Comparison of Iron Assays in Multivitamin Products Using an Automated Chemical Procedure and an X-Ray Emission Method

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Two methods are described for the determination of iron in multivitamin preparations, formulated with either ferrous sulfate, ferrous fumarate, or ferrous gluconate. In the first procedure, an automated colorimetric procedure, iron is determined with 2,4,6-tripyridyl triazine in a buffered solution. In the second procedure, an X-ray emission spectrometer is used. Samples, analyzed as solutions, are placed in liquid sample cells and counted for 100 sec. Following sample preparation, approximately 20 samples can be assayed per hour with the automated procedure as compared to four (in duplicate) by X-ray emission.

THE DETERMINATION of iron in multivitamin preparations requires the use of assay procedures that are not affected by other minerals, vitamins, or formulation excipients. In achieving this, X-ray emission spectroscopy has given satisfactory results in our laboratories over a long period of time. Requests for large numbers of iron assays in formulation studies and in single tablet or unit dosage assays, however, prompted the use of an automated chemical assay procedure.

This report describes two methods for the determination of iron in multivitamin preparations, formulated with either ferrous sulfate, ferrous fumarate, or ferrous gluconate. In the first procedure, an automated colorimetric procedure, iron is determined with 2,4,6-tripyridyl triazine in a buffered solution essentially as described by Zak *et al.* (1).

In the second procedure, a General Electric X-ray emission spectrometer is used. Samples, analyzed as solutions, are placed in a liquid sample cell and are counted for 100 sec.

METHODS AND MATERIALS

Automated Chemical

Instruments—Automatic analyzer components[1]: Sampler I, proportioning pump I, colorimeter, linear recorder, chart reader, assorted tubing, and glass fittings.

Reagents—Double distilled water used throughout the procedure; hydrochloric acid, A.R.

Acetate buffer, pH 4, 1.0 *M*, 27.22 Gm. of sodium acetate trihydrate is dissolved in approximately 200 ml. of water in a 1,000-ml. flask, 45.79 ml. of glacial acetic acid is added and the solution is made up to volume with water.

2,4,6-Tripyridyl triazine (TPTZ)[2]; 200 mg. of anhydrous TPTZ is dissolved in 0.5 ml. of HCl and transferred to a 1,000-ml. volumetric flask with the aid of 200 ml. of acetate buffer. After dilution to volume with water, the contents are thoroughly mixed and allowed to stand several days with periodic shaking. The solution is filtered before use and stored in light-resistant bottles.

Ascorbic acid, 2% aqueous solution. When refrigerated, it is stable for several days.

Iron Standard—Stock solution; 200 mcg./ml. 1.4045 Gm. of ferrous ammonium sulfate hexahydrate is placed in a 1,000-ml. volumetric flask with 1.0 ml. of HCl, gently mixed, and diluted to volume with water. The standard is stable indefinitely in a tightly closed, glass container.

Working Solutions; The iron stock solution is used to prepare solutions containing 0.75 mcg., 1.0 mcg., 1.25 mcg., and 1.50 mcg. iron/ml. in water. The diluted standards are kept in tightly closed volumetric flasks. The standards appear to be stable indefinitely; however, new standards should be prepared periodically.

Multivitamin Sample Preparations—For single-tablet assays, individual tablets are placed in 10.0 ml. of concentrated HCl and approximately 5 ml. of water. After shaking for approximately 4 hr., or placing on a steam bath for approximately 1 hr., the solutions are diluted to 100.0 ml. with water. An aliquot is transferred to a suitable volumetric flask and diluted with water to a theoretical iron concentration of approximately 1.0 mcg./ml. If

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[1] AutoAnalyzer, Technicon, Ardsley, N.Y.

[2] International Chemical and Nuclear Corporation, City of Industry, Calif.

