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# Determination of Iron in Spinach plants Grown in different areas by using HIMMI as a reagent

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## ABSTRACT

Spinach is considered to be a rich source of iron. Bis [3-hdroxyimino-5-methyl-N-methyl]-2-imine (HIMMI) was applied for determination of Iron in spinach grown in different areas by using solvent extraction method. This study shows that the amount iron in spinach cultivated in farms is as expected while it is much more and can become hazardous for health for plants grown in polluted areas.

Keywords: HIMMI, Iron, Spinach; Solvent extraction method.

### INTRODUCTION

The bioavailability of iron is dependent on its absorption. This is influenced by a number of factors. The type of iron found in spinach is non-heme, a plant iron, which is poorly absorbed by the body unless eaten with vitamin C. Iron is essential to nearly all known organisms. In cells, iron is generally stored in the centre of metalloproteins. Knowing the importance of Iron, a method highly sensitive, selective, simple, rapid, accurate and superior to reported methods in the literature [1-26] has been developed for its extractive spectrophometric determination[27]. This developed method has been satisfactorily applied for the determination of iron in the spinach grown in different polluted and non polluted areas.

## EXPERIMENTAL

The **HIMMI** was synthesized, characterized[28] and used for extractive spectrophotometric determination of Fe(II). A stock solution of HIMMI (Concentration 0.005%) was prepared in methanol.

**Iron (II) Solution:** A weighed quantity of ferrous sulphate was dissolved in double distilled water containing dilute sulphuric acid and then diluted to the desired volume using double distilled water. The iron solution was then standardized by o-phenanthroline method [29]. **Recommended procedure** 

A . Collection of Samples: Spinach plants grown at different areas were collected.

S1: Sample of spinach plant was collected from a farm situated in a pollution free area and water used for cultivation was non polluted.

S2: Sample of spinach plant grown near railway tract passing drainage nallah beside it and water used for cultivation was non polluted.

S3: Sample of spinach plant was collected from a farm situated in MIDC area and water used for cultivation was from the river where pharmaceutical industrial drainage pipeline was lead out.

S4: Sample of spinach plant was collected from a farm situated near the chemical industry and water used for cultivation was non polluted.

S5: Sample of spinach plant was collected from a farm situated near the Paint industry and water used for cultivation was from nearest well.

S6: Sample of spinach plant was collected from a farm situated next to the MIDC area and water used for cultivation was from the river where almost all industrial drainage pipeline was lead out.

S7: Sample of spinach plant was collected from a farm situated away from the MIDC area and water used for cultivation was from the river where almost all industrial drainage pipeline was lead out.

S8: Sample of spinach plant was collected from a farm situated away from the MIDC area and water used for cultivation was from the nearby well but river where almost all industrial drainage pipeline was lead out passes from one km of the firm.

S9 : Sample of spinach plant cultivated on good quality soil in the lab and water use for cultivation was non polluted but this cultivation tray has kept near the area of the paint industry where the air pollution is dominant.

S10 : Sample of spinach plant cultivated on good quality soil in the lab and water use for cultivation was non polluted and good air supply is from oxygen cylinder.

**B. Procedure of treatment Spinach plants:** The collected spinach plants are properly washed with water, then air dried and powdered. Then 10 gm of each sample is kept in a furnace at 800  $^{\circ}$ C for 3 hrs. The ash is treated with 1.0 ml of aquaregia and transferred into a 100 ml standard measuring flask and diluted with distilled water upto the mark

### **RESULTS AND DISCUSSION**

**Solvent Extraction**[28]: To the 1 ml of diluted solution, and 1 ml of 0.005% bis [3-hdroxyimino-5-methyl-N-methyl]-2- imine reagent in methanol was added. The pH of solution was adjusted to 6.0 with 0.1 N HCl. The solution was then transferred in a separating funnel and Fe is extracted twice using 5 ml

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of chloroform for each extraction. The organic layer was subjected to extractive spectrophotometric determination. The results are shown in Table 1.

<b>1</b>	
Parameters	Conditions
Absorption Maxima	580 nm
Solvent	Chloroform
pH range	5.5 - 6.5.
Equilibration time	1.0 min
Stability of Iron-HIMMI	12 h
Beer's range	1 to 8 $\mu$ g/ cm <sup>3</sup>
Molar absorptivity	6.86 X 10 <sup>3</sup> Lit mol <sup>-1</sup> cm <sup>-1</sup>
Sandell's sensitivity	$8.15 \text{ X } 10^{-3} \mu\text{g} / \text{cm}^2$
Mole Ratio of Fe : HIMMI	1 :2

**Table.1** Spectrophotometric determination of iron complex

**Preparation of calibration plot:** The calibration curve was prepared by taking known amount of iron which were treated as described in the procedure under the 'solvent extraction and spectrophotometric determination' and the graph of absorbance against concentration was plotted (Figure.1). The concentration of the unknown iron solutions is determined from the calibration plot.

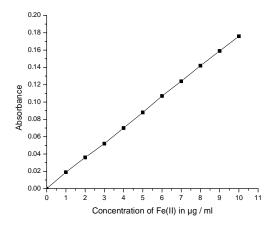


Figure.1 Calibration curve

The amount of iron present in various samples collected is determined and given in Table.2

Sample	Concentration of Iron in mg/100 g of Spinach
Controlled*	2.7
S1	2.8
S2	3.2

S3	4.5
S4	4.8
S5	5.0
S6	4.9
S7	4.0
S8	4.5
S9	4.8
S10	2.8

\*Source: USDA Nutrient database

#### APPLICATION.

This method can be applicable for the determination of iron present in other plants and also suggest the environmental problems.

#### CONCLUSIONS

The results obtained show that the method in which the reagent **HIMMI** was used, effectively used for quantitative extraction and estimation Fe (II). It shows that the metal amount in spinach cultivated in farms or in good environmental condition is as expected while it is much more and can become hazardous for health for plants grown in polluted areas.

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