Research Article

# Stabilization of Folic Acid in Liquid Dosage Form: Formulation Development, Method Validation and Comparative Analysis

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**ABSTRACT**

Vitamins are essential nutrients available as multivitamin combinations. The stability of individual vitamins remains a question since most of the vitamins are decomposed during storage. Folic acid is a water soluble vitamin and found to be unstable in the presence of water and also other vitamins. Due to this stability problem, this vitamin is not available alone in liquid dosage forms. This problem is mainly attributed to the change in pH. So, the present investigation aims to provide a stable liquid preparation of folic acid alone by the usage of suitable solvents and stabilizers in order to maintain the pH. Stability study has been conducted to find out the degradation rate and the shelf-life of formulation has also been determined. The obtained results have been compared with three different innovators multivitamin products containing the folic acid. The experimental studies clearly show that a stable pH range between 5 and 5.5 for folic acid could be maintained very well by using the mixed solvents of sorbitol, glycerine and propylene glycol and the degradation rate has been found to be significantly less in the present formulation as compared with innovator products. For the analysis of folic acid, High Performance Liquid Chromatography method has also been developed, optimized and validated for its precision, accuracy and specificity. The data obtained indicate the suitability of this High Performance Liquid Chromatography method for the analysis of folic acid. This report is the first of its kind in dealing with the stabilization of folic acid in liquid dosage form.

**Keywords:** folic acid, stabilization, formulation, validation, liquid dosage form.

## 1.0. INTRODUCTION

Formulation and stability problems arise more frequently with liquid pharmaceutical preparations than solid dosage forms, and for this reason many new drugs first reach the market as tablets or dry-filled capsules. Later, when the pharmaceutical problems are resolved, a liquid form of the same drug is generally marketed. Among the various liquid preparations, oral liquids are homogeneous preparations containing one or more active ingredients in a suitable vehicle. Multivitamin preparations are available in liquid formulations like syrups, suspensions, and elixirs. In these preparations, vitamins are known to lose their potency during storage even though most of the individual vitamins are stable and many are prepared into reasonably stable dosage forms by themselves [1]. Loss of vitamin activity in premixes and complete feeds during storage may account for hidden depressions in growth, feed efficiency and disease resistance due to sub clinical vitamin deficiencies [2]. Unfortunately, many vitamins are relatively unstable compounds and undergo significant deterioration under normal storage conditions. Some of the factors that are generally responsible for the instability of vitamins in multivitamin preparations are the pH of the medium, the presence of certain minerals like iron, copper, calcium, etc., and the concentration of water present in the preparation, the conditions of storage such as light, atmospheric oxygen,

temperature, etc.[3] and the incompatibility between vitamins.

Folic acid is available in multivitamin combinations in liquid formulations but it is not available alone in liquid dosage form due to its instability. Looking at this important problem, the present investigation aims to formulate, stabilize and thereby provide a liquid formulation for the oral delivery of folic acid alone and to stabilize the preparation against all the destabilizing factors as mentioned above. Basically, folic acid is stable in aqueous solutions [4] of pH in between 5 and 8. Thus, the present study of folic acid stability in liquid formulation is aimed with the help of laboratory solvents and stabilizers.

## 2.0. EXPERIMENT

### 2.1. MATERIALS

Folic acid was obtained from Sri Krishna drugs, Chennai, India. Glycerine, Sorbitol solution and Propylene glycol were supplied from Pan Century, Malaysia, Gulshan polyols and Manali petrochemicals, Chennai, India respectively. All the solutions and reagents were of pharmaceutical and analytical grade.

### 2.2. PREFORMULATION STUDIES

The preformulation studies[5] have been conducted in five divided stages, which include studies on purified water, sorbitol solution, glycerine, propylene glycol and sugar solution 50 % which were used as solvents and/or co-solvents for the solubility and stability of folic acid.

## 3.0. RESULTS AND DISCUSSION

### 3.1. pH and Determination of amount of folic acid in individual solvents

The pH and amount of folic acid were determined in the preformulation. The pH was determined using pH meter and the amount of folic acid was estimated using High Performance Liquid Chromatography technique[6]. The analysis was made during the initial day, 15$^{th}$ day and after 30$^{th}$ day of storage.