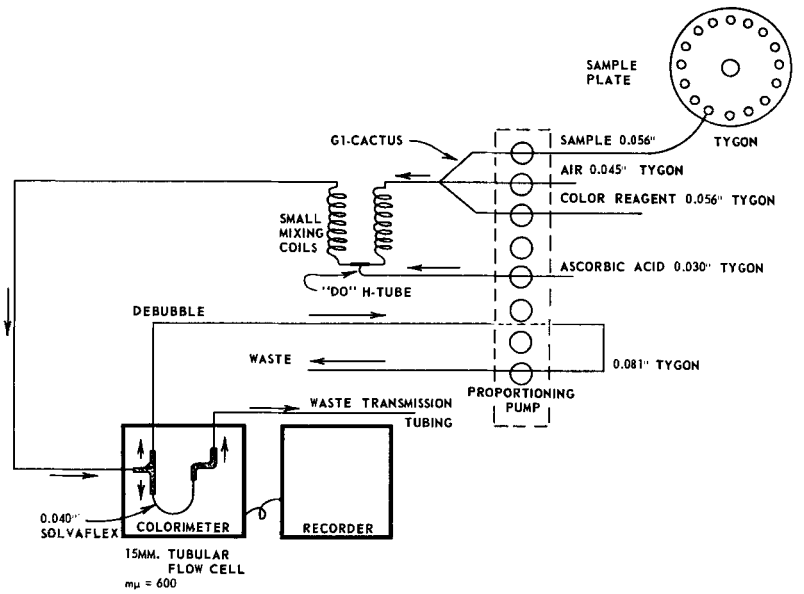

Fig. 1—Manifold-flow diagram for iron (ferrous) with the automatic analyzer.

necessary, the solutions are filtered prior to filling the automatic analyzer cups.

When a specified number of tablets are required in the initial sample preparation, the same ratios of tablet, acid and water are maintained as for single-tablet assays. For example, if a procedure calls for the assay of 10 tablets, they are placed in a 1,000-ml. volumetric flask with 100 ml. of concentrated HCl and 50 ml. of water. Following disintegration of tablets, the volume is adjusted to 1,000 ml. with water and an aliquot is further diluted to the desired concentration of iron.

Procedure—The automatic analyzer is standardized using the manifold-flow system of Fig. 1. As the figure shows, a 15-mm. flow cell is used in the colorimeter and measurements are made at a wavelength of 600 mμ. Standardization is achieved by pumping water through the tubes to establish a 99% transmittance base line, substitution of reagents for water, and adjusting to a new 99% transmittance base line. A known iron solution is continuously sampled to establish initial stability of the automated system. The four levels of iron standards (0.75, 1.0, 1.25, and 1.50 mcg./ml.) are placed in alternate sample cups followed by test preparations. The analysis is set at 40 samples/hr. with a water washout cup between each iron sample, standard as well as test preparation. At regular intervals a 1.0 mcg./ml. iron standard is inserted to compensate for the effect of instrumental or reagent variations.

Calculations—A transmission curve is plotted on the chart reader using recorded peak heights of the iron standards. The mcg./ml. of iron in each test preparation is determined from its corresponding transmittance peak using the chart reader and the iron standard curve. An adjustment factor, determined from standards placed periodically, is applied if necessary. The iron content of each product is obtained by multiplying the mcg./ml. by the proper dilution factors. To express results in terms of the proper ferrous salt (fumarate, gluconate, or sulfate), an additional factor based on the ratio of molecular weights is applied.

X-Ray Emission

Instruments—The General Electric XRD-5 spectrometer, equipped with a lithium fluoride analyzing crystal and a tungsten target X-ray tube operated at 50 kvp. and 45 ma., was used in the study. The flow proportional counter (GE No. SPG4) was operated at 2.100 kv. The spectrometer was equipped with a General Electric large sample holder. General Electric liquid sample cells, closed with 1/4-mil Mylar were used to hold the samples. The liquid sample holder is shown in Fig. 2.

Reagent—Hydrochloric acid, A.R., concentrated and 1:1000.

Iron Standard—A 1.0 mg./ml. iron standard is prepared by dissolving 7.0217 Gm. of ferrous ammonium sulfate hexahydrate in 1,000 ml. of 1:1000 HCl.

Multivitamin Sample Preparation—Sufficient tablets to provide approximately 100 mg. of iron are placed in a 250-ml. volumetric flask with 4 ml. of HCl and 20 ml. of deionized water and left at room temperature for at least 1 hr. or, if convenient, overnight. Another 180 ml. of water is added, the sample is heated on a steam bath for an hour; the solution is then cooled to room temperature and made to volume with water. This solution contains 0.4 to 0.5 mg. of iron per ml.

