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**Research Article****SPECTROPHOTOMETRIC ESTIMATION OF IRON CONTENT IN ORAL AND TOPICAL FORMULATIONS**

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**ABSTRACT**

Four raw materials, two lots of a single ingredient capsule and two lots each of four multi-ingredient capsule formulations along with two lots of topical cream were subjected to spectrophotometric estimation of iron content. Different dosage forms namely bhasma, capsules and cream were analysed for iron content by the well known spectrophotometric method. Variety of iron sources like Abhrak bhasma, Loha bhasma, Ferrous sulphate and Apple Juice Concentrate were examined for their iron content and used as a source of iron in preparation of various single and multi-ingredient capsule formulations as well as cream.

Iron content in different formulations at different concentration levels can be satisfactorily estimated by adopting easily available and comparatively cheaper spectrophotometric technique, using thiocyanate reagent. This method is proved to be reliable to determine the concentration of iron from about 0.100% in Prementrid capsules to more than 55.00% in Loha bhasma, by accurately adjusting the sample size and sample preparation with respect to the iron concentration, during the process of color development.

Iron content in all raw materials was found to be more than 100% with respect to the theoretical value. In all oral capsule formulations it was giving more than 90% results relevant to the corresponding composition. In topical category, "Viligo Derma" cream showed distinct red colored residue after igniting the sample. Iron content was found to be 1.46mg/g and 1.52mg/g in two lots of cream samples against the theoretical value 1.50mg/g.

Thiocyanate spectrophotometric method can be considered as a reliable method to determine the various concentration levels of iron in oral as well as topical formulations. Hence its application in batch to batch analysis can be recommended to supervise the quality of the product.

**KEYWORDS:** *Iron content, Spectrophotometric method, Thiocyanate, Oral, Topical Formulations.*

**INTRODUCTION**

All living things contain a variety of minerals. Overall eleven major elements and about thirty minor trace elements get incorporated into animal lives. Of these certain trace elements like copper, molybdenum, manganese, iron and cobalt have definite biochemical functions. Iron is the most plentiful trace element and is wide spread in nature. It has relatively high threshold limit of 10mg/lit. The therapeutic use of iron is known since 1500BC. Its medicinal uses have included treatment of acne, alopecia, hemorrhoids, gout, pulmonary diseases, excessive lacrimation, weakness, edema and fever. The primary therapeutic use of iron salt is in the treatment of iron deficiency anemia and is available

in nonprescription forms. Generally iron carbohydrate complexes, are used for treating iron deficiency. The oral absorption of iron is complicated, and the intestinal mucosa is the principle site for limiting the absorption of iron. Under normal circumstances the iron from food is not well absorbed in nonanemic person. Iron crosses the placenta and concentrates in the fetus. However, this concentration of iron serves as a valuable physiological purpose and prevents anemia caused by rapid growth. The excess of iron taken in the body is excreted in feces. However, small amounts may get accumulated. Some amount of iron may be excreted via bile. The excess of iron is also excreted by loss of

epithelial cells of gastrointestinal tract, which limit iron intake. The chronic excessive intake of iron can lead to hemosiderosis or hemochromatosis. Excessive dietary iron intake appears to be the cause of abnormal iron accumulation.

Iron is transported to the tissue and is utilized from iron containing compounds and stored as "Ferritin". It is stored in liver, spleen and bone marrow. At low level of iron storage, the ratio of ferritin to hemosiderin is relatively constant where ferritin predominates but with increase in iron storage, iron is stored primarily as hemosiderin. Hemosiderin is a relatively amorphous compound consisting mainly of ferric hydroxide. Iron gets accumulated at a rate of 2 to 4 mg per dm.

Iron plays a crucial role in the transport of oxygen from the lungs to the tissues, in the transport of CO<sub>2</sub> away from the tissues to the lungs, and in the process of cellular respiration. Haemoglobin, myoglobin, and the cytochromes are the iron containing proteins and enzymes mediating oxygen transport. There is evidence from animal studies<sup>1</sup> that in dietary iron deficiency the concentration of the respiratory cytochrome enzymes may drop before the haemoglobin level in the blood drops. Iron may play a role in the conversion of beta-carotene to vitamin A, the synthesis of purines, the clearance of blood lipids and the detoxification of drugs in the liver.

