Spectrophotometric Estimation of Iron (III) in Iron Polysaccharide Complex Capsule Formulation and its Accelerated Stability

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Summary: Iron plays a pivotal role in human physiology, while its deficiency may prove fatal in severe cases. Analytical methods for the quantitative determination of iron are thus very important. Herein, we report the estimation of iron in iron Polysaccharide complex (IPSC) using raw material and formulations, through a spectrophotometric analytical method. IPSC capsules were formulated and their stability was studied by developing a simple and validated analytical method. The process is based on the acid hydrolysis of IPSC and development of chromogen by the reaction of ammonium thiocyanate with IPSC. The chromogen was detected at 474nm. Beer's Lambert law (linearity response) was observed in the range of 10-20 µg/ml with excellent correlation coefficient of determination (R = 0.998). The quantification and detection limits were established to be 0.45 mcg/ml and 0.14 mcg/ml correspondingly. The recovery of IPSC analysis was 99.25 to 102.28 %. Percentage assay of IPSC capsules showed results around 102.34 %. The formulated IPSC capsule was stable under accelerated conditions for 6 months (% assay > 91.69). The dissolution profile over 60 minutes showed a better dissolution (94%) compared with the internationally marketed IPSC capsule (92%).

Key words: Iron polysaccharide complex, Method development, UV spectrophotometer, Estimation, Elemental iron and formulation.

Introduction

Iron in mineral form is involved in several important physiological functions, especially the transport of oxygen in the blood and other energy providing processes [1-2]. It is a cofactor of many important enzymes, like cytochromes which are concerned in transport of electron [3], necessary for the function and development of red blood cells [4-5]. According to WHO, around 2 billion people and 25% of worldwide is affected by iron deficiency [6-7], especially women's are at high risk during pregnancy [8-9]. Iron insufficiency causes an increase in morbidity and mortality rate [10]. The symptoms of anemia consist of headache, irritability, fatigue, and complexity in focusing. The fear related with moderate anemia is breath shortness and fragile nails. In superior iron deficiency anemia, the body becomes ravenous for oxygen. The major severe risk includes failure of organs, unbalanced heartbeat and ultimately heart failure. A varied and nutritious diet is needed to prevent anemia. Strengthening of suitable food vehicles with absorbable forms of iron is an extremely desirable tactic to control iron insufficiency [11].

Several iron supplements, available in the market, are used to prevent and treat iron deficiency [12-13].

However, iron is determined quantitatively by titration but the process is not very accurate when applied to pharmaceutical dosage forms like tablet, capsule and syrup. To our knowledge, there is no pharmacopoeial spectroscopic method or official method of analysis reported for iron (III) estimation from IPSC [14-15].

In this study, we formulated capsule dosage form formulation, performed accelerated stability studies and finally validated the simple, visible spectrophotometric method to determine the iron (III) from IPSC, dosage forms and validation done according to the guidelines of International Conference on Harmonization (ICH) [16-17].

Experimental

Chemicals and Reagents

Inactive pharmaceutical ingredients in the capsule formulation; Lactose anhydrous (diluents), microcrystalline cellulose (filler), magnesium stearate (lubricant), sodium starch glycolate (disintegrent), H.G capsule shells (brown color) were used and other facilities provided by Fozan pharmaceuticals Peshawar, Pakistan.

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Mixing was carried out in stainless steel cone mixer. The capsules were filled by a semi-automatic capsule machine. Blistering process was carried by alu-alu blister machine.

Agilent 8453 UV/visible spectrophotometer having quartz cells of 10 mm was used for checking the absorbance. Stability testing was carried out on a stability chamber (GKY-YQ, SERIES, and Elitetechnology Lahore, Pakistan). Weighing scale (Sartorius Germany) Analytical balance was used. Water bath (Germany memmerts) for heating purpose. Whatman 41 filter paper (Whatman, Maidstone, England) was used for filtration.

All reagents and chemicals, i.e., hydrochloric acid (Merck, Germany), and ammonium thiocyanate (Merck, Germany) were of analytical grade.

The active pharmaceutical ingredient in the capsule formulation, standard iron polysaccharide complex was purchased (39.65 % elemental iron, B# H14-IPSC-010) from chemiworld (pvt) ltd Peshawar Pakistan.

Formulation Development

Three different formulae of IPSC 150 mg capsules were prepared. The components used are IPSC, Sodium starch glycolate, lactose anhydrous, microcrystalline cellulose (MCC), and magnesium stearate. The detailed compositions of the three formulations are listed in Table-1.

