SPECTROPHOTOMETRIC MICRODETERMINATION OF FOLIC ACID IN PHARMACEUTICAL TABLETS OXIDATIVE VIA COUPLING REACTION WITH P-AMINOPHENOL IN THE PRESENCE OF POTASSIUM IODATE

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1. Introduction

Folic acid, 4-(2-amino-4-hydroxypteridine-6-yl) methylaminobenzoyl-l-glutimic acid, is usually employed in the treatment or prevention of megaloblatsic anaemia during pregnancy, childhood and other clinical situations often associated with alcoholism and diseases[1]. Methods for folic acid determination include liquid chromatography with different detectors[2-4], high performance liquid chromatography with different detectors[5-7], spectophotometry[8-10], polarography[11-12], voltammetry[13-15], flow injection chemiluminescence[16-17] and fluorimtric [18-19]. Oxidative coupling organic reactions were recently used for the micro determination of many organic compounds [20] and drugs[21]. As far as we know, no one used these reactions for folic acid determination. Therefore the objective of the investigation reputed in this paper was to evaluate spectrophotometric method for the determination of folic acid (FA) based on the reaction of FA with p-minophenol in acidic medium and in the presence of potassium iodate as oxidizing agent. A stable-soluble red colour product was formed which can be measured at 530nm. The method doesn't require temperature control or solvent exaction step and can be applied successfully to pharmaceutical tablets that containing FA.

2. Experimental

Apparatus:

All absorbance measurements were carried out Cecil CE 1021. Single–beam recording spectrophotometric using 1cm silica cells.

Reagents:

All chemicals used were of analytical reagent grade and folic acid (fluka).
Folic acid (FA) stock solution (1000 mg. ml-1), A 0.1 g amount of FA was dissolved in solution of sodium hydroxide (0.1M) and made up to 100ml volumetric flask with the same solution.
FA working solution (100 mg. ml^{-1}), prepared by diluting 10ml of stock to 100ml volumetric flask with distilled water.
P-aminophenal reagent ($5x10^{-3}$ M), prepared by dissolving 0.05445g of P-aminophenol regent in ethanol and made up to 100ml volumetric flask with the same solvent.
Potassium iodate solution ($1x10^{-2}$ M), prepared by dissolving 0.5350 g of potassium iodate in distilled water and made up to 250 ml volumetric flask with distilled water. **Procedure:** Into a series of 25ml calibrated flask, transfer increasing volume of FA working solution (100 mg /ml^{-1}) to convert the range of the calibration curve (50-1000 µg. ml^{-1})
In find volume of 25ml, add 4.5 ml of ($1x10^{-2}$ M) of potassium iodate solution and 1.5ml of ($5x10^{-3}$ M) of p-aminophenol and shake will, followed by 1ml(1M) of hydrochloric acid.
Dilute the solution to mark with distilled water and allow the reaction mixture to use for 40 min at room temperature. Measure the absorbance at 520 nm against a reagent blank prepared in the same way but containing no FA .The colour of the formed product instable for 120 min.
For the optimization of conditions and in all subsequent experiments, solution of 1000mg of FA in a final volume of 25ml was used.

3. Procedure for Pharmaceutical Tablets

Weigh and finally powder an enough number of tablets followed by extraction of an cutely weighed portion of the powder equivalent to about 0.1 of FA in a solution of sodium hydroxide (0.1M). Shake and filter the solution into a 100ml volumetric flask. Wash the residue and dilute to volume with solution of sodium hydroxide (0.1M) to obtain (1000µg. ml^{-1}) of FA, dilute 10ml of this solution to 100ml by distilled water to prepare (100µg. ml^{-1}) of FA. Use suitable volume of it solution for the colour formation with potassium iodate, p-aminophenol and acid a described under calibration procedure.

4. Results and Discussion

Absorption Spectra:

When Avery dilute aqueous solution FA was mixed with potassium iodate and p-aminophenal reagent and hydrochloric acid, an intense red colour forms after 5 min, which became stable after 40 min. the red product has a maximum absorption at 530 nm. [Fig: (1)] shows the spectra of the red product formed and of the reagent blank, the maximum absorption at 530 nm was used in all subsequent experiments.

