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DETERMINATION OF VITAMIN B9 (FOLIC ACID) IN SOME PHARMACEUTICAL PRODUCTS USING SPECTROPHOTOMETRIC AND CHROMATOGRAPHIC METHODS

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ABSTRACT

The present study is aimed principally to compare two different analytical tools, viz. UV spectrophotometry, reversed phase high performance liquid chromatography RP-HPLC in terms of selectivity and sensitivity for the determination of vitamin B9 (traditionally named folic acid) in the absence and presence of other active ingredients encountered in pharmaceutical products, viz. tablets, capsules and injection ampoules. The obtained results demonstrate which one of these analytical tool gave best results with high selectivity and sensitivity. The HPLC gave high sensitivity for separation of folic acid of the studied samples.

KEYWORD: UV spectrophotometry, viz. tablets, capsules.

INTRODUCTION

Folic acid 'FA' or B9 is a water soluble vitamin, initially identified as an anti-anemia and growth factor. It is produced by plants (green leaves, algae) and micro-organisms (bacteria, yeast). In mammals, folic acid and its derivatives, the folates, serve as acceptors and donors of carbon units and are involved in amino acid and nucleotide biosynthesis.[1, 2] Folic acid also prevents neural tube defects such as spina bifida, while its ability to lower blood homocysteine concentration, suggests that it might have a positive influence on cardiovascular disease and certain cancers.[3,4] A role for this B vitamin in maintaining good health may, in fact, extend beyond these clinical conditions to encompass several others disorders (birth defects, several types of cancer, dementia, affective disorders, Down's syndrome etc. This vitamin is essential for rapid cell growth like blood production, especially during pregnancy.[5]

Because of these health benefits, there is a trend to increase the daily recommended dietary folate intake either by fortification with synthetic folic acid in the form of pharmaceutical products or by biofortification in natural foods, e.g. yeast.

Hence, FA determination is important and essential in food & pharmaceutical industries and also in clinical laboratories. Chemically, folic acid is {N-[4-(2-amino-l,4-dihydro-4-oxo-6-pteridinyl)methyl)amino) benzoyl] –L glutamic acid}. The chemical structure of FA is given in scheme 1. This study was carried out on some different drugs products containing folic acid, to compare two different analytical methods, viz. spectrophotometry, and RP-HPLC in terms of sensitivity and selectivity for determination of folic acid available in pharmaceutical tablets, capsules and injection ampoules.

EXPERIMENTAL

Apparatus

Single beam DU800 Beckman Coulter spectrophotometer connected to PC computer. Perkin Elmer HPLC Series PE-200 (USA) equipped with a P200 pump, solvent degasser DGU-3A, an automatic sampler AS200, Rheodyne injector with 200 µL loop, UV/VIS detector Series 200 with controlled wavelength at 282 nm and communication Network chromatography Interface DotLink 600, a Brownlee BIO C18 reversed-phase analytical column, 5µm particle size, 250x4.6 mm dimension. Mettler Toledo pH meter. Mettler Toledo balance 200.

Reagents and Solutions

All reagents used were of analytical-reagent grade. Folic acid was purchased from FLUKA and it was used without any further purification. Standard solution of folic acid was prepared by dissolving 55 mg of this vitamin in 1 ml sodium hydroxide solution (0.01 M), shaking well and completing to 100 ml with distilled water. This stock solution was kept in a refrigerator. The supporting electrolyte used for both RP-HPLC was phosphate solution ($0.04 mol.L^{-1}$ KH_2PO_4) adjusted to pH 7 with sodium hydroxide. All tested pharmaceutical products were purchased from local pharmacies.

Procedure

UV Spectrophotometic analysis

An aliquot of standard folic acid solution (55-275 µg/ml) was placed in a 10 ml-measuring flask and completed to the mark with distilled water. The solution is then transferred to quartz cell with 10 mm path length and the absorbance is recorded against water blank at

wavelengths 261, 281 and 361 nm. The unknown samples were measured directly without further dilution and the concentrations were calculated according to the calibration curve as given in (Figure, 1).

