International Journal of Research in Pharmacy and Pharmaceutical Sciences
ISSN: 2455-698X
Impact Factor: RJIF 5.22
www.pharmacyjournal.in
Volume 3; Issue 2; March 2018; Page No. 107-110

Development and validation of UV-visible spectrometry method for water soluble vitamin Folic acid in pellet formulation

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Abstract
A simple, precise, accurate and cost effective UV-visible spectrometry method and validation for water soluble vitamin folic acid in the pellet formulation was developed according to ICH Q2 (R1) guideline. The absorption maxima of folic acid was found to be 283nm. The method represents correlation coefficient (R^2 =0.999) at concentration range of 1-3.5 µg/ml. The validation of method was carried out using linearity, accuracy and precision values. The percent relative standard deviation of inter-day and intra-day precision range (0.884-0.713) & (0.627-0.142) respectively shows the method was precise. The recovery of folic acid was found to be 99.66 - 99.36 %. This validated precise UV- visible spectrometry method can be applied for the estimation of folic acid with respect to the determination of assay content for the solid dosage form.

Keywords: folic acid, pellets, 0.01 M NaOH, validation

Introduction [1-3]

Vitamins are the essential, non-energy producing compounds that gets metabolize in the human body and must be supplied in the diet. Vitamins mainly play as important and primary role in the prevention and treatment of the various deficiency diseases. Various factors that affect the deficiency of vitamins include malabsorption, inadequate intake, increased excretion, genetic abnormalities. Vitamins can be categorized into fat soluble vitamins and water soluble vitamins.

Folic acid (FA) or vitamin B9 is water soluble vitamin chemically known as N-[4-[[(2-amino-1,4-dihydro-4-oxo-6-pteridinyl)methyl]benzoyl]-L-glutamic acid. FA is tasteless and odorless, yellowish in color. FA is used to treat the neural tube defects in newborns, cardiovascular disease and in case of anemia.

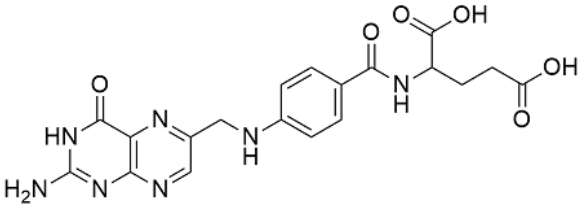

Fig 1: Chemical structure of Folic Acid

FA occurs as yellowish crystals which are less soluble in water but, its sodium salt is freely soluble in water. There are few reports demonstrating development of UV method for estimation of folic acid. Said methods were developed for the estimation of folic acid in bulk only. Moreover, there was use of strong alkali as one of the solvents.

The main aim of the present study was to develop and validate a simple and accurate UV method for the assay of folic acid raw material and its pellet dosage form with less aggressive solvent system.

Materials and Method [4-6]

Materials

Folic acid (FA) was obtained as gift sample from Micro lab, Sikkim, India. All chemicals were used was analytical grade of Merck.

Instruments Used

A double beam UV-visible spectrophotometer (UV-530 Jasco) with spectra manager software loaded in to computer system. 1 cm path length was used for spectral measurements with 3 cm matched quartz cells. Essae (Vibra HT) balance was used for weighing.

Preparation of standard stock solution

Accurately weighed 5 mg of FA was transferred to the 5 ml pre-calibrated volumetric flask and dissolved in to 1 ml of 0.01 M NaOH solution. Volume was made up to 5 ml with water to achieve a stock solution of 1000 µg/ml (Stock-1). From the Stock-1, 0.5 ml of solution was suitably diluted further with water to achieve solution of 100 µg/ml strength (Stock-2).

Determination of maximum wavelength (λ_{max})

From the Stock-2, solution of 2 µg/ml strength was prepared and used for determining the maximum wavelength. Full scan mode was used to obtain a complete scan between 800 nm to 200 nm with water as a blank.

Preparation of calibration curve

For the preparation of the calibration curve, Stock-2 was suitably diluted to achieve six different calibration standards representing 1, 1.5, 2, 2.5, 3 and 3.5 µg/ml strength. From the full spectrum measurement mode (Figure 1) of stock-2, 283 nm was identified as λ_{max}. Absorbance of each calibration standard was measured at 283 nm using fixed wavelength

measurement mode. The calibration curve of concentration vs. absorbance was plotted (Figure 2).

Estimation of Folic acid in Pellet formulation

Accurately weighed pellets equivalent to 10 mg of folic acid were dissolved in 2 ml of 0.01 M NaOH solution and transferred into the 10 ml volumetric flask. It was diluted suitably with water to achieve a solution of 1000 µg/ml. It was further diluted with water to achieve a solution of 100 µg/ml.

