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Development and Validation of New UV- Spectroscopic Method for Water Soluble Folic Acid

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Abstract : In the present work, six simple and sensitive spectrophotometric have been developed for the quantitative determination of folic acid. Method A is a UV spectrophotometric method in which Folic acid was dissolved in Water and exhibited absorption maximum at 281 nm and obeyed Beer's law in the concentration range of 2-10µg/ml. Method B is the first order derivative spectroscopy method adopted to eliminate spectral interference, in which derivative amplitude was measured at 281 nm with n=1.

The proposed methods are economical, simple, sensitive and accurate for quantitative determination of folic acid in bulk drug and pharmaceutical formulations.

Key words: folic acid; First order derivative; Area under curve.

Introduction

Vitamins are non-energy producing organic compound, essential for normal human metabolism that must be supplied in small quantities in the diet.[3-5] The importance of vitamins as drugs is primarily in the prevention and treatment of deficiency diseases. Some vitamins do have other empirical uses in pharmacological doses. Vitamin deficiencies occur due to inadequate intake, malabsorption, increased tissue needs, increased excretion, certain genetic abnormalities and drug-vitamin interaction.[1-2] Myths like 'they energies the body', 'any physical illness is accompanied by vitamin deficiency', 'vitamin intake in normal diet is precariously marginal', 'they harmless' are extensive. Vitamins are traditionally divided into two groups: fat-soluble and water-soluble.[5-8] Water-soluble vitamins (B-complex) are meagerly stored: excess is excreted with little chance of toxicity. They act as cofactors for specific enzymes of intermediary metabolism.[13-14]B-vitamins are essential for the functioning of the nervous system, which controls our stress response. Our stress response system determines how we feel in the face of everyday stress.[15-16]

The B-vitamin complex is made up of such B vitamins as:

Vitamin B9 (Folic Acid)

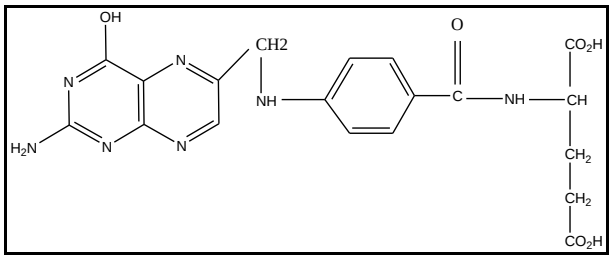

Figure 1:- Chemical Structure of Vitamin B9 (Folic Acid) [$C_{19}H_{19}O_6$]

Vitamin B9 (folic acid) is chemically, (2S)-[4-[(2-amino-4-hydroxypteridin-6-yl) methylamino] benzamiido] glutamic acid (Mol.Wt.441.4).[9-10] It is a yellow to yellowish-orange crystalline compound. It occurs as yellow crystals which are insoluble in water but its sodium salt is freely water soluble.[11-12] Folic acid itself is inactive.[1-2]In the body it is converted to folinic acid, which is its active form. Folinic acid (citrovorum factor, leucovorin) is 5formyl terahydrofolic acid. Folinic acid further participates in the production of purines and pyrimidines leading to the synthesis of deoxyribonucleic acid (DNA) and thus regulates cell division. Folic acid deficiency directly leads to megaloblasticanaemia.[17-20]

Material and Methods

Vitamin B Folic Acid LR purchased from research –lab fine chem industries, Mumbai 400 002(India). All the chemicals were of analytical reagent grade of Merck (Germany) unless otherwise specified.

Instruments Used

The UV Spectrophotometric estimation was done by using Shimadzu-1800 UV-spectrophotometer with 1 cm path length was used for spectral measurements with 1cm matched quartz cells. Shimadzu balance (BL-220H) was used for all weighing.

Method Development

Solubility test

Solubility test for the drug Folic Acid was performed by using various solvents, which includes various dilute acids & alkaline solutions, very slightly soluble in boiling water, practically insoluble in cold water & in most of the organic solvent. However, according to the percentage solubility distilled water was chosen as solvent for developing the method.