Steps of mixing

Raw materials were dispensed following the standard manufacturing protocol. The temperature was maintained at 24 ^{o}C and humidity was kept under 40% IPSC was sieved from mesh no. 14 in stain less steel container. Lactose, sodium starch glycolate, and microcrystalline cellulose were sieved from mesh no. 12 and mixed. Magnesium Stearate was sieved from mesh no 60 and mixed with the above materials. The mixture was transferred to a clean cone mixer, where it was mixed for 30 minutes. After analysis, formulated powder was filled in the capsules using a semi-automatic capsule filling machine. Upon completion of filling and analysis, capsules are blistered and packed.

Dissolution of formulated IPSC capsule

Dissolution was done according to USP and ICH guidelines, using USP apparatus 2 (paddle). The dissolution test was performed in distilled water. The dissolution apparatus was run at 100 Revolution per Minute and 37 ^{0}C for 60 minutes. One capsule was placed into each of the six dissolution vessels containing 900 ml of dissolution medium. 10 ml of the sample was withdrawn by syringes from each dissolution vessel after 60 minutes. The average reading of triplicate measurements was taken to calculate the percentage of dissolved iron (III) using the following formula:

$$\% \text{ of dissolved IPSC} = \frac{\text{Actual amount of released IPSC}}{\text{Theoretical amount of IPSC in capsule}} \times 100$$

Specification of the formulated capsules

The weight variation was done by weighing 20 capsules individually and the test was considered successful when it met the requirements set by the official pharmacopeia.

The disintegration time of the formulated capsule was performed according to USP. Disintegration time was recorded when all the capsules had completely disintegrated.

Capsule physical specifications like the thickness and diameter were determined and tested. The test was done on ten capsules; the average reading was assigned as the capsule thickness, and diameter specifications of the formulated capsules, as shown in Table-2.

Table-1: Compositions of three formulated capsules.

Component	Formula (f1) Quantity/capsule	Formula (f2) Quantity/capsule	Formula (f3) Quantity/capsule	Function
Iron polysaccharide complex (ipsc)	375.00 mg (81.52%)	375.00 mg (81.52%)	375.00 mg (81.52%)	Active
Lactose anhydrous	53.00 mg (11.52%)	55.00 mg (11.95%)	57.00 mg (12.39%)	Diluents
Microcrystalline cellulose (mcc)	14.00 mg (3.04%)	12.00 mg (2.60%)	14.00 mg (3.04%)	Filler
Magnesium stearate	2.00 mg (0.43%)	1.00 mg (0.21%)	2.00 mg (0.43%)	Lubricant
Sodium starch glycolate	16.00 mg (3.47%)	17.00 mg (3.69%)	12.00 mg (2.60%)	Disintigrant
H.g capsule shells size 01 # (brown)	80 mg	80 mg	80 mg	Shell
Weight of filled capsule		540 mg/filled capsule		

Table-2: Specification of the formulated capsules.

S #	Tests	Specification
1.	Description	Brown color capsule shells size # 1, unprinted, having dark brown color granular powder.
2.	Identification	Gives Absorbance maxima at λ = 474 nm
3.	Average Content weight	460 mg +7.5 %
4.	Weight Variation	±7.5 %
5.	Loss on drying	NMT 5.0 %
6.	Disintegration Test	NMT 30 minutes
7.	Dissolution	NLT 90 % to NMT 110% NLT 80%
8.	Assay (Iron polysaccharide complex)	90 – 110 %

Table-3: Accelerated stability data of formulated capsule, F– 01 (40 °C + 2°C & 75 % + 5%. RH).

S.No	Tests	Specification	Observation Value				
			0 Month	1 Month	2 Month	4 Month	6 Month
1	Appearance	Dark brown color powder filled in 1 size shells	Dark brown color powder filled in 1 size shells	Dark brown color powder filled in 1 size shells	Dark brown color powder filled in 1 size shells	Dark brown color powder filled in 1 size shells	Dark brown color powder filled in 1 size shells
2	Disintegration Time	NMT 30 mins	8 mins	8 mins	9 mins	9.5 mins	10 mins
3	Dissolution	NLT 75%	94.8%	94.5%	94.1%	94%	93.5%
4	Assay of IPSC	90% to 110%	102.3%	102.1	101.5	101.2%	101.1%

Accelerated Stability of formulated IPSC capsule

This part of the study was conducted in accordance with International Commission for Harmonization guidelines where, these capsule of selected formulation (F-1) were placed under accelerated conditions of relative humidity (75±5%) and temperature (40±2°C) in a stability chamber (GKY-YQ, SERIES, Elite technology Lahore, Pakistan) for 6 months' duration. Samples were tested for various required parameters that included appearance and assay at 0 times (pre-storage) and after 1, 2, 4, and 6 months by using the developed analytical test method. The percentage content of formulated IPSC capsules was calculated periodically as shown in Table-3.