Method Validation [7-9]

Developed UV method for estimation of FA was validated as per ICH guideline for evaluating different parameters like Linearity, accuracy, precision, robustness, ruggedness, limit of detection (LOD) and limit of quantification (LOQ).

Linearity

Linearity was established using estimation of absorbance of six different calibration standards and the calibration curve plot (Table no. 1 and Fig no. 2).

Accuracy

The accuracy of the above UV method was evaluated on the basis of recovery studies using standard addition method. Three different concentrations were prepared in the triplicate at level of 80%, 100% and 120% of original standard concentrations. The different level concentrations were added to the original concentrations and accuracy of the method was calculated on the basis of percent recovery. (Table no.2) following formula was used to calculate percent recovery.

% RC= [SPS-S/SP] × 100

Where, SPS= Amount found in the spiked sample

S= Amount found in the sample

SP= Amount added to the sample

% RC= Percent recovery

Precision

Intra- and inter-day precision of the method was established at three concentration levels. Intra-day precision was established by preparing nine different solutions of 1 µg/ml, 2.5 µg/ml and 3.5 µg/ml and its analysis at morning, afternoon and evening time. Deviation in results in terms of % relative standard deviation (% RSD) was calculated (Table no. 3).

Inter-day precision was established by analyzing the above mentioned solutions at three consecutive days (Table no. 4).

Robustness [10]

Robustness of the method was evaluated by changing the solvents. Three different solvents viz. 0.01 M NaOH, methanol and distilled water were used for dissolving FA and the absorbance of each was determined. FA levels in each sample were estimated using pre-defined calibration curve. Results were represented in terms of % RSD (Table no. 5).

Ruggedness

Ruggedness of the method was determined by carrying out the analysis of folic acid solutions (1, 2.5 and 3.5 µg/ml) at three different (25°C, 37°C and 60°C) temperatures and absorbance were noted and % RSD was calculated (Table no. 6).

Limit of Detection (LOD) [11]

The LOD (Table no. 7) of the developed UV method was calculated using the following formula

LOD=3.3×SD/S

Where, SD= standard deviation of Y- intercepts

S=Slope

Limit of Quantitation (LOQ)

The LOQ (Table no. 7) of the developed UV method was calculated using following formula LOQ=10×SD/S

Where, SD= standard deviation of Y- intercepts

S=Slope

Results and Discussion

Determination of maximum wavelength

The maximum wavelength (λ_{max}) was evaluated by scanning the full spectrum in the range of 200 nm-800 nm UV-visible ranges. (Figure no. 1)

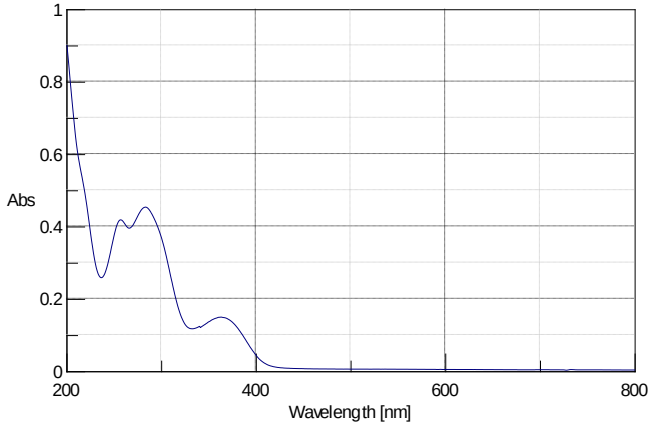

Fig 1.1: UV-visible spectra of Folic acid (FA)

Preparation of calibration curve

The calibration curve of Folic acid (FA) was performed and graph plotted concentration vs. absorbance. (Figure no. 2) The absorbance values of different concentration were noted. (Table no. 1)

Table 1: Calibration data of Folic acid

Concentrations (µg/ml)	Absorbance
1	0.2538
1.5	0.3862
2	0.5109
2.5	0.6413
3	0.7696
3.5	0.9132

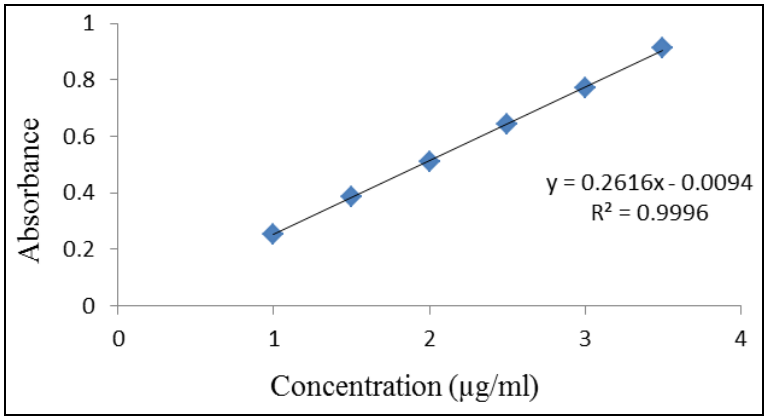

Fig 2: Calibration curve for Folic acid (FA)

Assay of Folic acid Pellet formulation

Assay study of pellet formulation containing Folic acid (FA) was carried out and the percent (%) assay was found to be 99.8 ± 0.2.