Iron deficiency is mostly due to inadequate intake of iron<sup>2,3</sup>. Oduro et al<sup>4</sup> have reported iron content in *M. oleifera* as 28.29mg/100g. Park and Britten<sup>5</sup> have reported iron content of a food cooked in iron utensil. Motegaonkar and Salunke<sup>6</sup> reported iron contents in carrot and tomato in Latur variety. Rane et al<sup>7</sup> reported iron content in various citrus fruit spreads.

Excessive amount of iron is toxic. Death occurs as a result of cardiac arrhythmia, congestive heart failure or hepatic failure. Acute iron toxicity can occur with ingestion of large quantities of iron supplements. Nausea, vomiting, and diarrhoea develop and in severe cases, hypotension, shock or coma are the common symptoms of iron toxicity. It is therefore essential to establish amount of iron intake through normal diet as well as additional supplements. Hence it is essential to monitor the content of iron in various Ayurvedic formulations for supervising their quality.

Various classical and instrumental techniques are available to quantify iron in different formulations. In present study spectrophotometric method is applied

where thiocyanate reagent is used to form the colored complex of iron.

Total four raw materials with various sources of iron that is two bhasmas namely Abhrak bhasma and Loha bhasma with different concentration of iron, Ferrous sulphate monohydrate and heptahydrate as well as Apple Juice Concentrate powder were taken for analysis. Various oral and topical formulations were prepared using these raw materials as one of the ingredients. For "Amla-Loha" capsule and "Formula to support recovery in Epilepsy" capsule, Loha bhasma was used in the formula. For "Antistroke" capsules Abhrak bhasma was used, whereas, in "Prementrid" capsules, Apple Juice Concentrate was used as a source of iron. However, synthetic iron in the form of monohydrate ferrous sulphate was also used to prepare "New hair and skin care" capsules and "Vitiligo Derma" cream. Two lots of each formulation including all the five raw materials that is overall 17 samples were analysed for their iron contents.

## MATERIALS AND METHODS

### Raw materials

Raw materials used for this study were procured from authorized vendors in India. Ayurvedic capsules and cream formulations under investigation were prepared in formulation laboratory of Piramal Phytocare Ltd.

### Reagents

All the chemicals and reagents used for manufacturing as well as testing purposes were of AR grade and were purchased from M/s Qualigens and M/s Merck India Ltd.

### Equipments and Instruments

All the glasswares used were well calibrated and were procured from M/s Borosil. Instruments used were weighing balance (M/s Shimadzu Corporation), hot plate, muffle furnace (M/s Pathak Electronics), UV-visible spectrophotometer (M/s Shimadzu).

### Estimation of Ash Content

Pharmacopoeial method<sup>8</sup> was applied to make ash of the samples. In all the raw materials about 1 to 2 g of uniform sample, in capsule formulations 1 to 2g of homogenous blend, and in cream about 5g of properly mixed soft mass was weighed accurately in a clean and previously weighed silica crucible. Samples were initially heated on a hot plate with gradually increasing the temperature. The fumes were allowed to cease and then the samples were subjected to ignition in a suitable muffle furnace with temperature controller. All these samples were

ignited at 450<sup>0</sup>C to 550<sup>0</sup>C for 10 to 12 hours. Content of Ash that is residue on ignition of individual samples was calculated and recorded by applying the formula:

$$\text{Ash content (Residue on ignition) \% w/w} = [\text{weight of the residue in (g) / weight of the sample in (g)}] \times 100$$

**Estimation of Iron by Spectrophotometric Method**  
**Calibration of Spectrophotometer:** Double beam spectrophotometer was calibrated using potassium dichromate solution as per the standard pharmacopoeial method<sup>9</sup>.

