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Research Article

Quantification of Iron Fe (II) in Formulations of Alternative System of Medicine

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Abstract

Present study involves the quantification of iron for three ancient kinds of formulations where iron found in the following contents: 1) Loha Bhasma, an ayurvedic drug, 2) Ferrous phosphoricum, a homeopathic drug and 3) Ferrum Phosphate, a biochemic salt. All of three are widely used in iron deficiency anemia, this ancient drug are claimed to be better absorbed gastro intestinally, and are also claimed to be devoid of the usual side effects associated with administration of the allopathic iron preparations, quantification for any quality preparation, is not found in the ancient and the modern literatures. Thus, an approach has been made to quantify iron in all the three kinds of formulations. A simple spectrophotometric method has been used for quantification of iron content.

Keywords: Anemia; Ayurvedic Formulations; Homeopathic Formulations; Biochemic Tissue Salts; Iron determination

Introduction

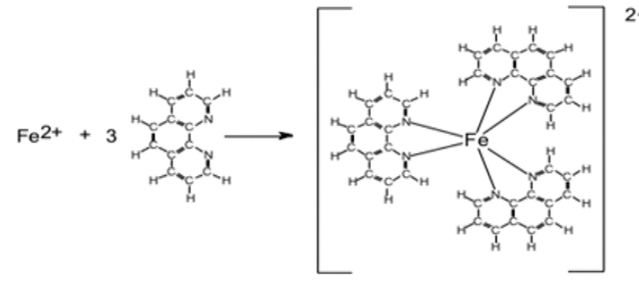
The iron deficiency anemia is the most common type of anemia [1-3]. The body is normally in a state of positive iron balance. When a negative iron balance occurs either due to blood loss, increased requirement, or impaired absorption; the deficit is made good by the iron mobilized from the tissue stores, thus an adequate for supply of iron for haemoglobin formation is maintained. However, when the tissue stores are exhausted, the supply of iron to the bone marrow for haemoglobin synthesis becomes in-adequate and hypochromic anaemia develops (Penington, 1984). Regarding the comparative efficacy of oral and parenteral iron preparation, McCurdy found the parenteral preparations except in case of defective iron absorption as in gastrectomy and in cases where iron salts may be irritating as in peptic ulcer, ulcerative colitis (Block, 1974) [1].

In the ancient system of medication, it is claimed that the oral iron preparation is better absorbed from gastrointestinal tract and is devoid of the side effects, generally associated with oral/parenteral iron preparations or the allopathic system of medication. Due to this claim, it would be interesting to quantify some of ayurvedic, homeopathic and biochemic preparations [4] which contain iron.

A rapid, accurate method for the determination of iron using hydroxylamine hydrochloride, sodium acetate buffer, and 1, 10-phenanthroline is carried out. No interference from pyrophosphates, copper and nickel was observed. The reagents used in this iron determination are stable and need not be refrigerated or stored in the dark. The order of addition of the sodium acetate buffer was not found to affect the intensity of the color produced [5-7].

Experimental Section

Principle [5,7]: Reaction of Ferrous and Ferric Iron with 1, 10-Phenanthroline. Ferrous Monophenanthroline Complex and the Colorimetric Determination of Phenanthroline.



- Iron can be quantitatively determined using o-Phenanthroline reagent.
- Fe (II) in presence of *o*- phenanthroline forms a coloured complex cation $[Fe\ (C_{18}H_8N_2)_3]^{+2}$ in faintly acidic solution.
- The coloration varies as per the concentration of iron present in the sample.
- Iron (III) has no effect and is reduced to bivalent state using hydroxylamine hydrochloride reagent.

$$2Fe^{3+}_{(aq)} + 2NH_2OH_{(aq)} + 2OH-_{(aq)}\ 2Fe^{2+}_{(aq)} + N_{2(g)} + 4H_2O_{(l)}$$

Instrument

UV-VIS spectrophotometer, UV-1700 pharmaspec model, from Shimadzu, Japan. This spectrophotometer is adequate and equipped with quartz cuvettes having the optical path of 10 mm.