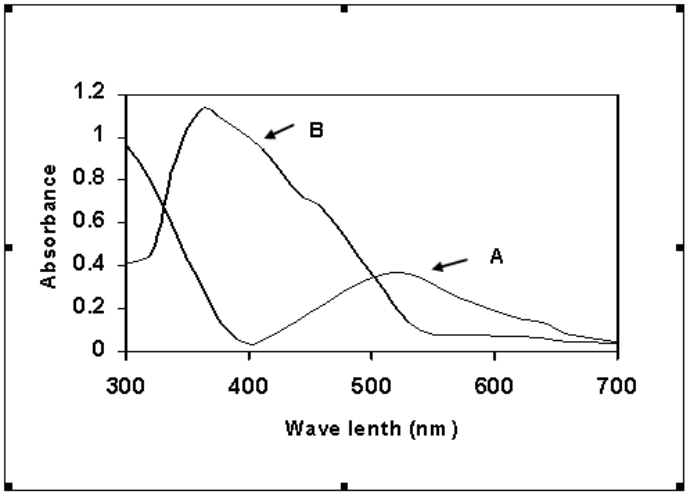


Fig. (1): Absorption Spectra of A (1000µg/ 25ml) of FA treated as described under procedure and measured against reagent blank and B the reagent blank measured against distilled water.

Study of the Optimum Reaction Conditions:

The effect of various parameters on the absorption intensity of the formed product were studied and the reaction conditions were optimized

Effect of Acid:

It was found experimentally that coloured product was formed only in acidic medium. Different acids were examined and these include hydrochloric, sulphureic, phosphoric and acetic acid, only hydrochloric acid was found optimum since it give a high sensitivity,

minimum blank value and high stability of the coloured product. The effect of the amount of hydrochloric acid was also investigated and 1ml of 1M was selected and was used in all subsequent experiments.

Effect of Reagent Concentration:

When various concentrations of p-aminophenal solution were added to fixed amount of FA solution, 1.5ml of ($5x10^{-3}$M) solution was found enough to develop the colour to its full intensity and give a minimum blank value and therefore considered to be optimum for the concentration range of (50-1000µg/25ml) of FA.

Effect of Oxidant Concentration:

The product formation reached maximum with about 4.5 ml of ($1x10^{-2}$ M) potassium iodate solution and remained at this maximum when (0.1 - 8ml) of the oxidant concentration was added to FA, 4.5 ml volume of the oxideizing agent solution was therefore used in the procedure since it give high sensitivity minimum blank value and ensure quantitative determination at the upper limit of calibration graph.

Effect of Order of Addition:

To obtain optimum results, the order of the addition of reagent should be followed as given under the procedure, otherwise a loss in the colour intensity and stability were observed.

Effect of Reaction Time:

The colour intensity reached a maximum after FA solution had been reached immediately with potassium iodate and p-aminophenl in acidic medium and became stable after 40 min, therefore 40 min development time was elected a optimum in the general procedure. The colour obtained was stable for 120 min.

Effect of Temperature:

The effect of temperature on the colour intensity of the product was studied. In practice the same absorbance was obtained when the colour was developed at room temperature ($20C^{o}$) but when the calibrated flask was placed in an ice- bath at ($5C^{o}$) or in a water-bath at ($40C^{o}$) a loss in colour intensity and stability were observed, it is

therefore recommended that the colour reaction should be carried out at room temperature(20C^{o}).

Accuracy and Precision:

To determine the accuracy and precision of the method, FA was determined at two different concentrations. The results shown in Table (1), indicate that a satisfactory precision and accuracy could be obtained with the proposed method.

Table (1): Accuracy and precision of the proposed method:

Amount of FA taken µg/ml	Recovery %*	Relative Standard Deviation*
20	100.73	1.25
40	100.03	0.99

*Average of five determinations.

