

Estimation of the Folic Acid Using Zero Order, Area Under Curve and First Derivative Spectrophotometric Methods in Pure and Marketed Tablet Formulations

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Abstract

Three simple, inexpensive and nontoxic spectrophotometric methods have been used for determination folic acid in pure and market formulation tablets. Linearity was founded in the range 6- 20 mg.l⁻¹ for the all three methods, the detection limits was found to be 0.261 for zero order method, 0.006 for AUC method and 3.800 for first derivative method. The RSD% was found to be less than 0.856 indicating a good accuracy and precision of three proposed methods. The results of zero order, AUC and first derivative methods were statistically compared with those obtained by the official standard method using the F-test and t-test and found to be a good agreement.

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Keyword: folic acid, zero order, AUC, first derivative, spectrophotometric.

Introduction

Folic acid(FA) as in fig.1 is an organic compound as a part of the vitamin B complex^[1], FA is formed by the some plants, microorganisms and FA can also be found in human and animals body like liver and bone marrow^[2]. There are many methods are used for the estimation folic acid including; determination FA in fruits, vegetable, cereal and pharmaceuticals tables by HPLC method^[3-7], FTIR spectroscopy^[8], capillary electrophoresis (CE)^[9], spectrophotometric^[10,11], Gold nano partical^[12], chemiluminescence^[13] voltammetric^[14] and flow injection^[15]. The aim of this study is developed a direct , sensitive and economic spectrophotometric method to estimation folic acid in pharmaceuticals tablets in Iraqi markets.

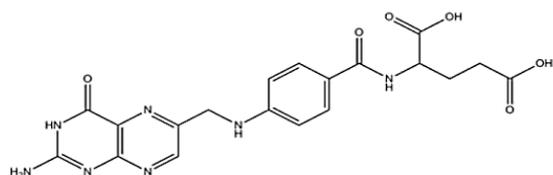


Fig.(1): Folic acid chemical structure.

Material and Method

Preparation of standard solutions

- 0.05 gm of pure folic acid (M.Wt=441.404 gm.mol⁻¹) was a gift from the state company for the drug industry Samara-Iraq (SDI), Folic acid was dissolved in 50 ml distilled water to obtain stock solution 1000 mg.l⁻¹, then was transferred 5 ml from stock solution to 50 ml volumetric flask and completed to the mark with distilled water to obtained concretions equal to 100 mg.l⁻¹.
- Series of standard solution were prepared using different volumes (3, 5, 7, 9 and 10 ml) from 100 mg.l⁻¹ solution and diluted to 50 ml with distilled water to obtained concretions of pure folic acid equal to (6, 10, 14, 18 and 20 mg.l⁻¹).
- Assay of market sample preparations: ten tablets of folic acid (5 mg, Actavis Barnstaple, Ex 328NS, US) were weighed and ground into a powder, the tablets powder 0.01 gm of folic acid market sample weighed and dissolved in distilled water and transferred to 100 ml volumetric flask to obtain concentration equal to 100 mg.l⁻¹; then was transferred (6 and 10 ml) from folic acid market sample 100 mg.l⁻¹ to 50 ml volumetric flask and

completed to the mark with distilled water to obtain concentrations equal to (12 and 20 mg/l).

Procedure

The standard solutions in three methods were estimated for folic acid using distilled water as a blank; then in zero order method the maximum absorbance was measured at 280 nm. For area under curve method (AUC), area was calculated by the computer software program depending on the theoretical equation^[16,17,18]:

$$AUC = \int_{306}^{296} Ad\lambda$$

A = absorbance of folic acid and $\int_{306}^{296} Ad\lambda$ = is area under curve between 296- 306 nm.

In the first derivative method the standard solutions for folic acid was scanned between 190-400 nm, the absorbance for first derivative method was measured at maximum peak at 264 nm and at valley peak 226 nm.