**Table I: pH and the amount of folic acid in various preformulations**

| S.No. | Test Solution | Initial Day | | 15$^{th}$ Day | | 30$^{th}$ Day | |
|---|---|---|---|---|---|---|---|
| | | pH | (%) | pH | (%) | pH | (%) |
| 1. | Folic acid (20mg) solution + Purified water | 5.33 | 99.35 | 7.34 | 19.26 | 8.48 | 8.86 |
| 2. | Folic acid (20mg) solution + Sorbitol solution | 5.35 | 99.97 | 5.33 | 94.6 | 5.32 | 93.09 |
| 3. | Folic acid (20mg) solution + Glycerine | 5.36 | 99.21 | 5.38 | 94.34 | 5.39 | 92.03 |
| 4. | Folic acid (20mg) solution + Propylene glycol | 5.37 | 99.06 | 5.36 | 95.75 | 5.35 | 94.08 |
| 5. | Folic acid (20mg) solution + Sugar solution | 5.33 | 98.34 | 4.98 | 67.17 | 4.06 | 9.78 |

From the above results, it could be observed that the pH range in between 5 and 5.5 was maintained by using sorbitol solution, glycerine and propylene glycol, but the pH was increased in purified water, whereas it was decreased in sugar solution. In addition, turbidities were also found in sugar solution as well as in purified water after 15 and 30 days respectively. In assay, folic acid was stable and stability was found to be high in sorbitol solution, glycerine and propylene glycol except purified water and sugar solution, which was well correlated with pH. Thus, sorbitol solution, glycerine and propylene glycol were taken for further studies.

### 3.2. pH and Determination of amount of folic acid in combined solvents

The sorbitol solution, glycerine and propylene glycol were combined together with folic acid at the same concentration and determined for pH and content of folic acid in every 15 days intervals.

**Table II: pH and the amount of folic acid in the combined solvents**

| S.No. | Test Solution | Initial Day | | 15th Day | | 30th Day | |
|---|---|---|---|---|---|---|---|
| | | pH | (%) | pH | (%) | pH | (%) |
| 1. | Folic acid (20mg) solution + Sorbitol solution + Glycerine + Propylene glycol | 5.34 | 99.97 | 5.35 | 95.92 | 5.35 | 94.78 |

Based on the above observations, it could be seen that the pH range in between 5 and 5.5 was maintained in the presence of combined solvents, i.e. sorbitol solution, glycerine and propylene glycol with folic acid. This supports that the folic acid is stable in the above combination and due to this the same solvents has been considered for the formulation development.

### 3.3. ANALYTICAL METHOD DEVELOPMENT AND OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS

Based on the optimization studies, the chromatographic parameters for the estimation of folic acid was as follows: Instrument setup -Isocratic; Column - Waters Symmetry 250 x 4.6 mm, 5 µm; Detection wavelength - 280 nm; Mobile phase - Methanol : Water : 1-Hexane sulfonic acid sodium salt in 30:70:0.15 and the pH was adjusted to 3.0 using orthophosphoric acid; Diluents - Dipotassium hydrogen orthophosphate (0.57 % in water) adjusted to pH 7.0 using orthophosphoric acid; Injection volume - 20 µl; Flow rate - 1.0 ml/min; Temperature - Ambient temperature; Run time - 10 min.

### 3.3.1. HPLC ANALYSIS

#### 3.3.1.1. Preparation of working standard solution

Weighed accurately 20 mg of folic acid working standard, transferred into a 50 ml volumetric flask, dissolved and made up to the volume using the diluent. Further, 5 ml of standard stock solution was diluted to 100 ml with diluent, which was mixed well and filtered using Millipore filter (20 ppm).

#### 3.3.1.2. Preparation of test solution

Each 2 ml of sorbitol solution, glycerine and propylene glycol was pipetted out, transferred into a 50 ml volumetric flask, dissolved and made up to the volume using the diluent, which was then mixed well and filtered using Millipore filter (20 ppm). The content of folic acid was estimated in the test solution using HPLC technique and percentage content was determined.

### 3.3.2. HPLC METHOD VALIDATION

Following analytical method validation parameters [7, 8, 9] were studied; system suitability, similarity factor, specificity, linearity & range, precision (repeatability) and accuracy.

**(A) System Suitability**

**Table III: System Suitability**

| Parameter | No. of replications | % RSD of Rt | % RSD of area | Acceptance criteria of % RSD |
|---|---|---|---|---|
| System suitability | 6 | 0.7 | 0.7 | < 2.0 |

**(B)** Similarity Factor

**Table IV: Similarity Factor**

| Parameter | No. of replications | Result obtained | Acceptance criteria |
|---|---|---|---|
| Similarity factor | 2 + 2 | 1.02 | 1 ± 0.1 |

**(C) Specificity**

There was no interference with blank, placebo and in the retention time of folic acid and thus this method was specific.