Procedure—Three separate GE liquid sample cells are used for the following solutions: (*a*) *Blank*, 6.0 ml. of 1:1000 HCl; (*b*) *Sample*, 4.0 ml. of multivitamin test preparation and 2.0 ml. of 1:1000 HCl; and (*c*) *Sample with iron standard*, 4.0 ml. of multivitamin test preparation and 2.0 ml. of iron standard (1.0 mg./ml.). The cells are sealed with 1/4-mil Mylar and the contents are mixed by gentle shaking.

If a thick syrup is to be assayed, a suitable aliquot of the product is transferred with a syringe into two glass-stoppered centrifuge tubes and 1:1000 HCl is added to give an iron concentration of approximately 0.3 mg./ml. An aliquot of iron standard is added to one of the tubes to make the total iron concentration approximately 0.6 mg./ml. An equal volume of 1:1000 HCl is added to the other tube. Six-milliliter aliquots are then pipeted

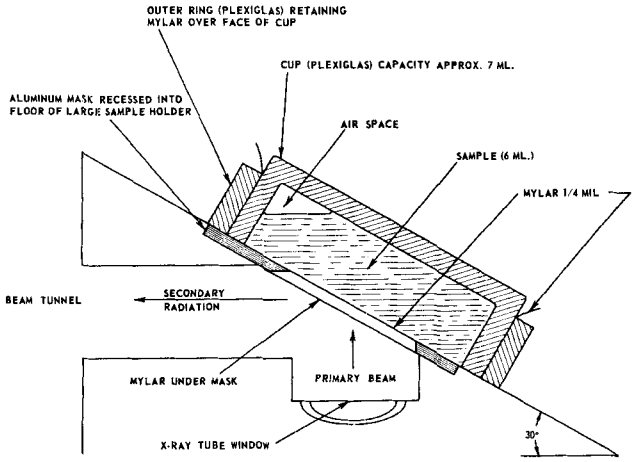

Fig. 2—Liquid sample holder used in X-ray emission analysis.

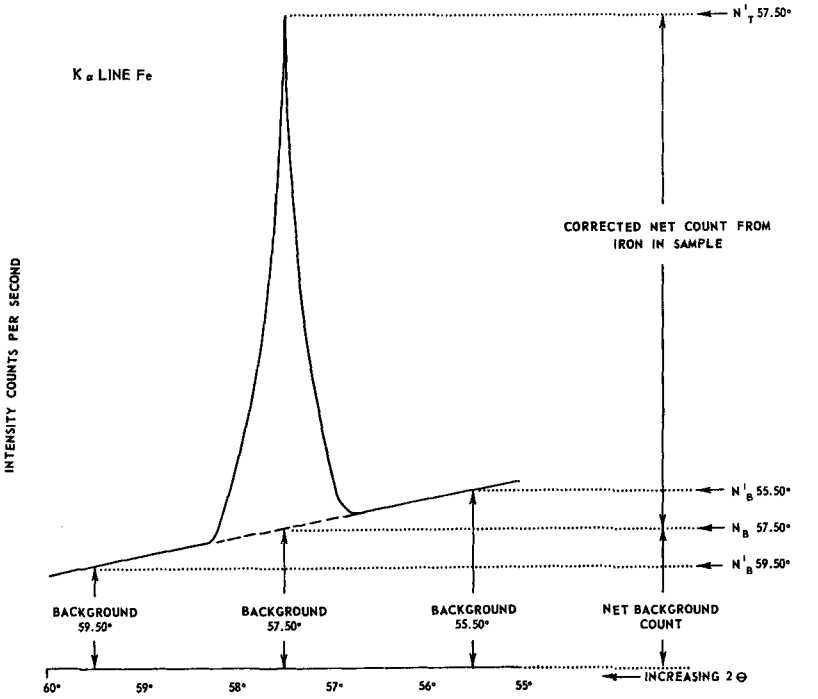

Fig. 3—Spectrum of the analytical line for iron showing background corrections.

into the GE liquid sample cells and sealed with 1/4-mil Mylar.

The cells are gently shaken, inverted, and placed in the large sample holder. Counts are made on each cell for 100 sec. with the spectrometer set for 2θ 55.50° (background), 57.50° (Kα emission line of iron), and 59.50° (background). A representative spectrum of the analytical line for iron showing background corrections is shown in Fig. 3. The average of the background counts taken 2° 2θ each side of the Kα emission line of iron at 2θ = 57.50° is the assumed background at this line and this value is subtracted from the count at 2θ = 57.50° to give the net count due to iron present.