Analytical Method Development

Each filled capsule contains Iron poly saccharide complex equivalent to 150 mg Iron (III).

Standard stock solution

45 mg of pure powdered IPSC was taken in a volumetric flask of 100 ml. 10 ml of conc hydrochloric acid were added to it and put in a water bath (70 ^{0}C) till pale yellow color appeared. The solution was cooled to 35 ^{0}C and the volume was marked up with 0.1 M hydrochloric acid.

Sample preparation

Twenty capsules from formulation F-1 were accurately weighed. The contents of capsules were removed as completely. Amount equivalent to 45 mg of pure powdered IPSC was transferred into 100 ml volumetric flask with addition of 10 ml of conc. hydrochloric acid to it and put in a water bath (70 ^{0}C) till pale yellow color appeared. The solution was cooled down to 35 ^{0}C and the volume was marked up to 100 ml with 0.1 M hydrochloric acid.

Reaction of iron and ammonium thiocyanate (Chromogen formation)

In a 50 ml volumetric flask 2 ml filtrate stock solution of standard was added to 2 ml 10 % (w/v) solution of ammonium thiocyanate. A red color chromogen was formed similar to sample. Volume was marked up with 0.1 M hydrochloric acid and shaken well.

Absorbance was measured at 474 nm. The percentages of IPSC were measured.

Blank preparation

2 ml of the 10 % ammonium thiocyanate was mixed with 48 ml of 0.1 M HCl in 50 ml volumetric flask to prepare the blank.

Calibration curve

In the range of 10-20 µg/ml IPSC calibration curve was plotted. Precisely measured standard solution of IPSC (10, 12, 14, 16, 18, and 20 µg/ml) was checked at 474 nm. Calibration curve data is shown in Table-4.

Table-4: Calibration data of IPSC.

S.No	Concentration IPSC(µg/ ml)	Mean Absorbance
1	10	0.439
2	12	0.529
3	14	0.616
4	16	0.704
5	18	0.792
6	20	0.881
7	Slope	0.04407
8	Intercept	-0.000904
9	Covariance	0.617
10	R^2	0.998

Results and Discussion

Precision

The method precision was determined by intermediate precision inter-day repeatability intraday. In intra-day, six samples of 18 µg/ml concentration of IPSC were checked in a day. For variation in inter-day studies, each set of 6 replicates of same concentrations was performed on two different days and RSD (%) was determined as shown in Table-5.

Table-5: Results of precision.

S. No.	Absorbance Intra-day	Absorbance Inter-day	Assay % Intra-day	Assay % Inter-day
1	0.797	0.79269	101.7	99.3
2	0.793	0.79368	101.3	98.2
3	0.792	0.79299	101.2	99.0
4	0.793	0.79473	101.7	99.0
5	0.792	0.79182	100.5	98.5
6	0.795	0.79024	101.2	100.4
Mean	0.7931	0.79269	101.26	99.06
SD	0.00196638	0.001548	0.441	0.76
% RSD	0.25%	0.20%	0.44	0.77

Accuracy (Recovery studies)

The accuracy of the developed method for the quantification of the IPSC in presence of different excipients were carried out by utilizing standard addition method (placebo).

Known amount of standard IPSC at 3 levels (80 %, 100 % and 120 %) were added to pre placebo and the obtained results were used to find % recovery are presented in Table 6.

Table-6: Results of Accuracy.

Concentration (µg ml-1)	IPSC added (mg)	IPSC Recovered (mg)	% Recovered	Mean % recovery	S.D (±)	% RSD (n=3)
14	35.5	35.22	99.21	98.99%	0.188	0.19%
		35.10	98.87			
		35.11	98.90			
16	40.5	40.13	99.08	99.58%	0.437	0.44%
		40.45	99.87			
		40.42	99.80			
18	45.5	45.10	99.12	99.45%	0.33	0.33%
		45.40	99.78			
		45.25	99.45			

Linearity and Range

The concentration ranges from 10-20 µg/ml of the sample solutions were prepared. The absorbance was measured at 474 nm in triplicate. The response of the IPSC was found linear in the investigation range, intercept y = 0.0003 with slope 0.043983 and RSD 0.130%. The absorbance is given in Table-7.

Table-7: Data of calibration curve.