**Sample Preparation:** Ash of individual samples was dissolved in 40ml aqua regia and boiled gently to concentrate to 10-15ml, on a hot plate. All these digested samples were cooled to room temperature and diluted to appropriate level with distilled water. Blank preparation was carried out in the similar

manner omitting the sample. These diluted aqueous solutions were then filtered through filter paper number 1 and further appropriate dilutions were made as per the concentration level of iron for color development. Final diluted solutions of each lot that is stock solution (A) and blank solution (B) were taken in respective 50ml volumetric flasks.

**Standard Preparation:** 703 mg of Ferrous Ammonium Sulphate was accurately weighed and dissolved in 100ml distilled water and then 5ml dilute sulphuric acid was added. To this solution 0.1N Potassium permanganate was added dropwise until pink color persisted. Then the volume was made to 100ml with distilled water. This solution ( C ) was well shaken and left overnight. Next day, 1ml of this solution (corresponding to 0.1mg iron) was pipetted out in 50ml volumetric flask for color development. Proportions of Reagents added for forming iron complex is briefed as:

Sr. No.	Reagents	Blank	Standard	Sample
1	Stock solution	1ml solution B	1ml solution C	**1/2/4/5 ml solution A
2	4M Nitric Acid (25% v/v)	3ml	3ml	3ml
3	Distilled water	5ml	5ml	5ml
4	0.1N KMnO <sub>4</sub> solution. (Drop wise)	Till persistent pink color	Till persistent pink color	Till persistent pink color
5	10% w/v Ammonium thiocyanate solution	10ml	10ml	10ml
6	Distilled water	Dilute up to 50ml	Dilute up to 50ml	Dilute up to 50ml

**In the table above, \*\*indicates that the aliquot of sample solution (A) may vary as per the concentration of iron present in the sample.**

The final dilutions were made up to 50ml, mixed properly by vigorously shaking the individual flasks and the absorbance of sample and standard was recorded after 5minutes, on previously calibrated suitable double beam spectrophotometer at 480nm, against the blank solution prepared as mentioned above. Filled average weight of the capsule formulations was determined by finding out the average of the total blend powder content of 20 capsules.

The concentration of iron in percentage for raw materials, in mg/capsules for oral capsule formulations and in mg/g for topical cream formulations was calculated by applying following formula:

$$\text{Content of Iron (\% w/w)} = (\text{Sample Absorbance} / \text{Standard Absorbance}) \times (0.0001/50) \times (100/\text{weight of the sample in g}) \times \text{Dilution Factor} \times 100$$

$$\text{Content of Iron in mg/capsule} = (\% \text{ w/w}) \times (1000/100) \times \text{Average filled weight of capsules in g}$$

$$\text{Content of Iron in mg/g} = (\% \text{ w/w}) \times (1000/100)$$

## RESULTS AND DISCUSSION

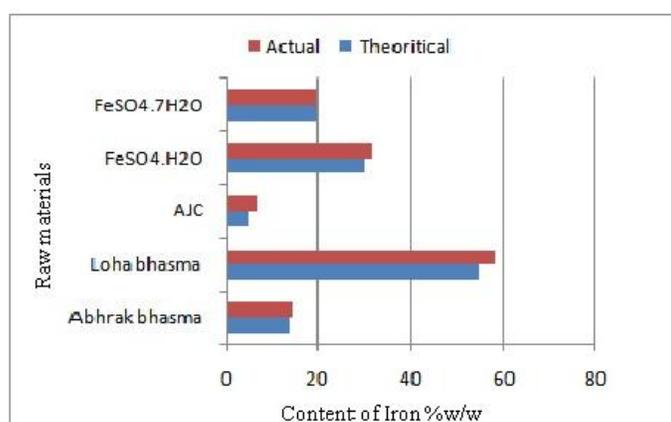
Different sources of iron were used as raw materials in preparing the oral and topical ayurvedic formulations. Table 1 shows the distinct iron content in bhasmas processed from ores that is Abhrak bhasma, Loha bhasma, natural source of iron like Apple Juice Concentrate (AJC), and synthetic form like Ferrous sulphate in two different hydrated forms that is monohydrate and heptahydrate.

