the findings, substances do not interact with the function of the true folic acid complex in a negative way.

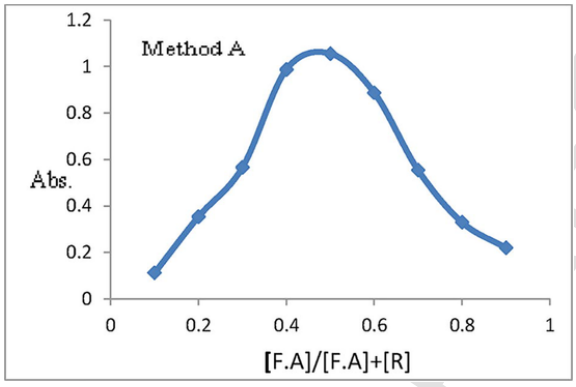


Fig. 8. In technique A, Job's approach of forming a product.

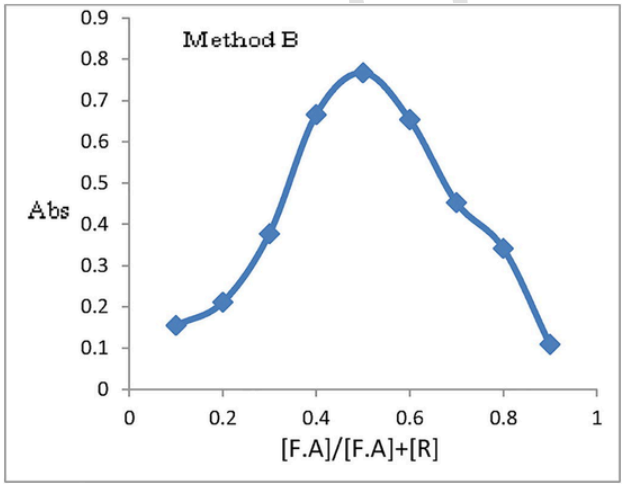


Fig. 9. In technique B, Job's approach of forming a product.

12. Stoichiometry of product:

Product stoichiometry is a measure of how well a product works. Equimolar densities of the drugs and reagents were used to study the stoichiometry of the reaction using the process of continuous variation (the lopes method). The stoichiometry of the reaction was found to be 1:1 by applying the process of continuous variation as illustrated in Figs. 8 and 9. The Figs. 8 and 9, reflect the results obtained according to the Job's technique.

As a result, the product is most likely formed for both procedures, as shown in scheme 1.

Schema.1: Probable product formation pathway for method A.

Using the equation [33,34], the stability constants of the products for the reaction between a folic acid medication and reagents yielding ML complex were determined under ideal conditions to be 4.1617105 L.mol1 and 2.9060105 L.mol1 for procedures A and B, respectively, for the reaction between a folic acid medication and reagents yielding ML complex.

Where:

α = degree of dissociation

C = concentration of colored product

Am = is the greatest value of the absorption

As = absorption value at the equivalence point (when the ratio of product 1:1)

The stability constant (log Kf) for colored products was 5.6192 and 5.4633, respectively, for procedures A and B, showing that the product is stable.

The Gibbs free energy change (G) [35] of the process was also calculated using the following equation:

$$\Delta G = -2.303\ R\ T \log Kf$$

Where: ΔG = Gibbs free energy change of the reaction (k.J. mol^{-1})

R = Universal gas constant (8.314 J)

The value of Gibbs free energy, are found to be -25.6071 k.J.mol^{-1} and -24.8963 k.J.mol^{-1} for colored product for method A and B respectively.

The higher K*f* and negative ΔG values obtained indicate very stable reaction products.