Materials and Reagents

1. Concentrated hydrochloric acid
2. Hydroxylamine hydrochloride solution: (10% w/v) Dissolve 10g H_2 N OH. HCl in water and dilute to 100 ml.
3. Acetate buffer solution: Dissolve 8.3g of anhydrous NaOAC (previously dried at 100˚C) in water, add 12 ml of glacial acetic acid and dilute to 100 ml.
4. Orthophenanthroline solution: (0.1% w/v) Dissolve 0.1g of O-Phenanthroline in 80 ml of water at 80˚C, cool and dilute to 100 ml with water. Keep in cool and dark place.
5. Formulations used for analysis were purchased from local market.

Preparation of Standard stock and working standard solution [7]

Iron standard stock solution: (0.01 mg Fe/ml) Dissolve 0.3512g Fe $(NH_4)_2$ $(SO_4)_2$.$6H_2O$ in water, add 2 drops of conc. HCl and dilute to 100 ml. Dilute 5 ml of above solution to 250 ml.(10µg/ml)

Working standard solution: Withdraw 0.1 ml, 0.2 ml, 0.4 ml, 0.8 ml, 1.6 ml, 3.2 ml and 6.4 ml from the above solution in 25 ml volumetric flasks.

Preparation of sample solution

Ayurvedic formulations

1) Loha bhasma (from Hakim and from Baidyanath): Take 100 mg and dilute up to 100 ml with water. Withdraw 1 ml from the above solution in 25 ml volumetric flasks.
2) Hemeto malt syrup: Take 1g and dilute up to 100 ml with water. Withdraw 4 ml from the above solution in 25 ml volumetric flask.
3) Tablets: Triturate 25 tablets and make fine powder. Weigh powder exactly as per the weight of one tablet and dilute up to 100 ml with water. Withdraw 1 ml from the above solution in 25 ml volumetric flasks.
4) Capsule: Triturate powder from 25 capsules and weigh powder exactly as per the weight of one capsule's powder and dilute up to 100 ml with water. Withdraw 1 ml from the above solution in 25 ml volumetric flask.

Homeopathic formulations

20 tablets of each Homeopathic Iron containing formulations were triturated and powdered. Equivalent weight of one tablet was taken in separate 25 ml volumetric flask. The powder is dissolved in 10ml distilled water and sonicated for 2 minutes and filtered.

Biochemic formulations

20 tablets of each biochemic formulations were triturated and powdered. Equivalent weight of one tablet was taken in separate 25 ml volumetric flask. The powder is dissolved in 10ml distilled water and sonicated for 2 minutes and filtered.

Selection of Analytical wavelength

Working standard solutions were scanned separately in the range of 400 - 800 nm. Maximum absorbance obtained at 510 nm. (Figure 1)

Procedure [7]

To 25 ml volumetric flasks, appropriate aliquot of working standard for calibration curve ranging from (0.04 - 2.56 µg/ml) and sample solutions were taken and 0.2 ml conc. HCl, 1 ml Hydroxylamine HCl were added. After 5 minutes, 5 ml Acetate buffer and 1 ml of 1, 10-phenanthroline solution were added. Volume was made with distilled water and measure absorbance at l_{max} i.e. 510 nm.

A blank solution was prepared by replacing working standard with 10ml distilled water and remaining reagents were added as per above procedure.

Recovery studies were performed by preparing a laboratory mixture of the homeopathic and biochemic salts with blank matrix [8,9].

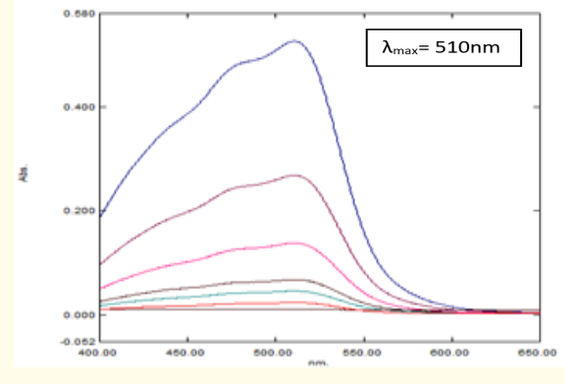


Figure 1: Overlay spectra of Calibration curve.

Result and Discussion

Validation parameters [10]

Linearity

The calibration curve was constructed with concentrations ranging from 0.04 - 2.56 µg/ml for Iron. The absorbance of Iron against Concentration was considered for plotting the graph (Figure 2). The linearity was evaluated by linear regression analysis which was calculated by the least square regression method.