S.no	Concentration µg/ml	Absorbance
1	10	0.440
		0.441
		0.445
2	12	0.528
		0.529
		0.524
3	14	0.616
		0.615
		0.612
4	16	0.704
		0.705
		0.701
5	18	0.792
		0.795
		0.794
6	20	0.879
		0.875
		0.874
7	% RSD	0.130
8	Coefficient of variant	0.337
9	Slope	0.043
10	r2	0.998
11	Intercept	0.0003

Robustness

Robustness was determined by analyzing IPSC by different analyst on altered days. The analysis showed % RSD less than 2 which indicates that the method established is robust. Table-8 shows the results of Robustness.

Table-8: Results of Robustness.

Parameter	λ max 1	λ max 2
Mean	0.883	0.890
SD	0.0026	0.0045
% RSD (n=6)	0.30%	0.57%

Ruggedness

Suggested % RSD is less than 2 by changing wave length +/- 2 nm and indicates that the method developed is rugged. The results obtained are shown in Table-9.

Table-9: Results of Ruggedness.

Parameter	Instrument-1	Instrument-2	Analyst -1	Analyst -2
Mean abs	0.883	0.890	0.79299	0.797
SD	0.00208	0.0045	0.002517	0.0026
% RSD (n=6)	0.24	0.51	0.051	0.33

Limit of Quantification (LOQ) and Limit of detection (LOD)

Limit of Quantification and Limit of Detection were calculated using the following formula.

LOQ = 10 x (SD) / S and LOD = 3.3 x (SD) / S, where SD is standard deviation and S is slope of the calibration curve. The results are shown in Table 10.

Table-10: Limit of Detection (LOD) and Limit of Quantification (LOQ).

Parameter	Results
Limit of Detection (LOD)	0.14
Limit of Quantification (LOQ)	0.45

The results clearly demonstrate that our formulated capsules comply with weight variation and the assay according to USP, weight variation test will pass only if not more than (NMT) 2 of the single weights deviate from ±7.5% and not any single deviates from double the percentage. Our results show that the variation for any of the tested capsules was not more than 2.5% from the mean weight. The IPSC dissolution profile of the formulated capsule showed a slight dissolution improvement over the marketed IPSC capsule. However, the result of similarity factor (f2) was >50 and the dissolution data revealed that there was no statistical difference (> 0.05) between the formulated capsule and the marketed one.

The developed analytical method showed good linearity, accuracy, precision, and specificity. This method recommends a simple, validated analytical test for pharmaceuticals; herbal and food supplement manufacturers to use in quality control of their products.

IPSC yields a distinctive curve in the scanned range between 400 and 800. The method shows maximum absorption at 474 nm of the red colored chromogen.

Correlation coefficient was found to be 0.998, signifying that a direct relation occurred between concentration and absorbance of the iron. Beer Lamberts law was obeyed in the concentration range 10-20 µg/ml. The precision was conceded out by known amounts of IPSC that were exposed to recovery readings in triplicate and estimated by means of the relative standard deviation (RSD) of the results, the lower R.S.D. of the outcomes make the process extra precise. Estimation of IPSC was done with various excipients like lactose anhydrous (diluents), microcrystalline cellulose (filler), magnesium stearate (lubricant), and sodium starch glycolate. No major change occurs between the added amount and recovered amount in the placebo. Thus, excipients have shown no interference in the estimation of iron (III). All results carried out for the validation are in complete agreement with the required limits and criteria. Therefore, the method is validated and suitable for its intended purpose i.e. quantification of active ingredients in the given product.

The spectrum shows absorption maxima, at 474 nm, adapted in our analytical method to avoid any absorption from the excipients. To examine absorptivity of the excipients at 474 nm, all the expected excipients which were included in formulation were dissolved in the diluents and their absorbance was measured. The results show that the absorbance at the selected λ-max is negligible relative to IPSC absorption at 474 nm. The result clearly demonstrates that there is no interaction between the excipients and IPSC active ingredient and the wavelength is selective for IPSC. Dissolution studies were carried out to compare *in vitro* dissolution profiles of formulated IPSC capsules. The results clearly demonstrate that formulation F1 has the best dissolution among three formulations. The disintegration of the capsule was performed using USP specified disintegration apparatus and all the capsules were found to disintegrate completely after 8 minutes. Stability studies were carried out for 180 days at 40 ^{0}C, revealing that the product was stable enough for the whole period.

