In conclusion, for identification of kaolin and bentonite, X-ray diffraction analysis was found to be more accurate, dependable, conclusive, and much less time consuming than the methods of identification presently employed in the NF XIII and USP XVIII monographs on kaolin and bentonite, respectively. Consequently, it is recommended that these monographs should be supplemented by X-ray diffraction analysis.

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Spectrophotometric Determination of Fe(II), Fe(III), and Total Fe

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Abstract □ The orderly use of two known reactions [Fe(II) with α,α'-dipyridyl and Fe(III) with ascorbic acid] allowed to take place successively in the same aliquot sample solution provides a procedure for the quantitative determination of Fe(II), Fe(III), and total Fe. The procedure is simple, rapid, and sensitive enough to cope with samples containing small amounts of iron or with samples of limited solubility. From a pharmaceutical point of view, the method is useful for the control of both hematinic raw materials or finished products, and it is especially useful for stability and content uniformity studies.

Keyphrases □ Fe(II), Fe(III), total Fe in aqueous solution—determination □ α,α'-Dipyridyl—color formation, Fe(II) determination □ Ascorbic acid—color formation, Fe(III) determination □ Hematinic salts—determination of iron content □ Colorimetric analysis—spectrophotometer

A simple procedure is presented for the simultaneous determination of Fe(II), Fe(III), and total Fe in a single aliquot from an aqueous solution of these components. This procedure can be readily adapted to content uniformity (1) and shelflife stability studies (2) on both hematinic raw materials and finished pharmaceutical products, especially those labeled to contain iron in its Fe(II) state. The titrimetric methods described by the USP (3) and NF (4) for total Fe on *finished products* are not feasible for single tablet or capsule analysis. Furthermore, these sources do not as yet provide methods for Fe(III) on finished products. These needs are met by the present procedure.

The procedure uses two well-known reactions [Fe(III) *versus* ascorbic acid to yield Fe(II), and Fe(II) *versus* α,α'-dipyridyl to yield a red complex] taking place in succession in the same aliquot solution. Initially, the intensity of the color produced by the reaction product of Fe(II) with α,α'-dipyridyl is measured against the blank to yield the Fe(II) content. Then solid ascorbic acid (10–15 mg.) is added to reduce any Fe(III) to additional Fe(II); the latter, in turn, reacts with the existing excess of α,α'-dipyridyl to produce an increase in color intensity. This intensity is then measured twice: once against the first colored solution yielding the Fe(III) content, and once against the blank yielding the total Fe content. Because of the small amount of solid ascorbic acid added, the volume increase of the final solution is negligible.

EXPERIMENTAL

Reagents and Instruments—The following were used: (*a*) α,α'-dipyridyl 0.1% solution in water; (*b*) buffer (pH 4.5) solution of 83 g. sodium acetate and 120 ml. acetic acid in water to a total volume of 1 l.; (*c*) ascorbic acid USP, crystalline powder; (*d*) Beckman DB-G double-beam spectrophotometer; (*e*) Beckman 25.4-cm. (10-in.) recorder for spectrophotometers; and (*f*) three matched cells, 1-cm. lightpath, marked as No. 1, No. 2, and No. 3.

Procedure—Using approximately 0.1 *N* HCl, dilute the soluble iron sample to an expected concentration of 10–300 mcg. Fe/ml. In the case of solid dosage forms, crush and powder the sample in a mortar, dissolve the iron salts with 0.1 *N* HCl, filter, and dilute as above. Transfer an aliquot, estimated to contain about 300–350

Table I—Analysis of Mixtures of Known Composition

Standard Mixtures[a]	Total Fe, —mcg./ml.— Taken	Found	Fe(II), —mcg./ml.— Taken	Found	Fe(III), —mcg./ml.— Taken	Found
Preparation A	2.60	2.58	0.81	0.82	1.79	1.76
Preparation B	2.83	2.84	1.63	1.62	1.20	1.22
Preparation C	3.19	3.16	2.26	2.21	0.93	0.95
Preparation D	2.94	2.97	1.69	1.70	1.25	1.27
Preparation E	2.43	2.45	2.43	2.44	0.00	0.01
Preparation F	2.33	2.36	0.00	0.02	2.33	2.34

[a] Prepared by dissolving six pairs of weighed amounts of ferrous and ferric ammonium sulfate. Single aliquots were then taken and diluted to indicated final concentration.