**Table 1: Content of ash and Iron in Raw Materials**

Sr.No.	Raw Materials	Content of Ash in %w/w	Content of Iron in %w/w	
			Specifications	Findings
1	Abhrak bhasma	99.028	Not less than 14.000	14.860
2	Loha bhasma	99.680	Not less than 55.000	57.850
3	Apple Juice Concentrate(AJC)	13.150	Not less than 5.000	6.924
4	Ferrous sulphate monohydrate	80.877	Not less than 30.000	31.413
5	Ferrous sulphate heptahydrate	51.497	Not less than 20.000	20.010

Since bhasmas are calcinated products, the loss on ignition is minimum that is less than 1%, hence ash value of them are more than 99%, whereas in Apple Juice Concentrate (AJC) and Ferrous sulphate, ash value is quite variable as shown in table 1 because of the presence of different iron complexes in the

corresponding raw materials. Practical findings of iron content in all the raw materials are more than theoretical value. Figure 1 shows the accuracy of actual findings of Iron content in the raw materials used under study with respect to the theoretical values.

**Fig 1: Content of Iron in Raw materials used**

Hence all these raw materials can be used as a source of iron in various formulations. Raw materials mentioned in table 1 were used for preparing various capsule formulations. These capsule formulations were tested for their ash and iron content. To record

the iron content in mg/capsule unit it was necessary to know the filled average weight of the capsule. Hence all the relevant findings of two lots of each capsule formulation are recorded in Table 2

**Table 2: Evaluation of Oral Capsule Formulations with reference to Ash and Iron Content**

Sr No	Oral Capsule formulations	Filled Average Weight in mg		Content of Ash in %w/w		Source of Iron (Raw materials used per capsule)	Content of Iron in mg/capsule		
		Lot 1	Lot 2	Lot 1	Lot 2		Theoretical value	Lot 1	Lot 2
1	Amla – Loha	400.40	513.80	14.300	12.680	30mg Loha bhasma	About 16.500	15.490	16.230
2	Formula to support recovery in Epilepsy	406.65	388.76	11.170	12.038	25mg Loha bhasma	About 13.750	15.770	15.391
3	Anti Stroke	461.83	456.60	14.204	14.108	25mg Abhrak bhasma	About 3.500	3.551	3.996
4	New Hair and Skin Care	370.05	373.76	17.334	17.534	50mg FeSO <sub>4</sub> .H <sub>2</sub> O	About 15.000	17.717	15.712

5	Prementrid	478.95	469.20	24.995	29.624	7.5mg Apple Juice Concentrate	About 0.525	0.589	0.547
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Since 30mg of Loha bhasma was used as a source of iron in “Amla- Loha” single ingredient capsule formulation, theoretically the iron content should be around 16.50mg/capsule, as iron in Loha bhasma is around 55%, both the lots here show around 93% and 98% of accuracies respectively against the expected values. Similarly since 25mg of Loha bhasma was used in “Formula to support recovery in Epilepsy” capsule formulation, expected content of iron is around 13.75mg/capsule. Findings of two lots are more than 100%.

Abhrak bhasma was used as a source of iron in “Anti Stroke” capsule formulation. 25mg Abhrak bhasma with around 14% iron content is used per capsule. Practical findings of iron content in two lots are 101% and 114% respectively with reference to the theoretical values of the corresponding formulation.

In “New Hair and Skin Care” capsule 50mg of monohydrate Ferrous sulphate per capsule was used, accordingly the expected iron content is around 15 mg/capsule. Our findings in both the lots are more than 100% of actual expectation.

Natural source of iron that is “Apple Juice Concentrate” powder standardized for not less than 5% iron content was used in “Prementrid capsule”. Actual iron content in apple juice concentrate is about 7% and 7.5mg of the same is added per capsule as per the formulation details. Expected value is 0.525mg/capsule. However in both the lots it gives more than expected value for iron content.

