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Determination of Total Iron in Hematinics by Atomic Absorption Spectrophotometry

HARRIS I. TARLIN and MARTIN BATCHELDER

Abstract □ The total iron content of six hematinic preparations was determined rapidly, precisely, and accurately by atomic absorption spectrophotometry. Hematinics comprising iron–carbohydrate complexes required ashing prior to assaying by atomic absorption spectrophotometry while those with an iron chelate or simple salt structure may be determined directly by atomic absorption spectrophotometry. A statistical evaluation of the data indicated that the atomic absorption spectrophotometry method was equivalent to the official colorimetric and volumetric methods and to a classical gravimetric procedure.

Keyphrases □ Iron in dosage forms—analysis □ Atomic absorption spectroscopy—analysis □ Colorimetric analysis—spectrophotometer □ Titration—iron analysis □ Gravimetric analysis—iron

Hematinic preparations generally fall into three structural categories: iron–carbohydrate complexes, iron chelates, and iron salts. Preparations consisting of iron–carbohydrate complexes are usually assayed for total iron by a lengthy colorimetric (1) or gravimetric procedure.[1] The usual USP (2) or NF (3) procedure for determining the total iron content of iron chelates and iron salts involves a sodium thiosulfate or ceric sulfate titration. Extensive studies (4, 5) indicate that atomic absorption spectrophotometry (AAS) offers a technique for assaying iron which is relatively free from interfering ions. To date, no studies have been reported in the literature concerning the assay of total iron in hematinics by AAS.

[1] The procedure used in this study was a slight modification of the gravimetric iron assay procedure described in most quantitative analysis textbooks. (See H. H. Willard, N. H. Furman, and C. E. Bricker, "Elements of Quantitative Analysis," 4th ed., D. Van Nostrand, Princeton, N. J., 1956, pp. 335, 336.)

EXPERIMENTAL

Instruments—A Perkin-Elmer model 303 double-beam spectrophotometer, equipped with an iron hollow cathode lamp and single-slot burner head, was used for all atomic absorption measurements. The instrument was optimized with a 10-p.p.m. standard iron solution. A sensitivity of 0.18 mcg./ml. for 1% absorption was achieved. Instrument parameters appear in Table I. All colorimetric measurements were carried out on a Perkin-Elmer model 202

Table I—Instrument Parameters

Wavelength	248.3 mμ
Hollow cathode lamp current	30 ma.
Fuel	Acetylene (flow meter at 9)[a]
Oxidizer	Air (flow meter at 9)[a]
Aspiration rate	1.8 ml./min.
Slit	No. 3
Meter response	No. 2
Recorder	Perkin-Elmer model No. 165

[a] Perkin-Elmer Burner Control Box No. 303-0240.

Table II—Analysis of Iron–Carbohydrate Complexes

Type of Sample	% of Labeled Amount[a]			
	Gravimetric	Colorimetric	Atomic Absorption—Direct	Atomic Absorption—Ashed
Iron–carbohydrate complex 1				
Batch 1	101.96	101.96	90.54	98.76
Batch 2	98.86	98.86	88.84	101.66
Batch 3	100.28	100.20	90.34	100.24
Batch 4	102.42	102.40	94.38	98.72
Batch 5	99.44	NA[b]	NA[b]	98.58
Batch 6	101.32	NA[b]	93.12	98.54
Batch 7	99.98	NA[b]	NA[b]	98.14
Iron–carbohydrate complex 2				
Batch 1	100.70	97.95	84.70	96.80
Batch 2	96.60	95.50	85.35	96.55
Batch 3	99.05	97.63	85.55	97.05
Iron–carbohydrate complex 3				
Batch 1	100.01	100.78	93.12	99.84
SD	±0.129[c]	±0.135[d]		±0.334[e]

[a] Based on a minimum of two assays. [b] NA = not analyzed. [c] Based on three determinations of iron–carbohydrate complex 1. [d] Based on six determinations of iron–carbohydrate complex 2. [e] Based on eight determinations of iron–carbohydrate complex 2.

double-beam UV-visible spectrophotometer using a 1-cm. Corex cell and a slit width of 25.

Reagents—Iron standard solution for atomic absorption spectroscopy was used.[2] All other reagents were ACS, USP, or NF grade. Distilled water was used for all solutions. Iron standard solutions used for calibrations were 7, 10, and 12 mcg. Fe/ml., respectively.

PROCEDURES

Colorimetric Method—The total iron content of the three iron–carbohydrate preparations was determined colorimetrically by the 2,2′-bipyridine method described in USP XVII (1). Calculation of the mg. Fe/ml. solution was according to the following equation:

$$100 \times \frac{A_u}{A_s} \times \frac{d}{W} = \text{mg. Fe/ml. solution} \qquad \text{(Eq. 1)}$$

where A is the absorbance of the sample, A_s is the absorbance of the standard; d is the density of the sample measured with a pycnometer at 20°, and W is the weight of the sample in grams.