Conclusion

A capsule formulation was developed with 150 mg of the IPSC. To conclude, a simple validated method was developed for the analysis and quantification of iron in IPSC. The product was tested with all necessary parameters, the results of which suggested the accuracy, precision and reliability of the developed method and the stability and efficacy of the formulated capsules. The dissolution profile of the formulated capsule was found to be better than the marketed IPSC capsule. The capsules were found to be stable enough during the testing period. The developed method has the advantage of analytical validation parameters such as, precision, intermediate precision, linearity, accuracy, recovery, sensitivity and system suitability. The response of the IPSC was established to be linear in the investigation (10-20 µg/ml) range. The %RSD value in accuracy studies was found to be less than 2% indicating that the method is more accurate. This is the first attempt to analyze IPSC through spectrophotometer. Thus the developed method would help the pharmaceutical industry for routine analysis of the raw material and formulated products.

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References

1. M. Sanklecha, Iron polysaccharide complex, Indian *Pediatr.*, **39**, 893 (2002).
2. J. Zheng, X. Yue, Z. Dai, Y. Wang, S. Liu and X. Yan, Novel iron–polysaccharide multilayered microcapsules for controlled insulin release, *Acta Biomater.*, **5**, 1499 (2009).
3. L. Ortega, J. M. Ladero, M. P. Carreras, T. Alvarez, C. Taxonera, M. P. Oliván, J. Sanz-Esponera, A computer-assisted morphometric quantitative analysis of iron overload in liver biopsies. A comparison with histological and biochemical methods, *Pathol. Res. Pract.*, **201**, 673 (2005).
4. J. Cui, Y. Li, P. Yu, Q. Zhan, J. Wang, Y. Chi and P. Wang, A novel low molecular weight Enteromorpha polysaccharide-iron (III) complex and its effect on rats with iron deficiency anemia (IDA), *Int. J. Biol. Macromol.*, **108**, 412 (2018).
5. P. Santiago, Ferrous versus ferric oral iron formulations for the treatment of iron deficiency a clinical overview, *Sci World J.*, **1**, 2012 (2012).
6. R. Gangopadhyay, M. Karoshi and L. Keith, Anemia and pregnancy: a link to maternal chronic diseases. *Int J Gynaecol Obstet.*, **115**, S11 (2011).
7. S. Taghavi, A. Amiri, A. Amin, A. Ehsani, M. Maleki and N. Naderi,Oral iron therapy with polysaccharide-iron complex may be useful in increasing the ferritin level for a short time in patients with dilated cardiomyopathy, *Res Cardiovasc Med.*, **6**, 9 (2017).
8. A. Krafft, R. Huch and C. Breymann, Impact of parturition on iron status in nonanaemic iron deficiency, *Eur. J. Clin. Invest.* **33,** 919 (2003).
9. J. P. Peña-Rosas and F. E. Viteri, Effects and safety of preventive oral iron or iron+ folic acid supplementation for women during pregnancy, *Cochrane Database Syst Rev.*, **4,** 4736 (2009).
10. A. R. Nissenson, L. T. Goodnough and R. W. Dubois, Anemia: not just an innocent bystander, *Arch Intern Med*, **163**, 1400 (2003).
11. Bereman RD, Berg KA. The structure, size and solution chemistry of a polysaccharide x (Niferex), *Inorganica Chim Acta.*, **155**,183 (1989).
12. K. Wang, Y. Zhang and L. Dai, Preliminary studies on physicochemical properties of Angelica sinensis polysaccharide-iron complex,*China j of Chinese materia medica.*, **31**, 747 (2006).
13. S. Hercberg, P. Preziosi, S. Briançon, P. Galan, et al, A primary prevention trial using nutritional doses of antioxidant vitamins and minerals in cardiovascular diseases and cancers in a general population the SU VI MAX study design methods and participant characteristics, *Contol Clin Trials.*, **19**, 336 (1998).
14. W. Liu, B. Etschmann, J. Brugger, L. Spiccia, G. Foran and B. McInnes, UV–Vis spectrophotometric and XAFS studies of ferric chloride complexes in hyper-saline LiCl solutions at 25–90 C, *Chem. Geol*. **231**, 326 (2006).
15. J. M. Powers, G. R. Buchanan, L. Adix, S. Zhang, A. Gao and T. L. McCavit, Effect of low-dose ferrous sulfate vs iron polysaccharide complex on hemoglobin concentration in young children with nutritional iron-deficiency anemia a randomized clinical trial, *JAMA.*, **317,** 2297 (2017).
16. I. H. T. Guidline, International conference on harmonization, Geneva, Switzerland, (2005).
17. USP 25-NF 20, United States Pharmacopeial Convention, Rockville, MD, (2002), 2256.