Results and Discussed

Zero order method

From the overlain spectra of the folic acid solutions, zero order method was scanned between 190- 400 nm as in Fig.(2) and a maximum peak was found at 280 nm. The linearity graph for folic acid solutions was plotted in concentrations range 6- 20 mg.l⁻¹, correlation coefficient was (R² =0.996) and LOD were found to be 0.261 mg.l⁻¹ as shown in Fig.(3) and table 1 respectively.

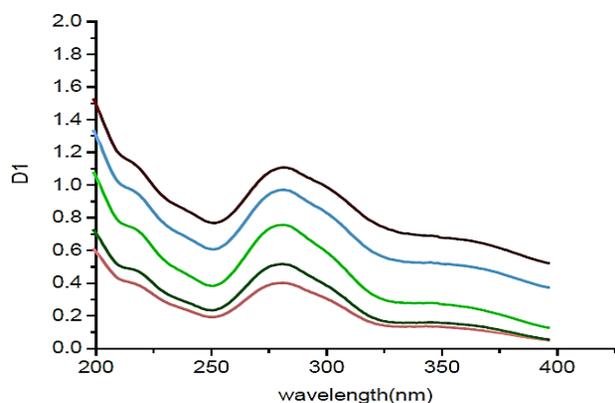


Fig.(2): Normal spectra of Folic acid 6-20 mg/l at peak= 280 nm.

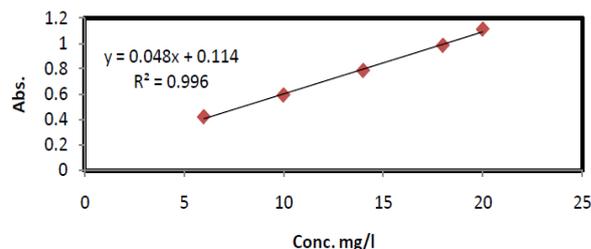


Fig.(3): Calibration curve of Zero-order spectra for Folic acid 6-20 mg.L⁻¹ at peak = 280 nm.

Area under curve method

From the spectra of the folic acid solutions, AUC method in the range 296-306 nm were selected for this study Fig.(4), the linearity graph was drawing in a concentration range 6-20 mg.l⁻¹, the regression equation was (Y= 2.028x+1.703) (R² = 0.994) and LOD was 0.006 as shown in Fig.(5) and Table (1) receptivity.