**(D) Linearity and Range**

A plot was made using area vs concentration and the value of $R^2$ was found to be 0.999. This clearly proves that the method was linear over the concentration range studied and within the specified limit.

**(E) Method Precision**

**Table V: Method Precision**

| Parameter | % RSD | | | % Content | |
|---|---|---|---|---|---|
| | Rt | Area | Acceptance criteria | Assay | Input |
| Method precision | 1.2 | 0.8 | 2.0 | 97.78 | 100.0 |

**(F) Accuracy**

**Table VI: Accuracy**

| Parameter | Assay (µg) | | % Recovery | |
|---|---|---|---|---|
| | Std Wt taken | Quantity found | Obtained | Acceptance criteria |
| Accuracy solution - 1 | 10.1 | 9.97 | 98.71 | 98.0 – 102.0 |
| Accuracy solution -2 | 20.2 | 20.24 | 100.19 | |
| Accuracy solution - 3 | 30.3 | 29.77 | 98.25 | |

The above method validation showed that the reported values were found to be within the acceptance criteria. So, this method was valid and thus could be implemented for further studies.

**3.4. FORMULATION**

**3.4.1. Preparation of Folic Acid Solution**

Folic acid 20 mg was weighed accurately into a clean and dried beaker. To this freshly prepared sodium hydroxide solution was added [10, 11] dropwise and was stirred well with a glass rod until it dissolves completely. Propylene glycol was weighed and poured into the above solution followed by glycerine with continuous stirring (Solution-I). Sorbitol was weighed and poured into that mixture and stirred well (Solution-II). Solution-II was added slowly into solution-I and mixed well with constant and continuous stirring. Methyl paraben and propyl paraben were added and act as a preservative. It was then filtered with muslin cloth; the strawberry flavor was added and transferred into a measuring cylinder, made up to the volume using purified water. This final solution was stored in an amber colored bottle and labeled after adjusting the pH to 5.31.

**3.4.2. Observation**

A clear, optimum viscous, yellowish colored, sweetened, flavored folic acid liquid was found and the pH was observed to be 5.31. This trial batch was analysed by HPLC and pH was periodically checked. The HPLC results and a stable pH clearly indicate an improvement of the developed formulation. Thus, the exact formula for this formulation have been achieved. After this, the formulation was analysed as per ICH guidelines and stability studies were performed.

**3.5. STABILITY STUDIES**

The accelerated stability study was conducted as per ICH guidelines at an accelerated temperature of 40±2 °C / 75±5 % RH and long term stability studies were conducted at a room temperature of 25±2 °C / 60±5 % RH for two years at periodic intervals. The shelf life of folic acid has been predicted by using the graphical method [12]. The results as shown in Table 7 and Fig.1 indicate the stability

maintained in the product at the periodic time intervals.

**Table VII: Stability studies for the formulated folic acid liquid**

| Storage condition | Months | Description | Wt. of working std folic acid (mg) | pH | Assay | | |
|---|---|---|---|---|---|---|---|
| | | | | | Label claim (µg) | Obtained | |
| | | | | | | µg | % |
| 25±2 °C / 60±5 % RH | 0 | A clear, viscous, yellowish colored, flavored folic acid liquid | 20.0 | 5.31 | 500 | 492.10 | 98.42 |
| | 1st | | 20.0 | 5.34 | 500 | 425.00 | 96.11 |
| | 2nd | | 20.0 | 5.36 | 500 | 478.70 | 95.74 |
| | 3rd | | 20.0 | 5.37 | 500 | 470.00 | 94.00 |
| | 4th | | 20.0 | 5.37 | 500 | 466.15 | 93.23 |
| | 5th | | 20.0 | 5.38 | 500 | 455.90 | 91.18 |
| | 6th | | 20.0 | 5.38 | 500 | 452.8 | 90.56 |
| | 9th | | 20.0 | 5.39 | 500 | 441.5 | 88.30 |
| | 12th | | 20.0 | 5.39 | 500 | 434.05 | 86.81 |
| | 18th | | 20.0 | 5.40 | 500 | 421.40 | 84.28 |
| | 24th | | 20.0 | 5.41 | 500 | 413.75 | 82.75 |
| 40±2 °C / 75±5 % RH | 0 | | 20.0 | 5.31 | 500 | 492.10 | 98.42 |
| | 3rd | | 20.0 | 5.36 | 500 | 464.70 | 92.94 |
| | 6th | | 20.0 | 5.37 | 500 | 428.05 | 85.61 |

