Biomedical & Pharmacology Journal Vol. **2**(2), 317-320 (2009)

Novel spectroscopic methods for determination of folic acid in pharmaceutical formulations

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(Received: May 20, 2009; Accepted: June 30, 2009)

ABSTRACT

Sophisticated analytical methods viz. HPLC and HPTLC which are being employed for analysis are relatively expensive and hence need for simple analytical methods arises, that are suggested in the proposed methods for routine determination of Folic acid in pharmaceutical formulations and bulk dosage forms. These methods are based on the formation of colored species on binding of Folic acid with Sodium nitroprusside and Ammonia reagent to produce a dark yellow colored chromogen (λ_{max} at 390) For Method A and MBTH and ferric chloride to produce a green colored chromogen (λ_{max} at 690) for Method B. Results of analysis were validated statistically and by recovery studies. Assay and recovery studies were also performed. The percent Relative Standard Deviation of these methods were found to be 1.37 for Method A and 0.77 for Method B. Based on these values these methods could be treated as simple, sensitive and reproducible based on the principle of absorption visible spectrophotmetry for the determination of Folic acid in pharmaceutical formulations.

Key words: Folic acid, Analysis, Spectroscopy, Molar abosrptivity, Beer's Law.

INTRODUCTION

Folic acid[1-4] also called folacin or pteroyl glutamic acid also designated, as B_9 is a water-soluble vitamin of high biological significance. The vitamin exists in the form of its coenzyme called tetrahydrofolic acid which is associated with the metabolism of one carbon compounds. It is concerned with the formation of RBC in the bone marrow and is an essential component in the synthesis of nucleotides, especially the purine nucleotides AMP and GMP. Foods with folic acid in them include leafy green vegetables, fruits, dried beans, peas and nuts. Enriched breads, cereals and other grain products also contain folic acid. Folic acid, a B vitamin, helps prevent birth defects of the brain and spinal cord when taken before the end of early pregnancy. A deficiency of folic acid after birth causes a kind of anemia, namely, megaloblastic anemia in which there is a paucity of red blood cells and those that are made are unusually large and immature.

Only a few methods viz, Spectrophotometric [5-11], HPLC [12-15] appeared in the literature for the determination of Folic acid[1-4] in bulk, formulations and dosage forms. As the vitamin is of immense biological importance, the number of available procedures for estimation that could be of utility to a small-scale industry is less and hence the author has presented these methods described below for the routine quality control analysis of folic acid in dosage forms.

MATERIAL AND METHODS

Instrumentation

After due calibration of the instrument,

spectral and absorbance measurements are made using Systronics UV – Visible Double beam spectrophotometer model 2201.

Reagents

All the chemicals used were of analytical grade. All the solutions were freshly prepared with double distilled water. Freshly prepared solutions were always used for analysis. In the proposed methods aqueous solutions of Sodium nitroprusside and Ammonia reagent to produce a dark yellow colored chromogen (λ_{max} at 390) for Method A and MBTH and ferric chloride to produce a green colored chromogen (λ_{max} at 690) for Method B were used.

Standard and Sample solution of Acyclovir

About 100 mg of Folic acid (formulation) was accurately weighed on a digital single pan balance and dissolved in 100 ml of water in a volumetric flask to prepare a solution that has a concentration equal to 1 mg/ml standard solution and further dilutions are made with the same solvent (100 µg/ml) for Method A and Method B.

Assay Procedure

Method A

Aliquots 1-5 ml of standard folic acid solution (100 µg/mL) was transferred to a series of 10 ml graduated tubes. To each tube 2 ml of sodium niotroprusside solution was added followed by 0.5ml of concentrated ammonia solution. The absorbance of the dark yellow colored chromogen was measured at 390 nm against reagent blank.

Method B

Aliquots 1-5 ml of standard Folic acid solution (100 µg/ml) was transferred to a series of 10 ml graduated tubes. To each tube 2 ml of MBTH solution was added followed by 0.5 ml of ferric chloride reagent. The absorbance of the green colored chromogen was measured at 690 nm against reagent blank. The amount of Folic acid was computed from the calibration curve. The absorption spetrum of Folic acid showing λ_{max} at 390 nm for Method A and 690 nm for Method B is represented in Fig.-1.1 and 1.2 The Beers law plots of Folic acid for methods A and B are shown in Fig 2.1 and 2.2.

RESULTS

The results of analysis for methods A and B were validated through systematic statistical analysis and the results are tabulated. The statistical analysis values are reported in Table-1 and assay and recovery results for these methods are tabulated in Table-II.

Table 1: Optical characteristics, precision and accuracy of the proposed methods for folic acid

Parameter	Method A	Method B
λ_{max} (nm)	390	690
Beer's law limit (µg/ml)	10-50	10-50
Sandell's Sensitivity (µg/cm^2/0.001 abs. unit)	0.00279	0.0975
Molar absorptivity(litre.mole^{-1}.cm^{-1})	4.7×10^4	0.4×10^4
Correlation coefficient (r)	9998.62	9999.23
Regression Equation (Y)* Slope a	1.421	0.0263
Intercept b	0.00024	0.000395
% RSD	1.37	0.77
%Range of errors(95%Confidence limit)		
0.05 level of Significance	± 1.1508	± 0.643
0.01 level of Significance	± 1.4236	± 0.7954

* Y= a + bx, where'Y'is the absorbance and x is the concentration of folic acid In µg/ml

** For six replicates.

Table 2: Estimation of Folic acid in Pharmaceutical formulations

Formulations	Labeled amount mg/Tablet	% Recovery by proposed methods	
		Method A	Method B
Tablet 1	250 mg	99.1	99.82
Tablet 2	250 mg	99.6	99.4
Tablet 3	250 mg	99.68	99.7
Tablet 4	250 mg	100.4	100.3

DISCUSSION

Method A

The proposed method is based on initial reaction of the drug with sodium nitroprusside and further reaction of the drug with ammonia to form a dark yellow colored complex. This method is a simple examples of oxidation followed by complex formation i.e., oxidation in the presence of Ferric Chloride.

Method B

The proposed method is based on initial reaction of the drug with Potassium ferri cyanide and further oxidation of the drug with ferric chloride in acidic medium to form a yellow colored complex with Ferric Chloride**.** This method is a simple examples of oxidation followed by complex formation i.e., oxidation in the presence of Ferric Chloride in acidic medium.

For these methods the optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and sandell's sensitivity, regression analysis using the methods of least squares, slope (a), intercept (b) and correlation coefficient (r) obtained from different concentrations are summarized in Table-1. The precision and accuracy were found by analyzing six replicate samples containing known amounts of the drug and the results are summarized in Table-1.

The accuracy of these methods in the case of formulations was thoroughly studied by recovery experiments and the results were presented in Table-2. Additional checks on the accuracy of these methods were analyzed by adding known amounts of pure drug to pre-analyzed formulations

CONCLUSION

Performance recovery experiments and percent recovery values obtained indicated the absence of interferences from the commonly encountered pharmaceutical additives and excipients. Thus the proposed methods are simple and sensitive with reasonable precision and accuracy and can be used as a standard method for the routine determination of Folic acid in quality control analysis.

ACKNOWLEDGEMENTS

The authors are grateful to the management of Koneru Lakshmaiah College of Engineering, Vaddeswaram, Guntur Dist. for their continuous support and encouragement and for providing the necessary infrastructure facilities for executing this work.

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