Reverse Phase High Performance Liquid Chromatographic Analysis:

The mobile phase was 0.04 mol L^{-1} KH_2PO_4 (pH = 7): acetonitrile, 60:40. The flow-rate was 1 ml min^{-1}. The column was operated at room temperature (25°C). The mobile was first degassed and 10 µL folic acid solution (55-275 µg/ml) was injected into 200 µL loop and the column elute was monitored with a UV detector at 282 nm. Identification of folic acid in a sample was ascertained by comparing its retention time with that of standard and its concentration was calculated from the calibration curve of integrated peak areas versus the corresponding concentrations of standard solutions as given in (Figure, 2).

RESULTS AND DISCUSSION

For direct spectrophotometric determination of folic acid three maxima wavelengths are selected which are at 254, 281 and 362 nm as shown in Figure 1. For separation and determination of folic acid in drugs, the best results were obtained by isocratic elution with mobile phase of 0.04 mol l^{-1} KH_2PO_4 (pH 7) methanol, 60:40, as a typical chromatogram shown in Figure 2. The retention time of folic acid was fixed at 2.25±0.12 min.

Linearity and Detection Limits

As seen from Table 1, correlation coefficients (r) were 0.9996 for spectrophotometric method at 281 nm wavelength, 0.996 for HPLC. The detection limits (S/N = 3).[6] were 1.84 and 1.76 µg/ml, respectively, while the limit of quantification (S/N = 10) were 6.137 and5.858 and µg/ml.

Precision

Six determinations of the standard solution (11 ug/ml folic acid) were performed using the same reagents and apparatus, repeatability, to evaluate the precision expressed by relative standard deviation and found that it is in order spectrophotometry > HPLC as given in Table I.

Accuracy

Accuracy is expressed as relative error E_r which is a measure of deviation of experimental average from true value according to the following equation:

$$E_r = \frac{x_i - x_t}{x_t} \times 100\%$$

Where x_t is the true concentration and x_i is the concentration of sample evaluated from the calibration curves constructed by spectrophotometric and HPLC methods for each drug sample (Table 2). The accuracy obtained were satisfactory, over the range 0.45 to 7.43 % by spectrophotometric, -0.05 to 12.03 % for HPLC.

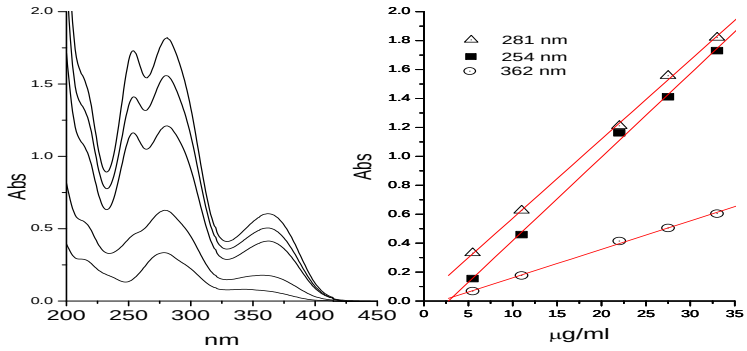

Figure1: Calibration curve of folic acid using spectrophotomtric method

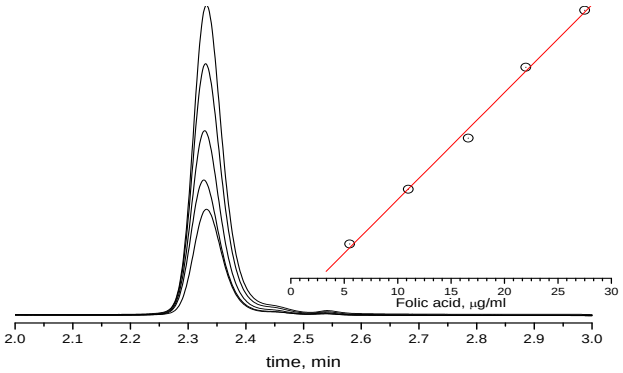

Figure (2): Calibration curve of folic acid using RP-HPLC

Table 1: Linearity of standard curves and concentration limits for determination of Folic acid using different analytical tools.