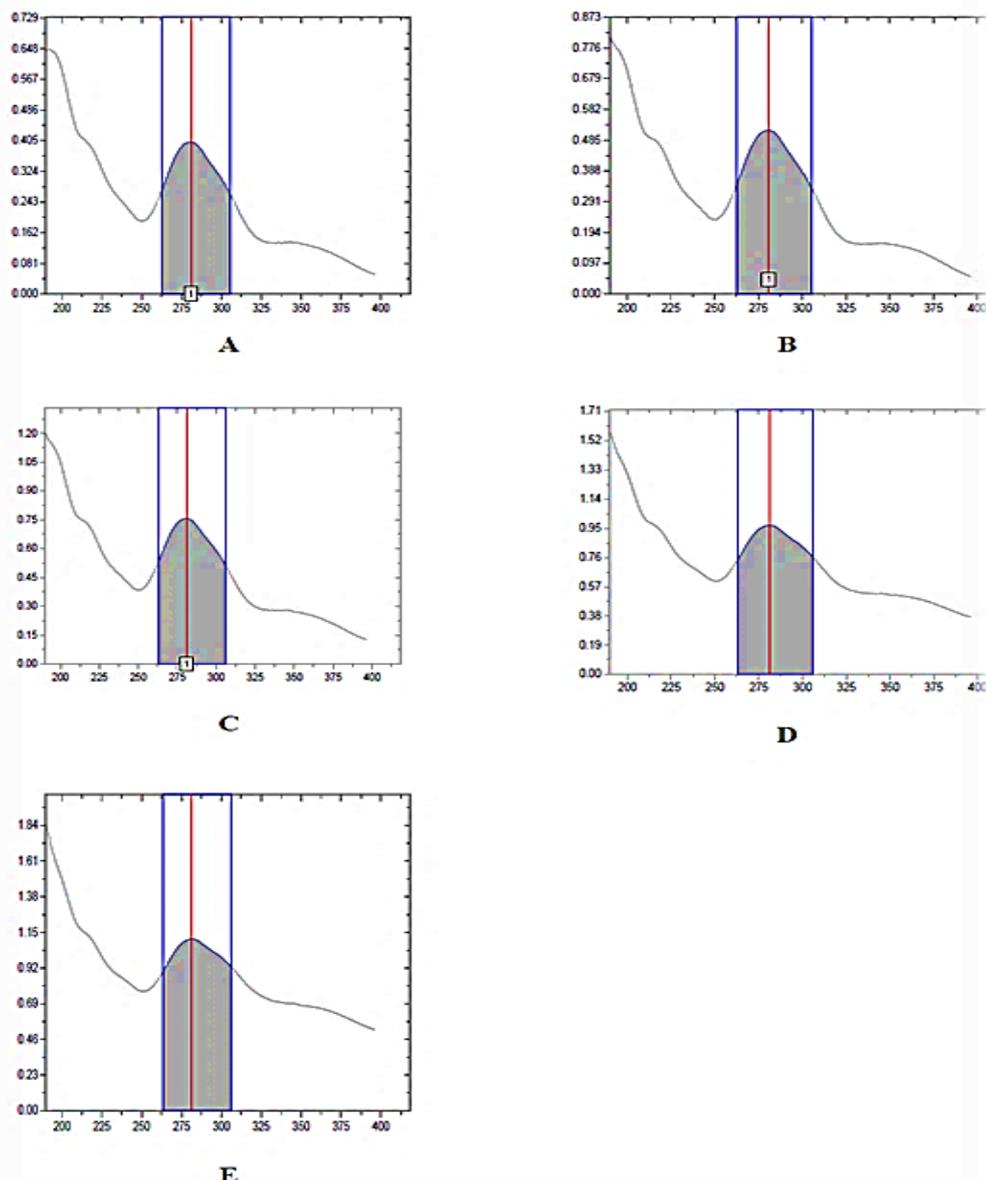


Fig.(4): Area under curve of Folic acid at concentration (A= 6 mg/l), (B= 10 mg/l), (C= 14 mg/l), (D= 18 mg/l) and (E= 20 mg/l).

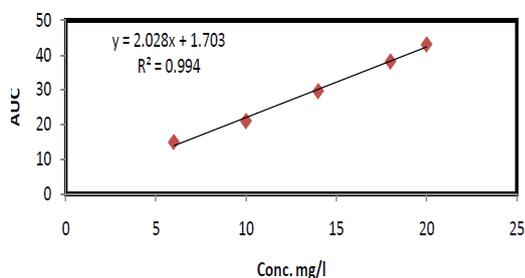


Fig.(5): Calibration curve of area under curve method for Folic acid 6-20 mg.L⁻¹ at area (296-306 nm).

First derivative method

The first derivative ($\frac{dA}{d\lambda}$) is calculated by the software program which was proportional

to the standard solutions concentration of Folic acid. In this study selected one maximum peak at 264 nm and one valley peak at 226 nm for estimation folic acid as shown in Fig.(6), the linearity graph for this method were 6-20 mg.l⁻¹ given in Fig.(7,8) respectively. The limit of detection were found to be 3.800 mg.l⁻¹ for D1 at peak 264 nm and D1 at valley 226nm are listed in Table (1).

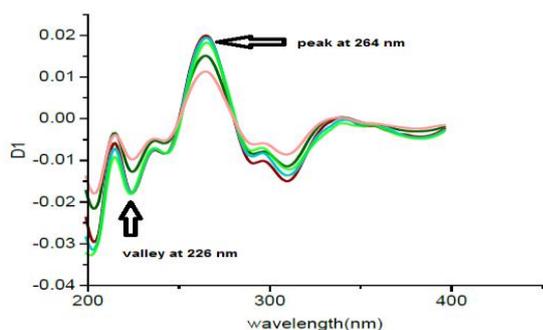


Fig.(6): First derivative spectra of pure folic acid at concentration 6- 20 mg.L⁻¹.