Titration Method—Tablet and sustained-release capsule preparations were assayed for iron following the USP XVII (2) sodium thiosulfate titration procedure. For the hard gelatin capsule, the NF XII (3) ceric sulfate titration procedure was followed.

Gravimetric Method—Accurately weigh a portion of iron–carbohydrate solution equivalent to about 90 mg. of iron into a platinum crucible. Ash the sample with a Meker burner for 30 min., and dissolve the ash in a 400-ml. beaker by boiling with 75 ml. of 6 *N* HCl. Dilute with water to 150–200 ml. and make alkaline with NH_4OH. Acidify with nitric acid and again make alkaline with an excess of NH_4OH. Heat to 70–80° for approximately 1 min. and allow to cool and stand for 2 hr. Filter through Whatman No. 41-H filter paper and wash the precipitate thoroughly with 1% NH_4OH. Ash the filtered precipitate in a tared platinum dish and determine the weight of the ash. Calculate the mg. total Fe/ml. solution as follows:

$$WA \times \frac{699.4}{W} \times d = \text{mg. total Fe/ml. solution} \qquad \text{(Eq. 2)}$$

where WA is the weight of the ash in grams, d is the density of the sample measured with a pycnometer at 20°, and W is the weight of the sample in grams.

ATOMIC ABSORPTION WITH ASHING[3]

Iron–Carbohydrate Preparations—Accurately weigh a portion of solution equivalent to about 90 mg. of Fe into a platinum crucible and ash for 30 min. over a Meker burner. Dissolve the ash in a 400-ml. beaker by boiling with 75 ml. of 6 *N* HCl. After the solution is cool, transfer to a 1-l. volumetric flask and dilute to the mark with water. Pipet a 6.0-ml. aliquot from the 1-l. volumetric flask into a 50-ml. volumetric flask and dilute to the mark with water. Aspirate the sample against a blank consisting of 100 ml. of 6 *N* HCl prepared according to the same dilution sequence as the sample. Calculate the mg. Fe/ml. solution as follows:

$$\text{mg. Fe/ml. solution} = \frac{(F_{AV})\,(A)\,(0.001)}{W/d \times 1/1000 \times 6/50} \qquad \text{(Eq. 3)}$$

where F_{AV} is the average mcg. Fe/ml./absorbance unit (determined from standards). F_{AV} was calculated as follows:

$$F \text{ (for a given standard)} = \frac{\text{concentration of standard, mcg. Fe/ml.}}{\text{absorbance}}$$

$$F_{AV} = \frac{\Sigma F}{\text{no. of standards measured}}$$

A = absorbance of the sample

d = density of the sample measured with pycnometer at 20°

Tablet—Weigh and finely powder 20 tablets. Weigh a portion of powder equivalent to about 90 mg. of Fe into a platinum crucible. Follow the procedure used for iron–carbohydrate preparations in the *Atomic Absorption with Ashing* section starting with "and ash for 30 min." up to "transfer to a 1-l. volumetric flask and dilute to the mark with water." Filter a portion of the solution through Whatman No. 2 filter paper. Pipet 6.0 ml. of the filtrate into a 50-ml. volumetric flask, and dilute to the mark with water. After aspirating the sample, calculate the mg. Fe/tablet as follows:

$$\text{mg. Fe/tablet} = F_{AV} \times A \times \frac{50}{6} \times \frac{1000}{1000} \times \frac{\text{average tablet weight, g.}}{\text{sample weight, g.}} \qquad \text{(Eq. 4)}$$

Table III—Ratio of Variance

	Critical, 5%	Calculated Value
Colorimetric *vs.* gravimetric *vs.* AAS with ashing[a]	3.88	1.88
Colorimetric *vs.* gravimetric *vs.* AAS with ashing[b]	6.94	3.63
AAS direct *vs.* AAS with ashing[c]	7.71	27.07

[a] Based on seven batches of iron–carbohydrate complex 1. [b] Based on three batches of iron–carbohydrate complex 2. [c] Based on five batches of iron–carbohydrate complex 1. These results showed significant difference at the 0.01 level.

[2] Fisher Scientific.

[3] In all the following AAS assays, the sample was aspirated against a blank consisting of the appropriate volume of 6 *N* HCl which was subjected to the same dilution sequence as the sample.

Table IV—Analysis of Tablet and Capsules

Type of Sample	Amount of Fe Declared	mg. Fe/Tablet or Capsule[a]		
		Titration	Atomic Absorption—Direct	Atomic Absorption—Ashed
Tablet	40 mg./tablet	39.25	40.48	38.95
Capsule (hard gelatin)	37.52 mg./capsule	39.48	40.54	38.95
Capsule (sustained release)	50 mg./capsule	50.11	52.23	51.71
SD		±2.10[b]	±1.79[b]	

[a] Based on a minimum of two assays. [b] Based on four determination of the sustained-release capsule.

where F_{AV} is the average mcg. Fe/ml./absorbance unit, and A is the absorbance of the sample.

