# METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF IRON IN FOLIC ACID (API) BY ATOMIC ABSORPTION SPECTROPHOTOMETER

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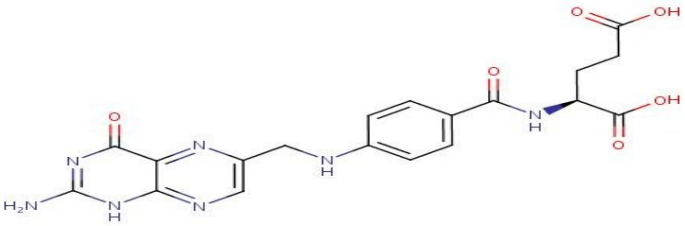
## Abstract

Atomic absorption spectroscopy was used to standardize a new technique for inline quality control in the measurement of elemental iron in chemically produced folic acid. The iron calibration graph was linear up to at least 0.0148 µg/ml, which is close to the detection limit. The new method was assessed in relation to validation criteria, and the ideal conditions for elemental iron determination were identified. The outcomes showed that the process may be effectively used to estimate the elements in folic acid.

## INTRODUCTION

Folic acid, as it is biochemically inactive, is converted to tetrahydro folic acid and methyl tetrahydrofolate by dihydrofolate reductase. Receptor-mediated endocytosis carries these folic acid congeners throughout cells, where they are required for purine and thymidylate nucleic acid synthesis, interconversion of amino acids, methylation of tRNA, formate generation, and maintenance of normal erythropoiesis. By using methionine synthetase to remethylate homocysteine to methionine, folic acid can regulate elevated homocysteine levels with the help of vitamin B12 as a cofactor. Folic acid can be found in vitamin B complex, dietary supplements, haematinics, and micronutrients. It comes in two forms: Folvite (100 mg ferrous ascorbate ≡ Elemental Iron-Oral) and Ferikind (100 mg Elemental Iron-Oral). Only a small number of methods reported using spectrophotometry, according to a thorough assessment of the literature.



Key words: Atomic absorption spectroscopy, haematinics

Fig1: Folic Acid

**Materials and Methods**

**Drugs and Reagents:** Folic acid was obtained as a Giftsamplebyalocalpharmaceuticalcompany, IronStandard obtained from Merckmillipore,Germany, RFCL, Mumbai, provided sulfuric acid, hydrochloric acid, and perchloric acid, while SDFCL, Mumbai, provided sodium hydroxide.

**Instrumentation**

Atomic adsorption spectroscopy was carried out by using ShimadzuAA-6300 with flame atomization, slit width of 0.2nm and lamp mode BGC-D2.

The optimum parameters for Atomizer/GasflowrateSetup are: Fuelgasflowrate2.2(L/min), SupportgasFlowRate15.0(L/min), FlametypeAir-$C_2H_2$, BurnerHeight3nm.

Optimal Measurementparameters are: Order 1, ZeroIntercept pass, ConcentrationUnit mg/l, RepetitionSequence SM-M-M, Pre-spraytime 3 sec, IntegrationTime 5 sec, Responsetime 1 sec.

**PreparationofStockSolution**

In this study, a 100 mL volumetric flask is pipetted with 1 ml of the 1000 ppm iron standard, which is then diluted with distilled water to create a 100 ppm iron standard solution. Additional dilutions were performed in order to get the 10 ppm concentration.

**StandardSolutions**

Standardsolutionsof0.375ppm,0.750ppmwerepreparedfrom 1 ppm standard solution where as 1.125 ppm, 1.500 ppm,1.875ppmand2.250ppmwereobtainedfrom10ppmstandardsolution.

**MethodValidationbyAAS**

**Linearity**

The linearity was determined by Six distinct concentrations between 0.374 and 2.250 mg/l were inhaled, and each concentration was analyzed in triplicate to ascertain the linearity.Plotting standard calibration curves involved using the y-axis for absorbance and the x-axis for conc. The standard deviation was computed for the standard curve's peak area, slope, intercept, and correlation coefficient.

**Limit of Detection and Limit of Quantification**

The relationship between the calibration curve's standard deviation (SD) and slope (S) was used to mathematically determine the limits of detection (LOD) and quantification (LOQ).

The LOD and LOQ were calculated from the following equations:

LOD = [3.3 x σ/S]

LOQ = [10 x σ /S]

**Accuracy**

The folic acid sample solution was made by triplicately adding a known quantity of contaminants at three different levels, and it was then examined using the test method's instructions. The samples were prepared according to the sample preparation, and the contaminants were spiked at 0.750 ppm, 1.500 ppm, and 2.250 ppm.