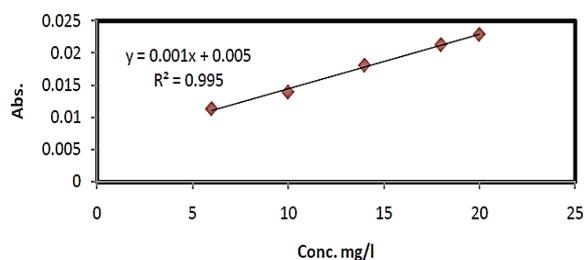


Fig.(7): Calibration curve of D1 spectra for Folic acid (6-20 mg.L⁻¹) at peak 264 nm.

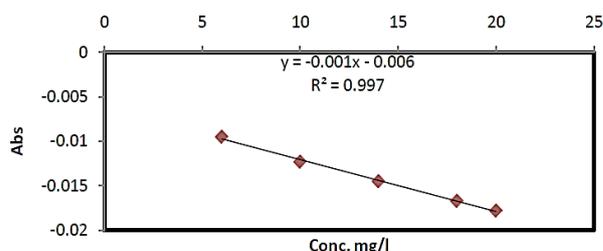


Fig.(8): Calibration curve of D1 spectra for Folic acid (6-20 mg.L⁻¹) at valley 226 nm.

The validation parameters was obtained from the linearity graph for three methods was recorded in Table (1), the small values for the most parameters (b, a, S_b and S_a) refer to the high reliable precision of the zero order, AUC and first derivative methods.

Table (1)

The validation parameters obtain from the linearity graph of folic acid.

	Method			
	Zero-order	AUC	D1 at peak 264 nm	D1 at valley 226 nm
Wavelength nm	208	296-306	193.7	210.6
R ²	0.996	0.994	0.995	0.997
Linearity range(mg/L)	6-20	6-20	6-20	6-20
Equation	Y=0.048x+0.114	Y=2.028x+1.703	Y=0.001x+0.005	Y=-0.001x-0.006
b	0.048	2.028	0.001	0.001
a	0.114	1.703	0.005	-0.006
S _b	0.002	0.088	0.00	-
S _a	0.023	1.278	0.00	-
ε (L. mol ⁻¹ . cm ⁻¹)	2.4946×10 ⁺⁴	-	-	-
Sandell's sensitivity (µg. cm ⁻¹)	0.0176	-	-	-
LOD (mg.l ⁻¹)	0.261	0.006	3.800	3.800

b = Slope, *a* = intercept, *S_b* = Standard deviation of the slope, *S_a* = Standard deviation of intercept,

ε = Molar absorptivity and Sandell's sensitivity = M.wt/ ε

LOD = limit of detection = 3.3×SD_b/S, SD_b=0.0038= is the standard deviation of the solvent (distilled water as a blank) (n=3), S is the slop of the corresponding linearity graph.

Precision and accuracy

To determination the precision and accuracy Table (2) of the zero order, AUC and first derivative methods, folic acid solutions at concentration 7 and 13 mg.l⁻¹ was analyzing

three time for each three methods, the recoveries were found to be more than 99.093 indicating the three proposed methods was reliable and accuracy.

Table (2)
Precision and accuracy for the three methods.

	Amount of folic acid (mg.L ⁻¹)		E*%	Rec*%=E%+100	Average of Rec.%	RSD*%
	Taken	found				
Zero-order	7	7.091	+1.30	101.300	100.196	0.660
	13	12.882	-0.907	99.093		0.856
AUC	7	7.153	+2.185	102.185	100.927	0.842
	13	12.957	-0.330	99.670		0.395
D1 at peak 264 nm	7	7.100	+1.428	101.428	101.056	0.574
	13	13.089	+0.684	100.684		0.784
D1 at valley 226 nm	7	7.210	+3.00	103.00	101.361	0.694
	13	12.964	-0.276	99.723		0.233

* Average of three time, $E\% = \text{relative error} = \frac{\text{found} - \text{taken}}{\text{taken}} \times 100$, $\text{Rec}\% = \text{recovery}$ and $\text{RSD}\% = \text{relative standard deviation}$.