Calibration Curve:

Employing the conditions described under procedure, a liner calibration curve [Fig:(2)] for FA was obtained ,which show that Beer's law was obeyed over the concentration range of (50-1000 µg/25 ml$^{-1)}$ or (2-40 ppm) with correlation coefficient of (0.9995) and an intercept of (0.0074). The conditional molar absorptivity of the red product formed with FA was found to be (4.8x10^{3} L. mol^{-1}.cm^{-1}) with reference to FA and a Sandell sensitivity was (0.092 µg cm^{-2})

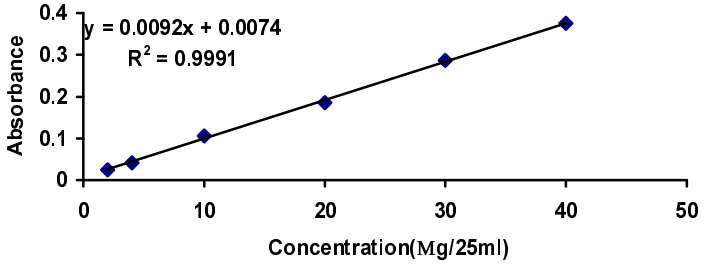


Fig. (2): Calibration curve for folic acid

Structure of the Product:

The stoichiometry of the reaction between FA and p-aminophenol was investigated using ratio method. The results obtained [Fig:(3)] show that a(1:1) product was formed between FA and p-aminophenol regent at 520 nm, therefore the formation of the product probably occurs as follows:

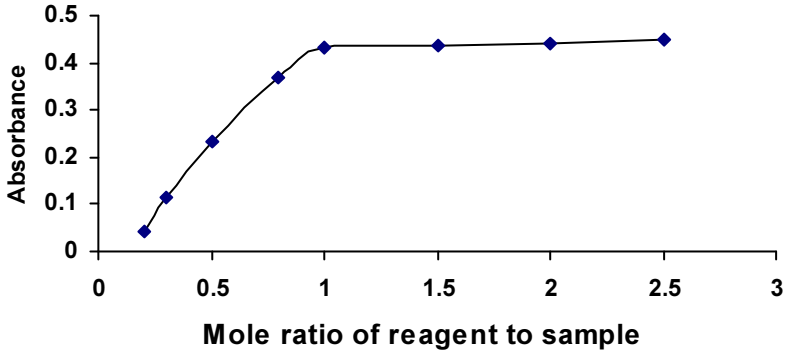
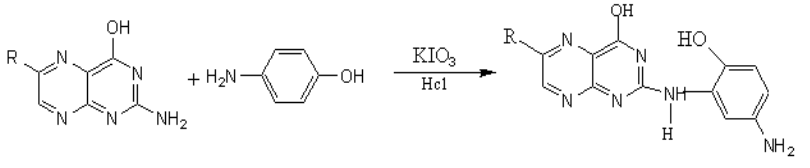
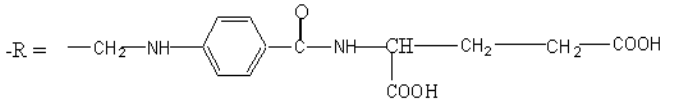


Fig. (3): Mole ratio of reagent to sample for the product formed

The concentration of both the sample and reagent are $9x10^{-3}$





The product formed was soluble in water. The apparent stability constant was calculated by comparing the absorbance of solution containing stoichiometric amount of FA and p-aminophenol with that of a solution containing a five-fold excess of p-aminophenol reagent.

The stability constant of the product in water under the described experiment of condition was ($9.1x10^4$ L. mol^{-1}).

Analytical Applications:

Three types of tablets containing FA have been analyzed and they gave a good accuracy and precision [Table (2)] the proposed method was compared successfully with the British pharmacopoeia standard method [Table (3)], since F-test and T-test showed that there was no significant differences between the proposed method and the standard method [22].

Table (2): Application of the proposed method for the determination of FA in pharmaceutical tablets.

Tablet sample	Wt of tablet (mg)	Wt of FA (mg)	Amount of FA taken(μg/ml)	R.S.D%*	Recovery%*
Folic acid	107.18	1.00	40	1.35	98.86
Furafolic	125.23	5.00	40	0.88	99.32
folmed	105.12	5.00	40	.98	99.70

*Average of five determinations.

Table (3): Comparison of the proposed method with standard method to determination of FA in pharmaceutical tablets.

FA tablet sample	Recovery%*	
	Proposed method	Standard method
Pure folic acid	100.00	100.00
Folic cid	98.86	98.49
Fur folic	99.32	99.20
Folmed	99.70	99.52

*Average of five determinations.

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