Concentration of Iron in various oral capsule formulations is well illustrated in Fig 2

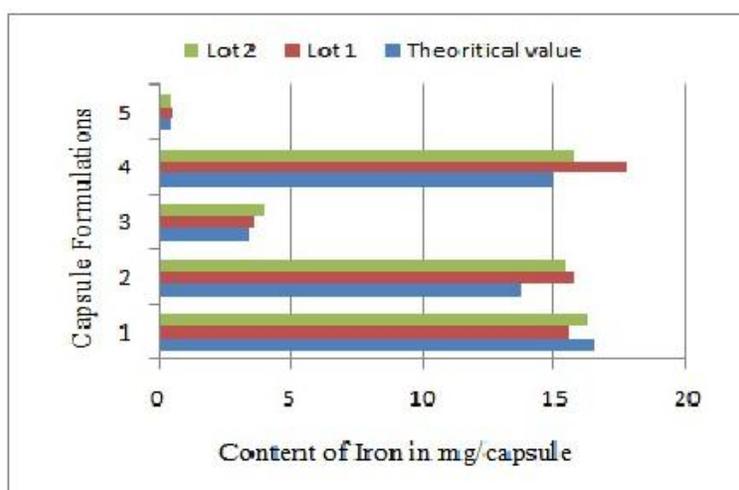


Fig 2: Content of Iron in various oral capsule formulations

Ash content of capsule formulations varies as various other active ingredients along with appropriate excipients are used in the blend during capsule preparation. Though iron content is less in topical

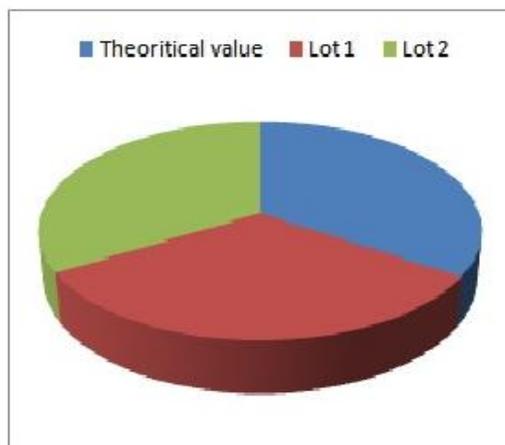
formulation like in “Vitiligo Derma Cream”, quantification is possible. Table 3 gives clear picture of the same.

Table 3: Evaluation of Topical cream formulation with reference to Ash and Iron content

Sr.No.	Topical cream formulation	Content of Ash in %w/w	Source of Iron (Raw material used)	Content of Iron in mg/g	
				Theoretical value	Actual Findings
1	Vitiligo derma cream Lot 1	0.642	0.500% Ferrous sulphate (monohydrate)	About 1.500	1.570
2	Vitiligo derma cream Lot 2	0.642			1.520

Ferrous sulphate monohydrate is used as a source of iron in blackish green colored “Viligo Derma” cream. The residue on ignition of 5g cream sample was found to be distinct red in color. The content of ash is about 0.642% w/w. As per the set up formula, 0.5% monohydrated Ferrous sulphate was used as a source of iron. Hence iron content in the cream should be around 1.5mg/g

Practical findings in two lots are 1.57mg/g and 1.52mg/g that is around 105% and 101% of the calculated values. Iron content in topical formulation can also be quantified with accuracy as shown in Figure 3



**Fig 3: Content of Iron in topical cream formulation**

Tripathi et al<sup>10</sup> reported iron contents in ground water from Satna. Vador et al<sup>11</sup> have given various spectrophotometric methods for standardizing ayurvedic formulation.

### CONCLUSION

All the results of iron content are found to be accurate. Thus the proposed thiocyanate spectrophotometric method can be successfully applied for estimation of iron from 0.1% to more than 50% concentration level. It gives reliable results not only for oral but also for topical formulations. Hence its application for batch to batch evaluation can be recommended to control and supervise the quality of the product.

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