Application

One market formulation of folic acid 5 mg tablets was analyzed using three proposed methods, there is no variance in spectra between the Fig.(2, 4 and 6) and spectra of market folic acid tablets Fig.(9, 10 and 11).

The average of recoveries and relative standard deviation were obtained from analyzed three time of two different concentrations of market Folic acid Tables (12) and 20 mg.l⁻¹ was summarized in Table (3).

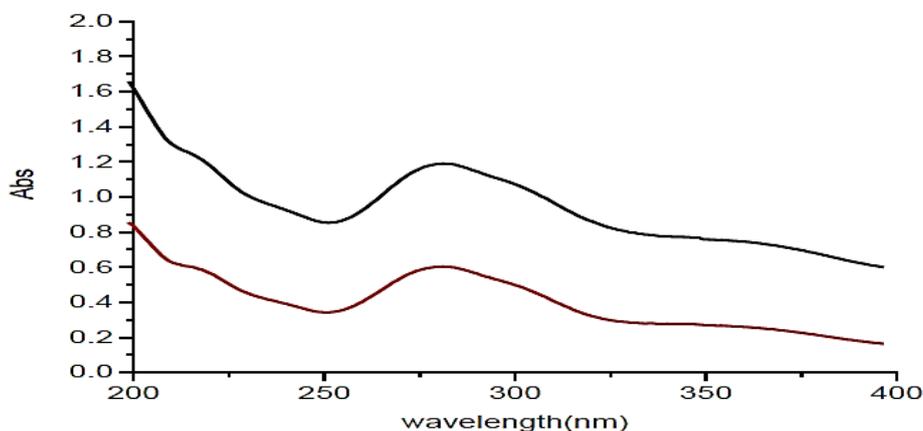


Fig.(9): Zero order spectra of market Folic acid tablet at 12 and 20 mg.l⁻¹.

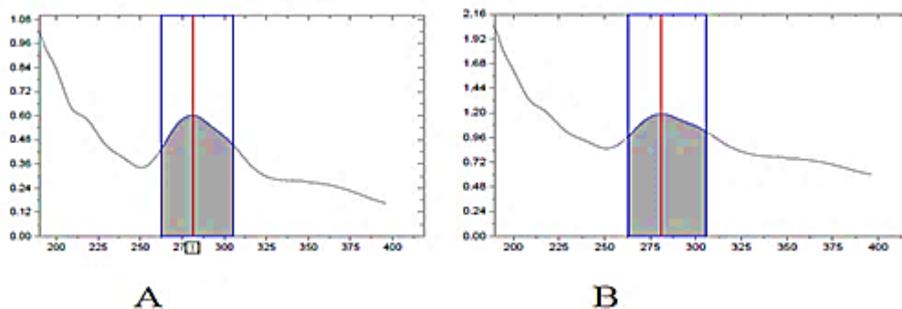


Fig. (10): Area under curve spectra of market Folic acid tablet at (A = 12mg.L⁻¹) and (B =20 mg.L⁻¹).

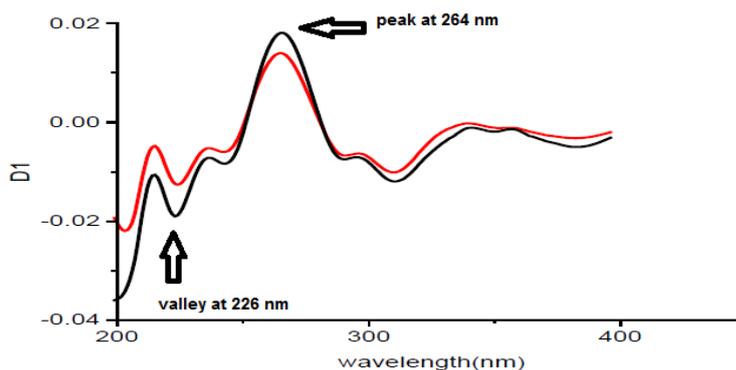


Fig.(11): First derivative spectra of market folic acid tablet at 12 and 20 mg.L⁻¹.