Preparation of stock solution

Weigh accurately 100mg of Folic Acid and transferred to 100ml volumetric flask. Then add small amount of distilled water and dissolve the drug. Then the final volume was made up with distilled water to get the solution having concentration 1000 µg/ml.

Preparation of working standard solution

From stock solution 0.1ml was further diluted to 10ml with distilled water to get the solution having concentration 100µg/ml.

Determination of λ max

From the above working standard solution, pipette out a sufficient amount concentration in µg/ml. Then the sample was scanned in UV-VIS Spectrophotometer in the range 400-200nm using distilled water as a blank and the wavelength corresponding to maximum absorbance (λ max) was found to be 281 nm.

Diluent preparation

Double distilled water was used as the diluent while 3 % dipotassium phosphate solution was used tofolic acid because it is insoluble in water.

Preparation of Calibration Curve:

From the working standard solution, pipette out 0.1ml, 0.2ml, 0.3ml, 0.4ml, 0.5ml, 0.6 ml, 0.7ml, 0.8 ml and 0.9ml then diluted up to 10ml using distilled water to produce 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml 50µg/ml, 60µg/ml, 70µg/ml, 80µg/ml and 90µg/ml solutions respectively. Then measure the absorbance of these solutions at the λ max of 281 nm using distilled water as the blank. Then, the calibration curve was plotted by taking concentration on X-axis and absorbance on Y-axis (fig: 1). The curve showed linearity in the concentration range of 10-90µg/ml. The correlation coefficient (r^2) was found to be 0.996.

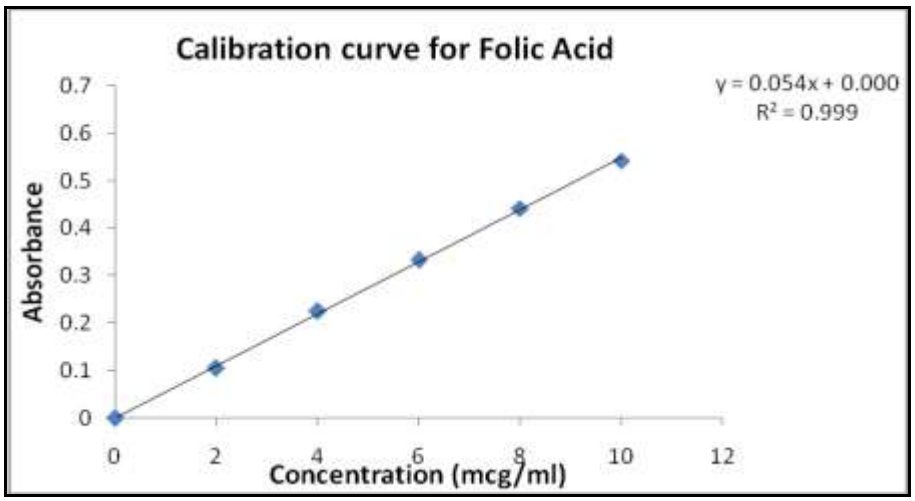

Figure 2: Calibration curve of Folic Acid

Method Validation

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. The method was validated as per ICH guidelines for different parameters like Linearity, Accuracy, Precision, Robustness, Ruggedness, Limit of Detection (LOD) and Limit of Quantification (LOQ).

Linearity

Various aliquots were prepared from the working standard solution (100µg/ml) ranging from10-90 µg/ml. The samples were scanned in UV-VIS Spectrophotometer using distilled water as blank. It was found that the selected drug shows linearity between the 10-90µg/ml (Table: 2).

Accuracy

The accuracy of the method was determined by spiking working standards of the nine vitamins into the placebo at different concentration levels: 80, 100 and 120% of target concentration of each of the vitamins. The resulting solutions were assayed in triplicate and results obtained were compared with the expected results and expressed as percentage. The mean recoveries (%) of vitamins B9 Folic Acid , found to be 98.84 respectively which are within the acceptance limit. (table: 1 & 4).