The trials and combinations used are: 50%Standard, 50%Standard+ sample, 100%Standard, 100%Standard+ sample, 150%Standard, 150%Standard+ sample.

**Precision**

System precision: By aspirating the solution three times, the analysis was carried out at the impurity specification level, and the percentage RSD of the impurity peak area was calculated.

Method Precision: A solution of folic acid which was spiked with impurity at specific level was prepared 6 times and analyzed as per the method for determining the method.

System Suitability: System suitability was performed by aspirating a blank followed by standard preparation of Iron 6 times.

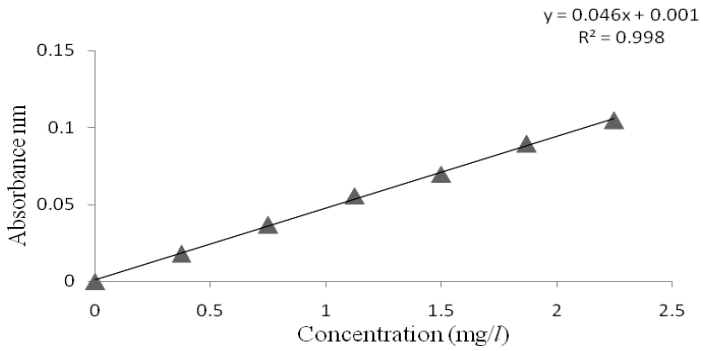
**Assay: EstimationofIronfrompharmaceuticaltabletdosageform**

Ten tablets were precisely weighed and ground into powder. Ten milligrams of iron were added to ten milliliters of 1M NaOH, and the mixture was sonicated for fifteen minutes. A Millipore filter paper was used to filter the mixture, and a 1M NaOH solution was used to completely remove any remaining residue. In a 10 mL volume flask, the filtrates and washings were mixed together and diluted with 1M NaOH to the appropriate level. At 248.3 nm, the absorbance of the resulting solution was measured in comparison to a blank. By filtering the response into the regression equation, the amount of iron in the pill was ascertained. Five iterations of the analysis process were conducted using the tablet formulation.

**Results and Discussion**

**Linearity**

Working standard solutions of iron with at least six distinct concentrations between 0.374 and 2.250 mg/l were analyzed to create the calibration curve; each concentration was aspirated and measured three times. Plotting




standard calibration curves involved using the y-axis for absorbance and the x-axis for concentration.

Fig2: Calibration curve

**Accuracy**

Recovery was within the range of 100±2%, which indicates accuracy of methods.

| Level Of Accuracy | True Vale | Amount found | % Recovery | %RSD |
|---|---|---|---|---|
| 0 | 100 | 100.19±0.0025 | 100.19 | 1.0009 |
| 50 | 0.750 | 0.7643±0.0006 | 101.9 | 1.58 |
| 100 | 1.500 | 2.0749±0.0017 | 102.4 | 1.71 |
| 150 | 2.250 | 3.0149±0.0025 | 100.1 | 1.56 |

Table 1- %Recovery of Iron

**Precision**

When the sample was analyzed on the same day and on subsequent days, the RSD values were clearly less than 2%, indicating that the procedure was accurate enough.

Table 2- Precision data obtained from analysis

| Conc. Trial | Trial 1 | Trial 2 | Trial 3 | Avg ± SD | % RSD |
|---|---|---|---|---|---|
| 0.75 | 0.0365 | 0.0369 | 0.0362 | 0.0365±0.0007 | 1.91 |
| 1.5 | 0.0688 | 0.0693 | 0.0695 | 0.0692 ±0.0011 | 1.57 |
| 2.25 | 0.1047 | 0.1042 | 0.1042 | 0.1043 ±0.0012 | 1.11 |

**Limit of Detection and Limit of Quantification**

The LOD and LOQ were 0.050µg/mL, 0.152µg/mL respectively.

**Assay**

Ferikind, 100mg Elemental Iron is used for assay and amount found was 0.19±0.0025, 1.0009.

Conclusion

The goal of the current study was to develop and validate an analytical method for measuring elemental iron in folic acid and other pharmaceutical dosage forms using atomic absorption spectroscopy. The method's linearity is demonstrated by the range of 0.374-2.250 mg/l.The commercial pills' assay revealed an iron content of 100.19 mg, falling within the range of 100±2%. When the sample was analyzed on the same day (n=3) and on successive days (n=3), the relative standard deviation values were less than 2%, indicating that the procedure was accurate enough**.**

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