ABSTRACT
A comparative study of the determination of iron in iron tablets was carried out using spectrophotometric and atomic absorption spectrometric method (AAS). Spectrophotometric method is based on the formation of ferrous tris-o-phenanthroline complex by boiling with hydroxylamine hydrochloride and subsequent addition with 1, 10 - phenanthroline at pH ~ 3±0.2. The work presented here reports on optimization of phenanthroline method. The maximum absorbance was found to be at 510 nm wavelength. A calibration curve was found to be linear up to the concentration range of 0.2 mg/L to 1.6 mg/L. Total four pharmaceutical samples from different pharmaceutical companies were analyzed and results were compared with the results obtained from atomic absorption spectroscopy. The study showed that the total iron content in pharmaceutical samples ranged from 50 to 54 mg per 60 mg. These values are in good agreement with pharmacopeial range.

Key words: Phenanthroline spectrophotometric method, iron tablets.

INTRODUCTION
Iron is the most abundant metal found in the earth crust, water as well as in different food stuffs naturally. Iron is also found in human body. The average adult human body contains 3 to 4 gram of iron. About 60 to 70 percent of total iron is present in haemoglobin of red blood corpuscles (RBC) of our body as circulating iron which plays a vital role in the transportation of oxygen from the lungs to the various tissues in the body and the rest 30 to 40 percent (1 to 1.5 g) as storage iron in liver, kidney, spleen and bone marrow [1]. Daily nutritional requirement of iron for adult male is 17 mg/d and for adult female is 21 mg/day [2]. A high level of iron needed for women is due to iron losses by the way of regular menstrual blood. Iron is required in the body for the formation of haemoglobin, brain development and function, muscle activity, regulation of body temperature, catecholamine metabolism etc. If the iron concentration is not enough in the body, it can cause anemia, cheilosis, koilonychia, dyspnea on exertion, irritability, impaired memory and concentration, increased susceptibility to infection etc. [3, 4]. In severe cases, it may lead even to the death of the patient. In such condition, iron supplementation is needed along with food stuffs like pork meat, fish, kidney, liver, boiled meat, egg yolks, goose, pheasants, wheat, apricots, rice, maize, cereals, lentils, spinach, rose mery etc. Iron supplementation can be administered in injection or solution or in tablet form. Iron content in iron tablets may vary from one pharmaceutical company to another pharmaceutical company. Pharmacopeial range of iron in iron tablet has been allocated from 48 mg - 54 mg per 60 mg of tablet [5]. Literatures revealed that there are several methods for the determination of iron [6-8]. Here, a simple, sensitive spectrophotometric method and atomic absorption spectrometric method (AAS) has been carried out for an investigation of total iron content in various iron tablets available in local drug stores.

MATERIALS AND METHODS
Materials
All the reagents used were of analar grade and were used without any further purification. Stock Mohr's salt [Fe(NH4)2(SO4)2.6H2O] (200 mg/L) solution was prepared by dissolving 1.404 gm in

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Distilled water. About 70 mL dilute sulphuric acid was added to it and final volume was made to 1000 mL in a volumetric flask. Calibration standard solutions were then prepared by taking appropriate volumes of stock iron solution. Hydroxylamine hydrochloride (NH$_2$OH.HCl) (1.5M) solution was prepared by dissolving 10 gm in 100 mL distilled water. Ammonium acetate buffer was prepared by taking 62.5 gm of ammonium acetate (NH$_4$C$_2$H$_3$O$_2$) in 40 mL distilled water and then 175 mL glacial acetic acid was added. Sodium acetate solution was prepared by weighing 25 gm of sodium acetate (NaC$_2$H$_3$O$_2$.3H$_2$O) and dissolved in 100 mL distilled water. Phenanthroline reagent was prepared in laboratory by dissolving 0.1 gm of 1 - 10 phenanthroline monohydrates (C$_{12}$H$_8$N$_2$.H$_2$O) in 100 mL distilled water and heating to 80°C. The 0.1 gm of hydroquinone was dissolved in 100 mL water to get hydroquinone solution.

**Apparatus**

WPA-S 104 Spectrophotometer and Fast Sequential Atomic Absorption Spectroscopy: AASR – Vari – AA 240 FS- 01 was used in present investigation.

**Methods**

**Phenanthroline Method** [9]

All the iron is converted into ferrous state by boiling with hydroxylamine hydrochloride. The reaction is as follows:

$$4\text{Fe}^{3+} + 2 \text{NH}_2\text{OH}.\text{HCl} \rightarrow 4\text{Fe}^{2+} + \text{N}_2\text{O} + 4\text{H}^+ + \text{H}_2\text{O}$$

The reduced iron chelates subsequently with 1, 10 - phenanthroline at pH 3.2 to 3.3 and forms an orange - red colour complex. The intensity of the colour is proportional to the amount of iron present in sample solution and follows Beer's law and therefore, can be determined spectrophotometrically.

**Determination of**

The λ$_{max}$ was obtained by recording absorbance at different wavelengths of 400 to 600 nm.