	UV spectrophotometery			HPLC
	254 nm	281 nm	362 nm	282 nm
slope	0.0577	0.05468	0.01968	33567
intercept	-0.1587	0.0261	-0.0359	60802
Correlation coefficient, r	0.9989	0.9996	0.9989	0.996
concentration range, µg/ml	5 - 33			5 - 33
Precision RSD, %	23.491	3.652	15.749	11.888
Limit of detection, µg/ml	1.499	1.841	0.523	1.757
Limit of quantification, µg/ml	4.995	6.137	1.743	5.858

Interference

Serious interference is shown on using UV spectrophotometric method due to presence of ascorbic acid or vitamin B6 as shown in Figure 3 while complete separation is observed in HPLC as demonstrated in Figures 4 & 5. The results also showed that the conditions which used in this study of HPLC showed good values comparing with U.V method.

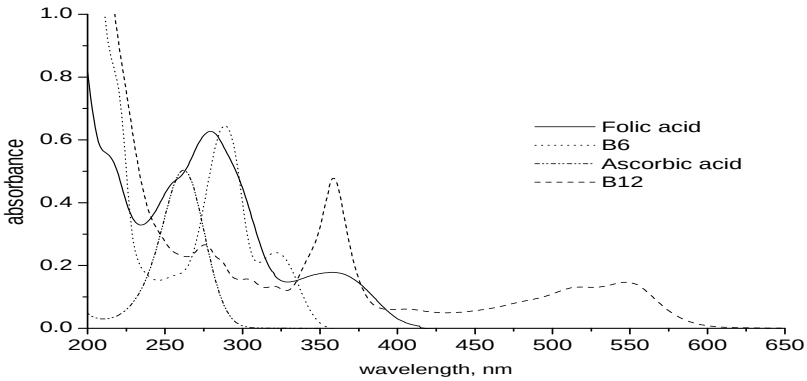

Figure (3): UV spectra of folic acid, B6, B12 and ascorbic acid

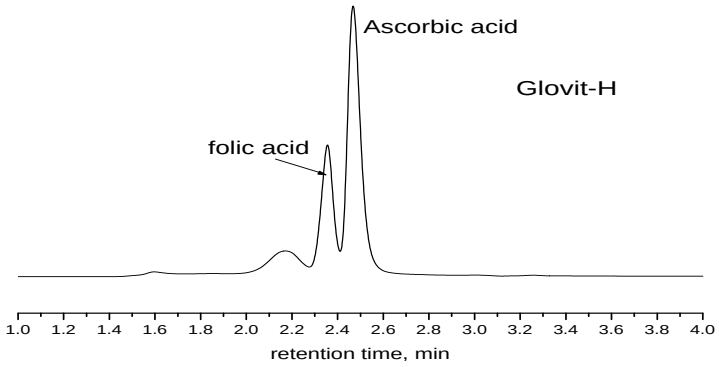

Figure (4): Chromatogram for determining folic acid in the presence of vitamin ascorbic acid

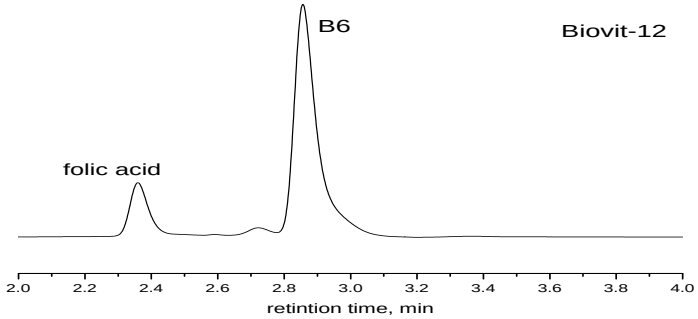

Figure (5): chromatogram for determining folic acid in the presence of vitamin B6

Table (2): Accuracy of methods for determining folic acid in different brands of pharmaceutical products

Trade name	Producer	content, mg		
			UV 281 nm	**HPLC**
Folic acid 1	Nile, Egypt	5	7.43	**8.29**
Folic acid 2	Wockhardt, UK	5	1.83	**0.05**
Biovit 12 Depot, ampoule	MUP, Egypt	1	0.45	**2.69**
Ferrofol, capsules	EIPICO, Egypt	0.5	serious interference	**8.19**
Glovit-H capsules	Globalpharma, UAE	0.5	serious interference	**12.03**

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