The diluent blank cell gives a net count over background which is a measure of the iron present in the X-ray tube, holder, mask, and cell. This blank is subtracted from the net count for the "sample" and "sample with standard" to give corrected net counts. A schematic of the procedure is shown in Fig. 4. The diluent blank is not counted for each sample; once a day is sufficient.

Calculations—The amount of iron present in the sample aliquot is calculated by applying the straight line-slope equations:

$$\text{Slope } (m) = \frac{\Delta y}{\Delta x} = \frac{\text{corr. net count sample with standard} - \text{corr. net count sample}}{\text{mg. Fe added to sample with standard} - \text{mg. Fe added to sample}}$$

and

$$\text{mg. Fe present in sample aliquot} = \frac{b}{m} = \frac{\text{corr. net count sample}}{m}$$

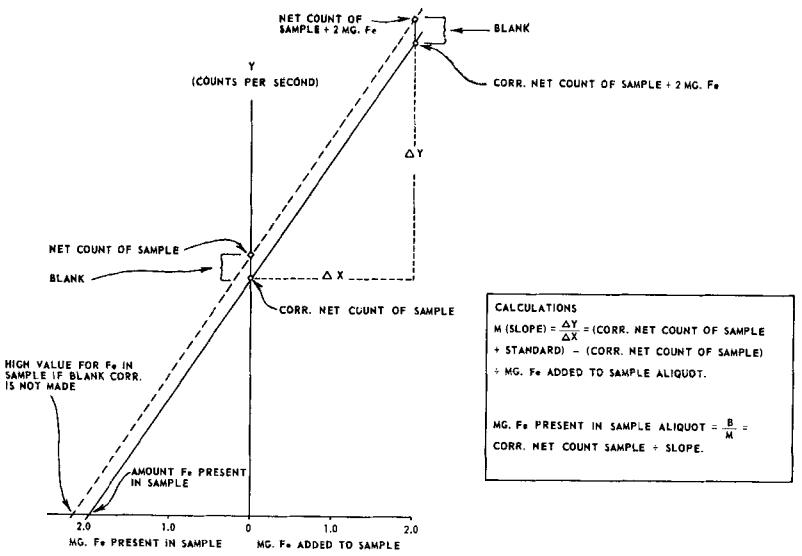

Fig. 4—Calculations used in X-ray emission analysis for iron.

RESULTS AND DISCUSSION

Initial experiments with the automated colorimetric procedure using a standard 10-mm. flow cell and dialysis gave satisfactory results for iron standards; however, product results were extremely variable from day to day. This was thought to be due to variable efficiency of the dialysis membrane or less than optimal sample preparation.

Installation of a 15-mm. tubular flow cell, elimination of dialysis, and the use of initially strongly acid solutions for sample preparation led to a satisfactory assay procedure. Because product excipients caused initial solutions to become basic when using water, the addition of acid was required; however, the volume of acid had to be regulated so that the capacity of the pH 4 acetate buffer was not exceeded.

A linear relationship exists for the log of transmittance and iron concentration in the range of 0.25 mcg. to 2.0 mcg./ml. with the automatic analyzer procedure. The assay has a percent coefficient of variation $\left(\frac{\text{standard deviation}}{\text{mean value}} \times 100\right)$ of approximately 1%.

To test the efficiency of the automated procedure, iron standards in varying amounts, were added to tablet sample preparations containing ferrous sulfate. Results of the study are shown in Table I, an average recovery of 100.3% was obtained, varying from a low of 98.8% to a high of 102.2%.

Table II gives automated assay results of individual multivitamin tablets containing ferrous sulfate. The average iron content was 10.33 mg., with an average tablet weight of 308.8 mg. Ten tablets of the same lot were assayed as a combined sample with an average iron value of 10.50 mg./tab. The average value compares favorably with the value of 10.33 mg./tab. for the single-tablet assay.

