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Research Article

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RP-HPLC Method of the Estimation of Folic Acid in Tablets

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Abstract A simple, specific, precise and accurate reverse phase HPLC method has been developed and described for the determination of Folic acid in solid oral forms, tablets. The chromatographic separation was achieved on column C18, 150 mmx4.6 mm, 3.5 µm, using PDA detector. The mobile phase was methanol and acetate buffer pH 5.2 (50:50 v:v), at flow rate 1.0 mL/min, detected at 240 nm, at 25ºC temperature for column. The retention time was at about 1.8 minutes. The method was validated according to the ICH guidelines with respect to specificity, linearity (r^2 =0.9999), accuracy (99 to 101%), precision (RSD<2%).

Keywords Folic acid, RP- HPLC, Validation

Introduction

Folic acid is necessary for the proper development of human bodies. This vitamin is involved in the production of genetic material and in a number of other functions. Folic acid is a human-made version of vitamin folate (also known as vitamin B9). Folate helps the body make healthy red blood cells and is in a specific feeding area. Folate and folic acid are water-soluble B forms. Folate occurs naturally in foods, and folic acid is a synthetic form of this vitamin [1, 2, 3]. The aim of this papaer is to to represent simple, specific, precise, accurate [4] and low cost RP-HPLC method for determination of folic acid, with very short chromatographic run time (less than 3 minutes). In addition, results for four marketing samples correspond to ICH 3AQ11a. Specification and Control Tests on the Finished Products [5].

Materials and Methods

Instrumentation HPLC system (Agilent technology) consisting of gradient pump, Auto sampler, column oven and photodiode array detector (PDA, Agilent technology) was employed for analysis. Chromatographic data was acquired using chemstation software.

Reagents and Materials

Folic acid was supplied by USP. Methanol (HPLC, Semikem), Sodium acetate (Merck) and Milli-Q Water.

Chromatographic Condition

Column C18, 150 mmx4.6 mm, 5 µm was used as a stationary phase. The mobile phase consisting methanol and acetate buffer pH 5.2 (50:50 v:v). The flow rate of the mobile phase was 1.0 mL/min. Detector signal was monitored at a wavelength of 240 nm. The column temperature was kept 25°C and injection volume was 20 µl.

Preparation of Standard Solutions

The standard stock solution was prepared in 10.72 mg/L. Solvent was mobile phase. From this solution was diluted to obtain final standard concentration of Folic acid from 3.22mg/Lto 12.86 mg/L, respectively (correspond to 30 – 120% from the target concentration).

Preparation of Sample Solutions (contains 5 mg of Folic acid per tablet)

Weigh mass of tablet correspond to 5 mg of folic acid in a 50 ml flask, add 30 ml of solvent (mobile phase), shake on ultrasonic bath for 15 minutes and dilute with solvent to volume. Centrifuge solution at 3500rpm/10min. Pipette 1 ml of this solution in a 10 ml flask and dilute with solvent to volume (10 mg/L).

Validation of the Proposed Method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines [4].

System Suitability

The system suitability test as per method should be performed and checked before performing any parameter (%RSD).

Linearity and Range

A standard linearity solution was prepared to different concentration of 30%, 50%, 80%, 100% and 120% of the target concentration.

Method Precision (Repeatability)

Five solutions were prepared from Folic acid. Then they were injected by one analyst and analyzed on same day.

Accuracy

The accuracy of the method was carried out at three levels in the range of 80-120% of the working concentration of sample. Calculated amount of Folic acidworking standards were added in placebo containing volumetric flasks to, prepare 80%, 100% and 142% level of the working concentration.

Specificity

A blank preparation and standard preparation were prepared and injected.

Result and Discussion

The method has provided very fast elution for Folic acid. Separation was obtained by using Column C18, 150 mmx4.6 mm, 5 µm as a stationary phase at 25°C temperature and using a mobile phase consisting methanol and acetate buffer pH 5.2, in the ratio of 50:50 (v/v), at a flow rate 1.0 ml/min and wavelength for detection was 240nm. Under these optimized conditions, Folic acid peaks were well resolved and free from tailing. The tailing factors was1.5and retention time at 1.8 minutes. Specificity of chromatograms shown in figure 1 and system suitability was established by injected standard solution and results are shown in table 1.