Table II—Analysis of Raw Materials[a] by This Method and USP or NF Method

Substance and Lot Number	Total Fe Found, %: USP or NF	Total Fe Found, %: This Method	Fe(II) Found, %: USP or NF	Fe(II) Found, %: This Method	Fe(III) Found, %: USP or NF	Fe(III) Found, %: This Method
Ferrous gluconate NHT-404	11.05	11.10	10.30	10.30	0.75	0.80
Ferrous fumarate LBB	32.05	31.96	29.15	29.02	2.85	2.94
Ferrous gluconate NET-2667	11.00	11.13	10.39	10.45	0.61	0.67
Ferrous fumarate H2177 × A12	31.60	31.71	29.75	29.71	1.85	1.95

[a] Raw material in "as is" condition.

mcg. of total iron, into a 100-ml. volumetric flask and add water to bring the volume to about 50 ml. Mix and add 15 ml. buffer solution and 6 ml. α,α'-dipyridyl solution. Bring the volume to the mark with water, mix, fill cell No. 2, and scan this solution so as to obtain an absorbance curve from 500 to 600 nm. against a blank in cell No. 1, prepared similarly but omitting the α,α'-dipyridyl. To the remaining sample in the flask, add an estimated 10–15 mg. of ascorbic acid, shake well, and fill cell No. 3; scan this solution through the same wavelengths, once against the contents in cell No. 2, and once against the contents in cell No. 1.

Under the conditions of this procedure, it was found that Beer's law is obeyed by the system and that the absorptivity of the red complex is 158 l./g.cm. at the wavelength of maximum absorbance. Therefore, the maximum absorbance readings (at around 523 nm.) from the first, second, and third graphs divided by 0.158 represent the micrograms per milliliter of Fe(II), Fe(III), and total Fe, respectively.

Since the sum of Fe(II) and Fe(III) is that for total Fe, it is obvious that one of the graphs may be omitted and the corresponding result be obtained by adding or subtracting the other two recorded results.

Table III—Analytical Results Obtained by This Procedure on Commercial Products

Commercial Products	Dosage Form	Claim, mg./unit	Found, mg./unit	Percent Labeled Found
A	Tablet	36.9 Fe(II) / T	38.6 Fe 35.7 Fe(II) 2.9 Fe(III)	104.9 97.0 7.9
B	Liquid	44 Fe(II) / 5 ml.	43.2 Fe 40.7 Fe(II) 2.4 Fe(III)	98.1 92.6 5.5
C	Tablet	40.0 Fe(II) / T	39.6 Fe 39.6 Fe(II) 0.0 Fe(III)	99.0 99.0 0.0
D	Liquid	200 Fe(III) / oz.	196.2 Fe 192.4 Fe(II) 3.8 Fe(III)	98.1 96.2 1.9
E	Tablet	65.7 Fe(II) / T	66.2 Fe 62.8 Fe(II) 3.6 Fe(III)	100.8 95.4 5.4
F	Liquid	10 Fe / 5 ml.	10.2 Fe 0.3 Fe(II) 9.9 Fe(III)	101.8 3.1 98.7
G	Tablet	> 160 $FeSO_4$ / T	173.2 $FeSO_4$ 0.0 Fe(III)	108.2 0.0
H	Tablet	100 Fe / T	107.2 Fe 6.3 Fe(II) 100.9 Fe(III)	107.2 6.3 100.9

RESULTS

Various known concentrations of Fe(II) and Fe(III) mixtures were prepared and analyzed by this method. The results in Table I demonstrate the validity of the method and the degree of its accuracy. Furthermore, various raw material hematinic salts were analyzed; the results reflected in Table II show no appreciable difference from those obtained by the USP and/or NF methods. Finally, a variety of commercial hematinic tablets and elixirs was tested by this method at the level of their unit dosage form. The results in Table III are of comparative value only, because the manufacturer's overage and the actual input in the unit were, of course, unknown.

DISCUSSION

Many hematinic preparations tested by this procedure were colored from caramel or other sources but presented no difficulty. This was due either to the subdilutions or to the decolorizing properties of ascorbic acid. Any persisting color could be taken into account in the blank. Some other hematinic products were found to require time and warming of the solution for the reduction of Fe(III) to Fe(II) by ascorbic acid. Products with an excess of ascorbic acid or other reducing agents present will not show difference in Fe(II) and total iron content, because, if present in elixirs, they maintain the iron in the ferrous state or react with any existing Fe(III) in the tablet or capsule to yield Fe(II) during the first step of dilution in this procedure.

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