Method validation

Linearity

For the linearity of the Folic acid (FA), six point calibrations curve were plotted in a concentration range of 1-3.5 (µg/ml). From the linearity study it was observed that the drug was found to be linear in the concentration range and the linear regression equation was y = 0.2616x-0.0094 with correlation coefficient 0.9996. (Table no. 1 and Fig no. 2)

Accuracy

Accuracy of the proposed UV method was verified by conducting the recovery studies by using standard addition method. Standard drug concentration at three different percent levels was added to known amount of folic acid taken from pellets. The percent recovery of added standards was calculated (Table no.2). The results showed better % mean recovery for respective percent levels. The % mean recovery values are closer to 100% showed high accuracy of the proposed UV analytical method.

Table 2: Evaluation data of accuracy study

Concentration (%)	Origin level (µg/ml)	Amount added (µg/ml)	% Recovery	Mean % Recovery	% RSD
80	1	0.8	100.00	99.66	1.527
80	1	0.8	98.21		
80	1	0.8	101.42		
100	2.5	2.5	99.20	98.92	0.220
100	2.5	2.5	99.00		
100	2.5	2.5	98.76		
120	3.5	4.2	100.00	99.36	0.719
120	3.5	4.2	99.42		
120	3.5	4.2	98.57		

Precision

Intra-day and inter-day precision study of drug were evaluated for the 1µg/ml, 2.5 µg/ml and 3.5 µg/ml. Absorbance mean, percent assay and percent RSD were calculated for the intra-day as well as inter-day precision study. (Table no. 3 and Table no. 4)

Table 3: Evaluation data for Intra-day study

	Morning			Afternoon			Evening		
Concentration Range (µg/ml)	Mean	% Assay	% RSD	Mean	% Assay	% RSD	Mean	% Assay	% RSD
1	0.2541	101.03	0.2166	0.2543	101.26	0.4740	0.2577	101.57	0.627
2.5	0.6440	99.23	0.7757	0.6470	99.84	0.5708	0.6455	99.75	0.372
3.5	0.9146	98.12	0.1433	0.9158	98.20	0.1992	0.9142	98.64	0.142

Table 4: Evaluation data for Inter-day study

	Day 1			Day 2			Day 3		
Concentration Range (µg/ml)	Mean	% Assay	% RSD	Mean	% Assay	% RSD	Mean	% Assay	% RSD
1	0.2548	101.34	1.721	0.2503	101.64	1.825	0.2521	101.50	0.884
2.5	0.6434	99.43	0.639	0.6405	99.76	0.145	0.6453	99.12	0.523
3.5	0.9153	98.37	0.555	0.9167	98.51	0.535	0.9176	98.37	0.713

Robustness

Robustness study was evaluated by using three different solvent. The method was found to be robust as indicated by the % RSD values which are less than 2%. (Table no. 5)

Table 5: Evaluation data for Robustness

Concentration (µg/ml)	Solvents	Absorbance	% RSD
2.5	0.01 M NaOH	0.6457	0.936
2.5	Methanol	0.6435	1.26
2.5	Water	0.6398	0.863

Ruggedness

Ruggedness study of drug was carried out at the three different temperature levels. From the results it was found that the method was rugged showing the % RSD value less than 2%. (Table no.6)

Table 6: Evaluation data for Ruggedness

Concentration (µg/ml)	Temperature (°C)	Absorbance	% RSD
2.5	25	0.6410	0.724
2.5	37	0.6578	1.56
2.5	60	0.6569	1.23

Limit of Detection (LOD) & Limit of Quantification (LOQ)

Form the results it was found that LOD & LOQ are in the sub-microgram level, which indicates the sensitivity of the method. (Table no.7)

Table 7: Evaluation data for LOD & LOQ

LOD	0.118 µg/ml
LOQ	3.593 µg/ml

Conclusion

A simple, precise and accurate UV-visible spectrophotometric method was developed for the estimation of FA in bulk as well as pellet formulation. Said method was developed using less aggressive solvent.

Acknowledgement

Standard folic acid as a gift sample by MicroLab, Sikkim is highly acknowledged.

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