13. Application of analytical techniques

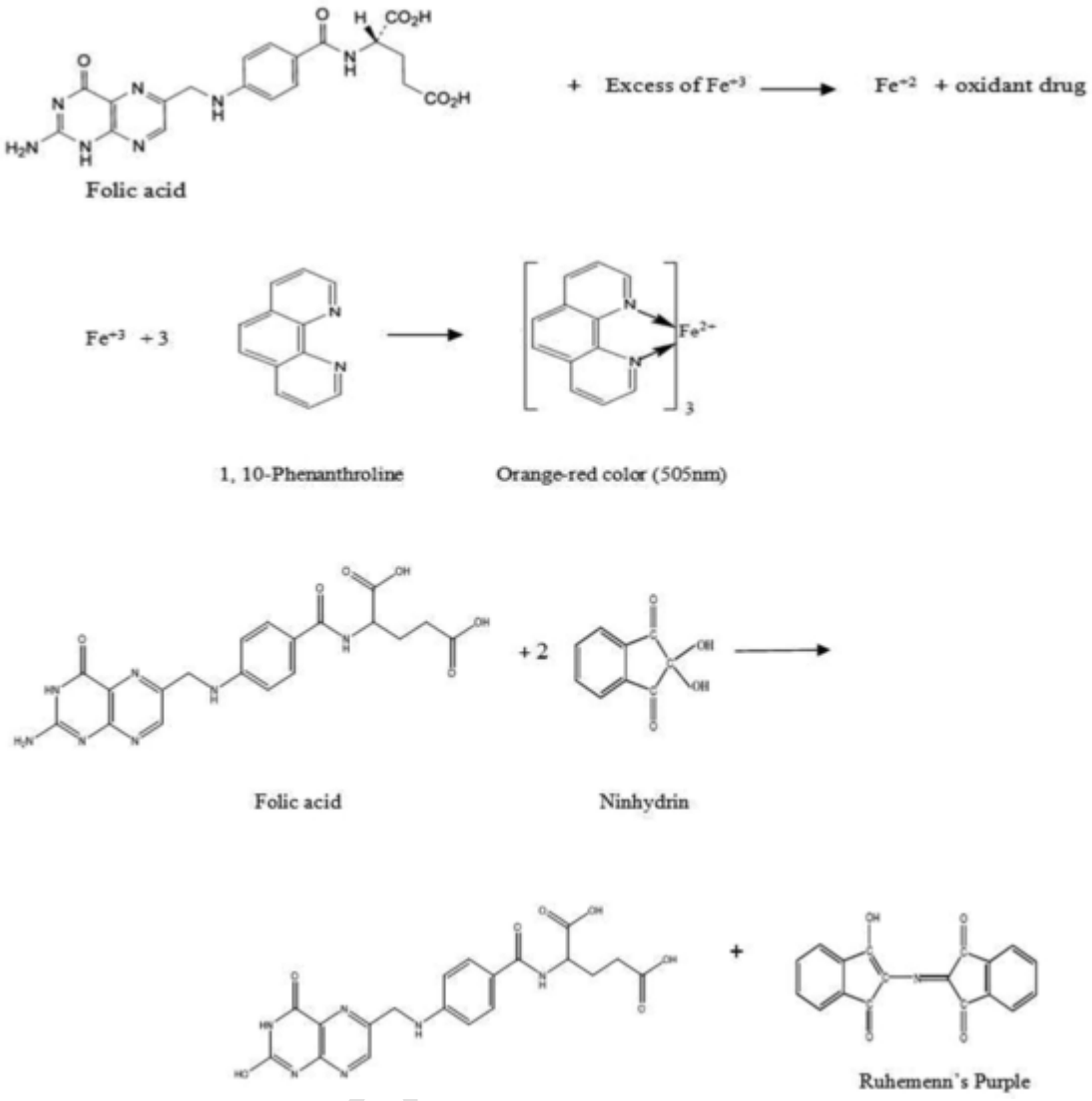
The results in table 4 show a low RSD percentage (1%) for methods A and B, indic

ating good precision and recovery precision ranges (101.00, 98.80), (100.60–99.25) for methods A and B, indicating good accuracy. This showed no interaction of the excipients and the excellent sensitivity of the method signed to capable applied the developing methods successfully to the determination of folic acid in pharmaceutical preparation.

14. Conclusions

This proposal uses spectrophotometry to detect folic acid, and the proposed method has the benefit of being simple, precise, accurate, and cheap. When the drug was oxidized with ferric ion, method A required a specific formation complex between the 1,10-phenonthroline reagent and Fe to be created. 505 nm absorbed the color product. Method B includes using Ninhydrine as an acid catalyst to form a purple-colored product, and further spectrophotometric measurements are completed under a saturated solution of NaHCOs at a wavelength of 575 nm without using any organic solvents. With this process, you do not have to use solvent extraction, and the technique is also quite simple to implement.

As a result of the changes made to the various aspects of the system, the linear range was increased from 0.1 to 35 µg/L (from 0.0001 to 10 µg/L), the molar absorptivity was increased from 2.331 × 10 L/mol/cm to 6.771 × 10 L/mol/cm, and the sensitivity was increased



Scheme 1. For procedures A and B, this is a possible product creation pathway.

from 1.944 × 10–5 to 1.6728 × 10–6 μg cm2. There is also a breakdown of the LOD (a very high value in this case; in this case, 0.1 μg/L) and the LOQ (a low value in this case; in this case, 0.600 μg/ml) for both methods A and B. In addition, research into the Gibbs free energy (ΔG) and the Kf constant (stability constant) has been carried out, indicating that the reaction products are highly stable. Compared to the currently available methods for quantifying folic acid, the new techniques can be characterized as environmentally friendly because they do not require organic solvents to achieve the desired results. In addition, the device is inexpensive, which increases the method's feasibility even further. The accuracy of this low-cost and environmentally friendly spectrophotometric method for determining folic acid concentrations in bulk and fixed-dose combination tablets is high, and it can be used for quality control of folic acid in fixed-dose combination tablets as well.

15. Thanks and appreciation:

All of the employees in the Department of Pharmaceutical Chemistry at the College of Pharmacy, University of Basrah, Iraq, and the College of Pharmacy, Al-Ayen University/ Thi-Qar, Iraq, deserve our gratitude.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Table 4
shows how the approaches can determine the amount of folic acid in pharmaceutical formulations.

Proposed methods	Conc.(µg/ml)	Commercial Formulations analyzed					
		Folmed (5.0mg/ tablet)			Kanafolicc (5.0mg/ tablet)		
Method A	Taken conc. (µg/ml)	5.0	10.0	15.0	5.0	10.0	15.0
	Found conc. (µg/ml)	5.05	10.07	15.04	5.03	9.88	15.06
	Recovery %*	101.00	100.70	100.26	100.60	98.80	100.40
	RSD%*	0.99	1.02	0.96	1.05	1.07	1.10
Reference method	Recovery% ± SD**	100.96±0.12			101.07±0.08		
Method B	Taken conc. (µg/ml)	2.0	4.0	8.0	5	10	20
	Found conc. (µg/ml)	1.98	3.97	7.97	5.03	9.96	19.88
	Recovery %*	99.00	99.25	99.63	100.60	99.60	99.40
	RSD%*	1.17	1.22	0.99	0.95	1.33	1.18
Reference method	Recovery% ± SD**	104.25±0.13			101.00±0.09		

*Average of three determination, ** Average of five determination ,RSD= Relative standard deviation , SD= standard

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