Precision

Precision of the method was demonstrated by intra-day and inter-day variation studies. In intra-day variation study, 3 different solutions of same concentration that is 10µg/ml were prepared and analyzed two times in a day i.e. morning, afternoon and the absorbance's were noted. The result was indicated by % RSD (table 1). In the inter-day variation study, 3 different solutions of same concentration (10µg/ml) were prepared and analyzed three times for three consecutive days and the absorbances were noted. The result was indicated by % RSD (table 1).

Robustness

Robustness of the method was determined by carrying out the analysis at three different Solvents [i.e. Solvent-A (0.1 N NaOH) Solvent-B (Methanol), Solvent-C (Distilled water]. The respective absorbances were noted and the result was indicated by % RSD (table: 1).

Ruggedness

Ruggedness of the method was determined by carrying out the analysis at different temperatures and the respective absorbances were noted. The result was indicated by % RSD (table: 1).

Limit of Detection (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample. The LOQ was calculated using the formula involving standard deviation of response and slope of calibration curve (table: 1).

LOD= 3.3xSD/S

Where, SD = Standard deviation of Y-intercepts

S = slope

Limit of Quantification

The LOQ is the concentration that can be quantified reliably with a specified level of accuracy and precision. The LOQ was calculated using the formula involving standard deviation of response and slope of calibration curve (table: 9).

LOQ= 10xSD/S

Where, SD = Standard deviation of Y-intercepts

S = slope

Results And Discussion

The developed method was found to be precise as the %RSD values for intra-day and interday were found to be less than 2%. Good recoveries (99.30% to 100.3%) of the drug were obtained at each added concentration, which indicates that the method was accurate. The LOD and LOQ were found to be in sub-microgram level, which indicates the sensitivity of the method. The method was also found to be robust and rugged as indicated by the %RSD values which are less than 2%. Summary of validation parameters of proposed Spectrophotometric method is shown in table: 1

Table: 1. Summary of Validation

Parameter	Result
Linearity indicated by correlation coefficient	0.999
Precision indicated by %RSD	0.0307
Accuracy indicated by % recovery	100.3
Limit of detection (LOD), µg /mL	7.193
Limit of quantification (LOQ), µg /ml	21.798
Linear regression equation	Y=0.0556x
Robustness indicated by %RSD	
1) Solvent-A	0.42
1) Solvent-B	1.34
2) Solvent-C	5.53
Ruggedness indicated by %RSD	
1) At-5°C	0.5
2) At-25°C	0.3
3)At-37°C	31.9
4) At-60°C	0.5

Table: 2. Linearity table of Vitamin B9 (Folic Acid)

Sr. No.	Concentration	Absorbance
1	2	0.105
2	4	0.2251
3	6	0.3321
4	8	0.4421
5	10	0.5412

Table: 3. Optical Characteristic of Vitamin B9 (Folic Acid)

Optical characteristics	Result
Beer's law limit (µg/ml)	15-100
Molar extinction coefficient (L/Mol. cm)	16023.45
Correlation coefficient (r2)	0.9999
Regression equation	Y =0.0556x
Slope b	0.0485
Slope c	0.532

Table: 4. Accuracy Studies of Vitamin B9 (Folic Acid)

Sr. No.	Vitamin B9 (Folic Acid) %
1	99.65
2	99.78
3	98.99
Mean	98.84
±SD	0.3667
RSD	0.3685
% RSD	99.51

Summary And Conclusion

An attempt has been made to develop the validated and UV- Visible method for estimation of Vitamin B9 (Folic Acid) in API. As the literature survey revealed that few methods are available for estimation of Vitamin B9 (Folic Acid) in API but there is a need for a simple, economical and proper method for estimation of Vitamin B9 (Folic Acid) in API. Results for the recoveries of selected drug were found within the limits (99.3-100.3%). These indicate that the proposed method was accurate for the analysis. The developed UV method for the determination Assay of selected drug is simple, accurate, precise, robust and economical. The solvent used in the proposed method is simple to prepare and economically reliable. The sample recoveries were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. The method can be used in laboratories for the routine analysis of selected drug. Since the system validation parameters UV method used for estimation of selected drugs in pure and have shown satisfactory, accurate and reproducible results (without any interference of excipients) as well, it is deduced that the simple and short proposed methods be most useful for analysis purpose.

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