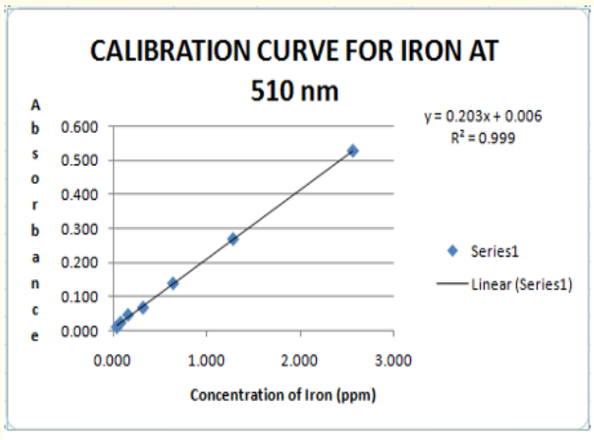


Figure 2: Calibration curve for Iron.

Precision

Conc. of Iron (µg/ml)	Intraday precision		Inter day precision	
	Abs. (mean ± SD) n=3	% RSD	Abs. (mean ± SD) n=3	% RSD
0.04	0.01074 ± 1.5×10^{-4}	1.35	0.01065 ± 8×10^{-5}	0.745
0.32	0.06779 ± 1.7×10^{-4}	0.243	0.06766 ± 6×10^{-5}	0.093
2.56	0.52694 ± 6.4×10^{-4}	0.121	0.52708 ± 5.8×10^{-4}	0.109

Table 1: Precision studies of Iron.

LOD and LOQ

From the standard deviation (SD) of response and slope curve, it was possible to calculate the detection and quantification limits. The LOD and LOQ for both drugs were shown in Table.

These low values indicated the good sensitivity of the methods proposed.

LOD =3.3 σ/S

LOQ =10 σ/S

Where 'σ 'is the standard deviation of intercepts and 'S' is the slope of response

Iron	Intercept (mean ± SD), n=3	Slope (mean ± SD), n = 3	LOD (µg/ml)	LOQ (µg/ml)
	0.0066 ± 1.0×10^{-4}	0.2035 ± 1.5×10^{-4}	0.00162	0.00492

Table 2: LOD and LOQ for Iron determination.

Summary of validation parameters:

Parameters	Iron by O-phenanthroline
λ_{max}, nm	510 nm
Calibration range, µg/ml	0.04 - 2.56 µg/ml
Regression Equation (y = bx + c)	y = 0.2035x + 0.0066
Slope (b ± SD)	0.2035 ± 1.5×10^{-4}
Intercept (c ± SD)	0.0066 ± 1.0×10^{-4}
Regression Coefficient (r^2)	0.9996
Intraday precision (%RSD)	0.121-1.35
Inter day precision (%RSD)	0.093-0.745
LOD, µg/ml	0.00162
LOQ, µg/ml	0.00492

Table 3: Summary.

Accuracy Studies

Recovery studies were carried out from pre-analyzed sample at three different level of standard addition 80%, 100% and 120% in prepared laboratory mixture.

Homeopathic Formulations

Sr. No	Name of Sample	Mean % Recovery	% RSD
1	Ferrum Phosphoricum 30x-SBL	100.87 - 102.81	1.52
2	Fe-min Tablets-SBL	100.47 - 101.81	0.53
3	MIG AID Tablets-Bakson's Homoeopathy	100.53 - 101.87	0.94
4	ALLER AID Tablets-Bakson's Homoeopathy	100.915-102.01	0.53
5	THROAT AID Tablets-Bakson's Homoeopathy	100.71 - 102.07	0.75
6	FIVE PHOS-SBL	99.11 - 100.07	0.41

Table 4: Accuracy Results of Homeopathic Formulations.

Biochemic Formulations

Sr. No	Name of Sample	% Spiking	Amt. of Fe Std added (ppm)	Avg. Amt. of Iron recovered (ppm)	Avg % Recovery ± SD	% RSD
1	Biochemic 1	80	0.355	0.365	102.81 ± 0.344	0.335
		100	0.44	0.451	101.41 ± 1.545	1.523
		120	0.533	0.538	100.87 ± 1.861	1.845
2	Biochemic 15	80	0.362	0.364	100.47 ± 0.720	0.717
		100	0.453	0.456	100.57 ± 0.233	0.231
		120	0.544	0.554	101.81 ± 0.250	0.245
3	Biochemic 16	80	0.362	0.364	100.53 ± 0.889	0.883
		100	0.452	0.461	101.87 ± 0.441	0.433
		120	0.543	0.552	101.63 ± 1.163	1.145
4	Biochemic 24	80	0.363	0.366	100.915 ± 0.139	0.137
		100	0.454	0.463	102.01 ± 0.133	0.131
		120	0.545	0.550	101.07 ± 0.852	0.843
5	Biochemic 28	80	0.360	0.363	100.71 ± 1.081	1.074
		100	0.459	0.460	101.97 ± 0.054	0.043
		120	0.540	0.552	102.07 ± 0.510	0.500