X-ray emission analysis (also called X-ray fluorescence analysis) provides a rapid procedure for the determination of iron in pharmaceutical materials. Approximately 25,000 counts are recorded in the 100-sec. period for each mg. of iron present in the cell. Total iron is measured without regard to valence or sample color and there is little interference from other elements. Tedious acid digestion of fluid samples containing sugar is unnecessary. The use of an internal standard for each sample minimizes absorption effects of various excipients. The assay has a coefficient of variation of approximately 1.2%. Four samples can be

Table I—Recovery of Iron (mcg.) from Solutions Prepared from Multivitamin Tablets Using the Automatic Analyzer

Iron from Tablets	Iron Added	Theoretical Amt. Iron Present	Iron Found	Recovery (%)
5.025	4.00	9.025	9.05	99.7
5.100	4.00	9.100	9.30	102.2
5.175	4.00	9.175	9.30	101.4
5.250	4.00	9.250	9.25	100.0
5.250	2.50	7.750	7.75	100.0
5.250	3.75	9.000	9.20	102.2
5.250	5.00	10.250	10.30	100.5
5.250	6.25	11.500	11.40	99.1
5.250	7.50	12.750	12.60	98.8
5.300	4.00	9.300	9.20	98.9
Av. recovery:				100.3%

Table II—Weight and Iron Content of Individual Multivitamin Tablets Assayed with the Automatic Analyzer
(Theory: 10 mg. Iron/Tab.)

Tablet No.	Tablet Wt., mg.	Iron/Tab., mg.
1	299.2	10.05
2	304.2	10.50
3	314.0	10.60
4	305.8	10.20
5	308.0	10.35
6	313.6	10.04
7	308.5	10.05
8	308.5	10.00
9	315.3	11.05
10	310.9	10.50
Averages	308.8	10.33
Coefficients of variation, %	1.60	3.25

TABLE III—WEIGHT AND IRON CONTENT OF INDIVIDUAL MULTIVITAMIN TABLETS ASSAYED WITH X-RAY EMISSION (Theory: 10 mg. Iron/Tab.)

Tablet No.	Tablet Wt., mg.	Iron/Tablet, mg.
11	280.1	10.183
12	278.2	10.207
13	281.6	10.303
14	284.1	10.619
15	274.8	10.547
16	283.2	10.162
17	277.7	10.133
18	277.6	10.141
19	280.4	9.979
20	284.1	10.185
21	279.8	10.690
22	286.0	10.518
23	279.7	10.397
24	277.5	10.234
25	275.7	10.017
26	279.9	10.272
Averages	280.0	10.287
Coefficients of variation, %	1.12	2.04

assayed in duplicate in an hour; however, it is possible to automate the assay with accessories now available commercially.

A large number of single-tablet assays of multivitamin tablets from studies involving formulation and tableting process changes were carried out using X-ray emission. Data from a study of this nature are shown in Table III. The coefficients of variation for both tablet weight and iron content are lower than that found with an earlier lot of tablets assayed by the automated colorimetric procedure (Table II). Sixteen additional tablets from the same lot were individually weighed and assayed on each of 2 days with the X-ray emission method. The coefficients of variation were 0.82 and 1.05% for tablet weight and 1.94 and 2.34% for iron content, respectively. These data indicated that a uniform tablet had been produced, from the standpoint of weight and iron content.

Assay results of pulverized tablets obtained by both procedures are shown in Table IV. Weighed amounts of the ground tablets approximating the average tablet weight were analyzed. More deviation in iron content was found with the X-ray procedure than with the automated colorimetric method; however, the average of the results agree quite favorably. Satisfactory assays with the two

TABLE IV—IRON CONTENT OF SEPARATE WEIGHINGS OF PULVERIZED MULTIVITAMIN TABLETS, 10 mg. IRON/TABLET

Automatic Analyzer		X-Ray Emission	
Sample No.	Iron/Tab., mg.	Sample No.	Iron/Tab., mg.
1	10.18	11	9.74
2	10.43	12	9.87
3	10.10	13	10.37
4	10.17		
5	10.19		
6	10.01		
7	10.19		
8	10.35		
9	10.11		
10	10.07		
Averages	10.18		9.99
Coefficient of variation, %	1.24		

procedures have been performed on numerous additional multivitamin preparations, including capsules, tablets, syrups, and powder mixes.

SUMMARY

An automated chemical method and an X-ray emission procedure have been described for the determination of iron in multivitamin preparations. Following sample preparation, approximately 20 samples can be assayed per hour with the automated procedure as compared to four (in duplicate) by X-ray emission.

REFERENCE

(1) Zak, B., Cohen, J. S., and Williams, L. W., *Microchem. J.*, **6**, 67(1962).

Keyphrases

Automated analysis—iron
Iron–multivitamin preparations—analysis
Colorimetric automated analysis
Diagram—manifold flow system, automated
X-ray emission spectrometry—analysis, nonautomated