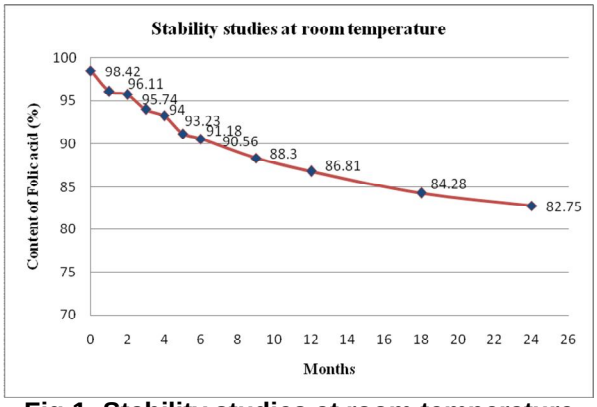


**Fig.1: Stability studies at room temperature**

### 3.5.1. Prediction of Shelf-Life from Stability Data

This accelerated stability testing is useful in providing the information from which to assess the probable stability of a new product but it should be conducted in conjunction with long-term stability studies at the maximum recommended storage temperature for the duration of the nominated shelf-life. The possible shelf-life of the present formulation was obtained using the long term stability studies for two years as shown in Fig. 1.

### 3.5.2. Comparative Analysis with Innovator Products

Among the innovator products of multivitamin combination of folic acid in liquid formulation, three of them have been selected for this study as shown in Table 8. The innovator products had been analyzed accordingly as per the analytical procedure followed in the present study and the percentage content of folic acid was determined in these preparations compared with our formulation at the room temperature storage condition

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**Table VIII: Comparative data of innovator products**

| Sl. No. | Name of the Product | Storage Condition | Description | Month of Analysis | pH | % content of Folic acid |
|---|---|---|---|---|---|---|
| 1. | Present Folic Acid liquid formulation | 25±2 °C / 60±5 % RH | A clear, optimum viscous, yellowish colored, sweetened, flavored liquid | 24th | 5.41 | 82.75 |
| 2. | Multivitamin liquid product - I | | A viscous, dark brown colored, flavored liquid | 24th | 5.83 | 51.25 |
| 3. | Multivitamin liquid product - II | | A viscous, very dark blackish colored, flavored liquid | 24th | 6.03 | 47.95 |
| 4. | Multivitamin liquid product - III | | A viscous, dark brown colored, flavored liquid | 24th | 6.33 | 38.45 |

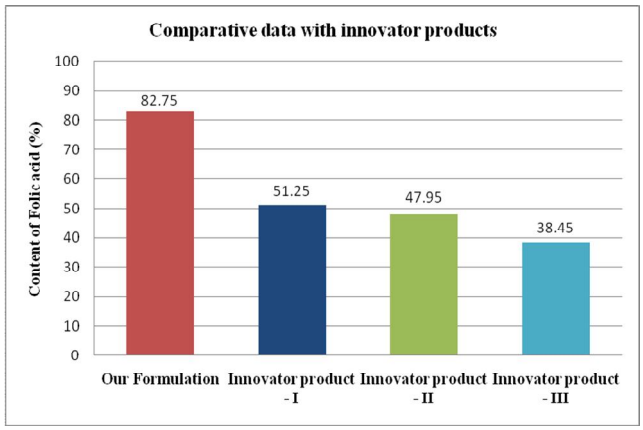


**Fig.2: Comparative analysis with innovator products**

At room temperature (25±2 °C /60±5 % RH), the present folic acid liquid formulation has been compared with that of 3 different innovator products. The percentage content and stability of folic acid has been found to be good in the present formulation, whereas the content was found to be very less in the innovator products. Based on the above observations, the pH ranges and folic acid stability was determined. When compared to marketed products, the present formulation was found to be stable.

## 4.0. CONCLUSIONS

The present investigation has provided a stable liquid preparation of folic acid in the combined solvents of sorbitol solution, glycerine and propylene glycol with stabilizers. The pH of the said preparation has been adjusted and maintained in between 5 and 5.5. This formulation was stable with no significant degradation even when the liquid preparation was stored for 2 years at room temperature. From the stability data, it has been observed that the folic acid degradation rate was drastically increased due to the presence of water in the formulation. Replacement of this water by sorbitol solution, glycerine and propylene glycol significantly improved the folic acid stability in liquid formulation, which was indicated by the high percentage of folic acid in the present formulation compared to innovator products.

## 5.0. ACKNOWLEDGEMENTS

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