**Preparation of Calibration Curve**

Calibration standards were prepared by appropriate dilution of stock iron solution. The absorbance of each 1 to 10 mg/L standard solutions were measured. A calibration curve was then obtained by plotting the absorbance as a function of concentration of iron in mg/L.

**Sample collection**

The samples (iron tablets) were collected from local drug stores of Kathmandu valley, Nepal and random sampling technique was adopted for the selection of study samples.

**Procedure for the determination of iron in iron tablets**

The samples collected from the drug stores were pulverised into fine powder. The samples were then dried. For ferrous sulphate samples namely ‘Stalferic tablet’, ‘Haema tablet’ and ‘Fecontin-Z’ tablet, appropriate amount of powder was taken in a volumetric flask and dissolved in distilled water and acidified with (0.1N) HCl. Then the volume was made up to the mark with distilled water. For ferrous fumarate sample, ‘Ferofolic tablet’, appropriate amount of powder was taken in a conical flask. About 50 mL (6N) HCl was added to it and boiled for 30 minutes. The solution was cooled to room temperature. After cooling, the solution was filtered and transferred into a 1000 mL volumetric flask. The distilled water was added up to the mark. Small amount of (about 6 mL) phosphoric acid was added to it.

For spectrophotometric technique, 50 mL of the sample solution was taken in conical flask, 1 mL hydroxylamine hydrochloride solution was added. Then the mixture sample was heated for about half an hour with glass beads in it. The sample was allowed to cool and transferred to 100 mL volumetric flask. The conical flask was rinsed several times with small portion of distilled water and each rinse was added to volumetric flask. Then 10 mL ammonium acetate buffer and 2 mL phenanthroline solution were added into it. The volume was made up to the mark and left for at least 10 minute for maximum colour development. The absorbance was then measured using spectrophotometer.

**RESULTS AND DISCUSSION**

**Determination of λ$_{max}$**

Absorbance of a calibration standard was plotted as a function of wavelengths ranges from 400 to 600 nm. The curve was shown in the (Fig. 1). As can be seen in the curve, λ$_{max}$ was obtained at 510 nm of wavelength. The 510 nm of wavelength was then chosen for entire work.
Calibration curve for the determination of iron
Absorbance of series of standard iron solutions (0.2, 0.4, ……..1.6 mg/L) were obtained at 510 nm wavelength and calibration curve was plotted by taking absorbance as a function of concentration of iron solution (mg/L) (Fig. 2).

As can be seen in Fig. 2, curve is found to be linear from the concentration range of 0.2 mg/L to 1.6 mg/L obeying Beer’s law. This calibration curve was utilized for the quantification of iron.

Determination of Iron (II) in iron tablets
Total amount of iron found in iron tablets were tabulated in (Table 1).

<table>
<thead>
<tr>
<th>S. No</th>
<th>Iron Tablets</th>
<th>Iron conc (mg per 60 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Fecontin-Z</td>
<td>50.60</td>
</tr>
<tr>
<td>B</td>
<td>Haema</td>
<td>50.60</td>
</tr>
<tr>
<td>C</td>
<td>Ferrofolic</td>
<td>52.40</td>
</tr>
<tr>
<td>D</td>
<td>Stalferic</td>
<td>54.00</td>
</tr>
</tbody>
</table>

The results revealed that the iron content in iron samples namely ‘Stalferic tablet’, ‘Haema tablet’, ‘Fecontin-Z tablet’ and Ferrofolic tablet from four different pharmaceutical companies by phenanthroline spectrophotometric method were found to be 50.6, 50.6, 50.4 and 54.00 mg / 60 mg respectively.

The iron content of four pharmaceutical samples were also analysed using atomic absorption spectrophotometry (AAS), the data obtained are shown in (Table 2).

<table>
<thead>
<tr>
<th>S. No</th>
<th>Iron tablets</th>
<th>Iron conc (mg per 60 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Fecontin-Z</td>
<td>50.64</td>
</tr>
<tr>
<td>B</td>
<td>Haema</td>
<td>50.80</td>
</tr>
<tr>
<td>C</td>
<td>Ferrofolic</td>
<td>52.66</td>
</tr>
<tr>
<td>D</td>
<td>Stalferic</td>
<td>53.78</td>
</tr>
</tbody>
</table>

As can be seen from the table, the results are quite comparable with the results provided by Zest lab and Taurus Pharma and were in good agreement with pharmacopeial range of 48 to 54 mg / 60 mg tablet [8].

CONCLUSION
The iron contents in iron tablets were successfully measured by phenanthroline spectrophotometric method and atomic absorption spectroscopic (AAS) method. The iron concentration in iron tablets were found to be comparable with results provided by Zest lab and Taurus Pharma and were in good agreement with pharmacopeial range of 48 to 54 mg / 60 mg tablet.

ACKNOWLEDGEMENT
We would like to acknowledge Trichandra Multiple Campus, Tribhuvan University,
Kathmandu, Nepal, for providing laboratory facilities to carry out the research work.

REFERENCES


2. ICMR (2010), Nutrient Requirement and recommended Dietary allowances for Indians. A Report of the expert Group of the ICMR.