Table 5: Accuracy Results of Biochemic Formulations.

Assay results

The method was applied to the determination of Iron in formulations. Each product was prepared by procedure given in sample preparation and analyzed in six replications (n = 6).

Ayurvedic formulations

SR. NO.	NAME OF FORMULATION	TYPE OF FORMULATION	LABEL CLAIM	AVERAGE CONTENT OF IRON (in mg) ± SD, n = 6	%RSD
1	Loha bhasma (From Hakim)	Bhasma	Not available	2.35 mg/g ± 2.514	1.070 %
2	Loha bhasma (Baidyanath)	Bhasma	Not available	1.48mg/g ± 1.006	0.679 %
3	Hemeto malt	Syrup	Loha bhasma: 10 mg/10 g	0.25 mg/10 g ± 0.377	1.521 %
4	Hemojay	Tablet	Loha bhasma: 30 mg/tablet Mandur bhasma: 20 mg/tablet	1.18 mg/tablet ± 6.745	0.574 %
5	Saptamrit loh	Tablet	Loha bhasma: 100 mg/tablet	0.37 mg/tablet ± 5.028	1.368 %
6	Abhraloha	Tablet	Loha bhasma: 125 mg/tablet	0.28 mg/tablet± 5.028	1.788 %
7	Feroliv forte	Capsule	Dhatri loh bhasma: 80 mg/capsule Navayaas loh bhasma: 80 mg/capsule Tapyadi loh bhasma: 80 mg/capsule	0.32 mg/capsule ±5.028	1.560 %

Table 6: Assay of ayurvedic formulations.

Homeopathic formulations [11]

Sr. No.	Name of sample	Ferrum phosphoricum	weight of tablet(mg)	Conc. found (mean ± SD) n=6, (µg)	% RSD
1	Ferrum Phosphoricum 30 x -SBL	30x	0.1	16.136 ± 0.101	0.627
2	Fe-min Tablets-SBL	3x	0.25	22.986 ± 0.04	0.172
3	MIG AID Tablets-Bakson's Homoeopathy	3x	0.55	25.179 ± 0.062	0.245
4	ALLER AID Tablets-Bakson's Homoeopathy	3x	0.55	22.931 ± 0.032	0.142
5	THROAT AID Tablets-Bakson's Homoeopathy	3x	0.55	26.329 ± 0.140	0.534
6	FIVE PHOS-SBL	3x	0.1	17.063 ± 0.011	0.065

Table 7: Assay of homeopathic formulations.

Biochemic formulations [12]

Sr. No.	Name of Sample	Type	Obtained Concentration (n=6) ± SD (mg/tablet)	%RSD
1	Biochemic 1	Formulation	34.529 ± 0.342	0.990
		Lab Mixture	11.115 ± 0.077	0.689
2	Biochemic 15	Formulation	20.801 ± 0.292	1.405
		Lab Mixture	11.336 ± 0.091	0.803
3	Biochemic 16	Formulation	19.867 ± 0.161	0.810
		Lab Mixture	11.316 ± 0.041	0.365
4	Biochemic 24	Formulation	14.643 ± 0.217	1.480
		Lab Mixture	11.304 ± 0.052	0.463
5	Biochemic 28	Formulation	16.386 ± 0.237	1.449
		Lab Mixture	11.270 ± 0.0853	0.757

Table 8: Assay Of Biochemic Formulations.

Conclusion

The Formulations containing Iron were quantified as per the ICH guidelines. The results affirm that these formulations can be analysed by accustomed method without the interference of the matrix. Re-validation of the method warrant simple, precise, rapid and sensitive determination of iron in formulations of alternative system of medicine. These kinds of formulations have no particular label claim of content of Iron (II) and thus such quantification is helpful for the general public health.

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