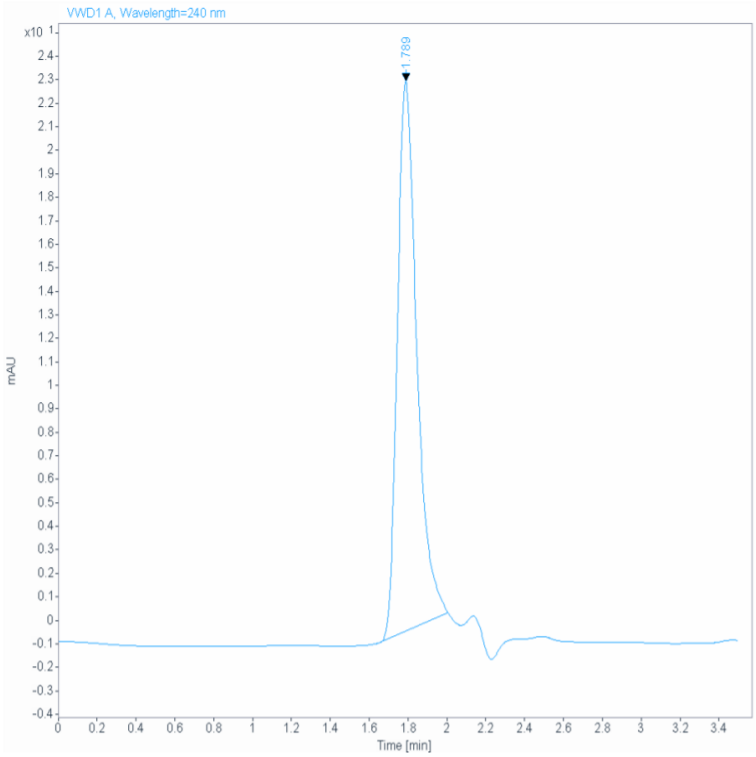

Figure 1: Chromatogram of Folic acid peak

Table 1: System suitability parameters

Component	Area	Symmetry	Plate
Folic acid	607	1.5	8259

Specificity of the chromatograms was checked for the appearance of any extra peaks. No chromatographic interference from tablets excipients was found.

Linearity of Folic acid was performed in the range of 3.22–12.86mg/L. The data for the peak area against the concentration were treated by linear regression analysis and the correlation coefficient value obtained was 0.9999.

The accuracy was expressed as the percentage of analytes recovered by the assay method. It was confirmed from results that the method is accurate (Table 2).

Table 2: Accurace data

Level (%)	RSD	
	Folic acid	Mean RSD (%)
80	99.4	100.1
100	100.0	
120	101.0	

In Precision, the relative standard deviations (RSD) was 1.1, which are well within the acceptable limit of 2.0%. Results are presented in table 3.

Table 3: Precision recovery data

Sample	Recovery (%)
1	100.0
2	97.3
3	99.7
4	98.9
5	98.4
Average (%)	98.8
RSD (%)	1.1

Results content for four samples of finished product are presented in table 4.

Table 4: Results for finished products

Sample No.	**Folic acid**	
	Found (mg/tablet)	**Found (%)**
Sample 1 (5 mg tablets)	4.97	99.%
Sample 2 (5 mg tablets)	5.05	101.0%
Sample 3 (5 mg tablets)	5.00	100.0%
Sample 4 (5 mg tablets)	4.95	99.0%

Conclusion

Proposed RP-HPLC method is specific, accurate and precise for the determination of Folic acid in tablets. The newly developed method is simple and cost effective. The elution is in three minutes only. The method was validated as per ICH guidelines. All other parameters such as specificity, linearity, precision and accuracy, passes the criteria set forth by ICH guidelines. The described method is suitable for routine analysis and quality control.

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