Table (3)
The relative error, recovery and relative standard deviations of the market Folic acid tablet at 12 and 20 mg.L⁻¹.

Pharmaceutical market tablet	method	Conc. of folic acid mg. L ⁻¹		E* %	Rec.*%	Average of Rec.%	RSD%
		taken	found				
Folic acid 5 mg tablets, Actavis, Barnstaple, EX328BS, UK.	Zero-order	12	11.733	-2.225	97.775	98.678	0.471
		20	19.908	-0.418	99.582		0.360
	AUC	12	12.270	+2.25	102.250	101.343	1.487
		20	20.096	+0.436	100.436		0.934
	D1 at peak 264nm	12	11.988	-0.1	99.90	99.657	0.511
		20	19.871	-0.586	99.414		1.289
	D1 at peak 226 nm	12	11.836	-1.366	98.634	98.956	0.778
		20	19.841	-0.722	99.278		0.806

* Average of three time, E% = relative error, Rec% =recovery and RSD%=relative standard deviation.

The results of three spectrophotometric proposed methods in Table (4) are compared with official standard method^[19], by using apply the F-test and t-test at 95% confidence level. The calculated of F value and t value for the zero order, AUC and first derivative

methods did not exceed the theoretical F value 19.0 and theoretical t value 4.303, refer to a high precision and accuracy and also indicated there is no difference between three proposed methods in estimation Folic acid drug in tablet preparation.

Table (4)
The comparison of the Zero order, AUC and First derivative Spectrophotometric methods with official standard method.

		Statistical parameters	Pharmaceutical preparation	
			Folic acid pure	Folic acid 5 mg tablets
suggest methods	Zero-order	Rec.%	100.196	99.678
		S**	0.888	
		F*	0.093	
		t*	0.292	
		s ₁ ²	0.134	
	AUC	Rec.%	100.927	101.343
		S**	0.875	
		F*	0.059	
		t*	0.237	
		s ₁ ²	0.086	
	D1 at peak 264nm	Rec.%	101.056	99.657
		S**	1.100	
		F*	0.676	
		t*	0.635	
		s ₁ ²	0.978	
	D1 at peak 226 nm	Rec.%	101.361	98.956
		S**	1.472	
		F*	2.003	
		t*	0.816	
		s ₁ ²	2.893	
Standard method ^[19]	Rec.%	99.850	98.151	
	s ₂ ²	1.445		

$S^{**} = \text{pooled standard deviation} = \sqrt{\frac{(n_1-1)s_1^2 + (n_2-1)s_2^2}{n_1+n_2-2}}$, (n_1-1) and (n_2-1) = number of degrees of freedom for suggest methods and official standard method, respectively

$F^*_{\text{calculated}} = \frac{s_1^2}{s_2^2}$, $F_{\text{theoretical}} = 19.0$, $F_{\text{calculated}} < F_{\text{theoretical}}$ at 95% confidence level,

$T_{\text{theoretical}} = 4.303$, $T_{\text{calculated}} < T_{\text{theoretical}}$ at 95% confidence level,

$S^2_1 = \text{variation} = \frac{\sum(x_i - \bar{x})^2}{n_1 - 1}$, $S^2_2 = \frac{\sum(x_i - \bar{x})^2}{n_2 - 1}$, $t^* = \frac{|\bar{x}_1 - \bar{x}_2|}{s^{**} \sqrt{\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$

Conclusion

Three simple spectrophotometric methods have been developed for the estimation of Folic acid in the marketed tablet preparation. three proposed methods require neither toxic material nor solvent extraction and nor pH and temperature control. So, the all three proposed methods are a favorable for the evaluation of folic acid in tablet formulation.

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