Hard Gelatin Capsule and Sustained-Release Capsule—Weigh the contents of 20 capsules, mix thoroughly, and weigh a portion equivalent to about 90 mg. of Fe into a platinum crucible. Follow the procedure used for iron–carbohydrate preparations in the *Atomic Absorption with Ashing* section starting with "and ash for 30 min." up to "transfer to a 1-l. volumetric flask and dilute to the mark with water." Filter a portion of the solution through Whatman No. 2 filter paper, pipet 6.0 ml. of the filtrate into a 50-ml. volumetric flask, dilute to the mark with water, and aspirate. Calculate the mg. Fe/capsule as follows:

$$\text{mg. Fe/capsule} = F_{AV} \times A \times \frac{50}{6} \times \frac{1000}{1000} \times \frac{\text{average net fill weight, g.}}{\text{sample weight, g.}} \quad \text{(Eq. 5)}$$

where F_{AV} is the average mcg. Fe/ml./absorbance unit, and A is the absorbance of the sample.

ATOMIC ABSORPTION BY DIRECT DILUTION

Iron–Carbohydrate Preparations—Accurately weigh a portion of solution equivalent to about 90 mg. of Fe into a tared beaker. Quantitatively transfer the solution to a 1-l. volumetric flask with 30 ml. of water, add 100 ml. of 6 *N* HCl, and dilute to the mark with water. Pipet a 6.0-ml. aliquot into a 50-ml. volumetric flask, dilute to the mark with water, and aspirate. Calculate the mg. Fe/ml. as described in the section entitled *Atomic Absorption with Ashing: Iron–Carbohydrate Preparation.*

Tablet, Hard Capsule, and Sustained-Release Capsule—Weigh 20 tablets or the contents of 20 capsules. Weigh a portion of tablet powder or capsule content equivalent to about 90 mg. of Fe and transfer to a 1-l. volumetric flask with 30 ml. of water. Add 100 ml. of 6 *N* HCl,[4] and dilute to the mark with water. Filter a portion of the solution through Whatman No. 2 filter paper, pipet 6.0 ml. of filtrate into a 50-ml. volumetric flask, and dilute to the mark with water. Aspirate the sample against a blank. Calculate the mg. Fe/tablet or mg. Fe/capsule as described in the section entitled *Atomic Absorption with Ashing: Tablet* or *Atomic Absorption with Ashing: Hard Gelatin Capsule and Sustained-Release Capsule.*

In the case of individual sustained-release capsules, the following procedure is used. Add the contents of one capsule to a 500-ml. volumetric flask. Add 50 ml. of 6 *N* HCl and 100 ml. of water, heat until solution is effected, and dilute to the mark with water. Filter a portion of the solution through Whatman No. 2 filter paper, pipet 6.0 ml. of the filtrate into a 50-ml. volumetric flask, and dilute to the mark with water. Aspirate the sample against a blank and calculate the mg. Fe/capsule as follows:

$$\text{mg. Fe/capsule} = F_{AV} \times A \times \frac{500}{1000} \times \frac{50}{6} \quad \text{(Eq. 6)}$$

[4] It was necessary to heat the contents of the sustained-release capsule to effect solution after adding 6 *N* HCl. The insoluble material observed was no doubt due to the coating excipients commonly used in sustained-release preparations.

Table V—Recovery Studies

Type of Sample	Amount of Fe[a] Added, mcg.	% Fe Recovered[b] by Atomic Absorption
Iron–carbohydrate complex	100	98[c]
Capsule	100	101.6[d]
Tablet	100	100.4[d]

[a] In all cases, the final concentration of the added iron was 2 mcg./ml. [b] Average recovery for two trials. [c] Ashed before analysis. [d] Assayed directly without ashing.

where F_{AV} is the average mcg. Fe/ml./absorbance unit, and A is the absorbance of the sample.

Recovery Study—Known amounts of iron were added to each of the three dosage forms studied, and the iron content was determined by AAS.

RESULTS AND DISCUSSION

The assay results for the three iron–carbohydrate preparations are summarized in Table II. These results show that there is a significant difference between iron–carbohydrate preparations assayed directly by AAS and those ashed prior to assay by AAS. Although the standard deviation of the AAS ashed method is greater than the colorimetric or gravimetric method, the analysis of variance shown in Table III indicates that there is no significant difference between the colorimetric, gravimetric, and AAS with ashing method. The assay results summarized in Table IV indicate that the tablet and capsules studied may be analyzed directly for iron by AAS. Table V summarizes data from the recovery study.

It is recommended that an initial study of a hematinic preparation should consist of an ashed and a direct AAS assay procedure. This generally will give a measure of interferences caused by organic molecules that complex or chelate the iron or excipients which may refract or absorb light and thus interfere with the assay.

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