Standard Test Method for
Iron in Trace Quantities Using the FerroZine Method[1]

1. Scope*

1.1 This test method covers the determination of iron in the range from 0.01 to 0.2 µg/g using FerroZine[2] reagent solution. The range may be extended through the use of a 5- or 10-cm cell or by suitable dilution of the sample solution.

1.2 This test method is intended to be general for the final steps in the determination of iron and does not include procedures for sample preparation.

1.3 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.4 Review the current Safety Data Sheets (SDS) for detailed information concerning toxicity, first-aid procedures, and safety precautions.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* For specific warning statements, see 7.4.

2. Referenced Documents

2.1 *ASTM Standards:*[3]

D1193 Specification for Reagent Water

E60 Practice for Analysis of Metals, Ores, and Related Materials by Spectrophotometry

E180 Practice for Determining the Precision of ASTM Methods for Analysis and Testing of Industrial and Specialty Chemicals (Withdrawn 2009)[4]

E200 Practice for Preparation, Standardization, and Storage of Standard and Reagent Solutions for Chemical Analysis

E275 Practice for Describing and Measuring Performance of Ultraviolet and Visible Spectrophotometers

3. Summary of Test Method

3.1 This test method is based upon a photometric determination of the FerroZine[2] complex with the iron (II) ion.[5,6] The sample is dissolved in a suitable solvent and the iron is reacted with FerroZine reagent solution which will convert the dissolved iron compounds to form a magenta color iron (II) complex. The iron content of the sample solution is determined by measurement of the magenta color at 560 nm using a suitable photometer.

4. Significance and Use

4.1 This test method is suitable for determining trace concentrations of iron in a wide variety of products, provided that appropriate sample preparation has rendered the iron and sample matrix soluble in water or other suitable solvent. Each sample matrix must be investigated for suitability using this test method.

4.2 This test method assumes that the amount of color developed is proportional to the amount of iron in the test solution. The calibration curve is linear over the specified range.

5. Interferences

5.1 Any ion that absorbs light at 560 nm will interfere with the determination. Anionic interferences include oxalate in concentrations over 500 µg/g, cyanide, and nitrate.[5]

5.2 Of copper, cobalt, calcium, magnesium, lead, silver, molybdenum, aluminum, nickel, zinc, arsenic, manganese, hexavalent chromium, trivalent chromium, divalent cobalt and monovalent copper are the only metals other than iron that form colored species with FerroZine under test conditions. At least 1000 mg/L of the alkali metals and the alkaline earths had no effect on the determination. Many heavy metals will react

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[1] This test method is under the jurisdiction of ASTM Committee D16 on Aromatic Hydrocarbons and Related Chemicals and is the direct responsibility of Subcommittee D16.15 on Industrial and Specialty General Standards.

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[2] FerroZine is a trademark of Hach Chemical Company.

[3] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[4] The last approved version of this historical standard is referenced on www.astm.org.

[5] Stookey, L. L., "FerroZine—A New Spectrophotometric Reagent for Iron," *Analytical Chemistry*, Vol 42, No. 7, June 1970, pp. 779 – 781.

[6] Gibbs, C. R., "Characterization and Application of FerroZine Iron Reagent as a Ferrous Iron Indicator," *Analytical Chemistry* , Vol 48, No. 8, July 1976, pp. 1197–1201.

***A Summary of Changes section appears at the end of this standard**

with FerroZine in competition with iron, but with the excess reagent used in the test there is no effect on the results.[5]

5.3 The pH range of the final solution should be from 4 to 9 to give the best test results.[5,6]

5.4 All glassware used in this test method must be iron-free and scrupulously clean by precleaning with dilute hydrochloric acid and FerroZine reagent solution followed by a water rinse.

6. Apparatus

6.1 *Photometer,* capable of measuring light absorption at 560 nm and holding a 5-cm or 10-cm cell. Check the performance of the photometer at regular intervals according to the guidelines given in Practice E275 and the manufacturer's manual.

6.2 *Absorption Cells,* 5-cm or 10-cm light path.

NOTE 1—A discussion of photometers and photometric practice is given in Practice E60.

7. Reagents

7.1 Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.[7] Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean Type II or Type III reagent water conforming to Specification D1193.

7.3 *Iron, Standard Solution,* 1 mL = 1 µg Fe (see Notes 2 and 3)—Dissolve 0.1000 g of iron wire in 10 mL of hydrochloric acid (HCl, 1 + 1) and 1 mL of saturated bromine water (400 mL water + 20 mL bromine). Boil until the excess bromine is removed. Add 200 mL of HCl, cool, and dilute to 1 L in a volumetric flask. Dilute 10 mL of this solution to 1 L.

NOTE 2—The preparation of this reagent is also described in Practice E200.

NOTE 3—As an alternative, the standard iron solution may be prepared by diluting 1.00 mL of commercially available iron standard stock solution (1000 mg iron/L) to 1 L with water.

7.4 *FerroZine Reagent Solution*—Contains FerroZine color reagent [3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine, monosodium salt, monohydrate], buffer, and a reducing agent. (**Warning—**This solution contains thiols as reducing agents. Wear butyl rubber or neoprene gloves when handling the solution and avoid inhalation of the vapors.)

7.4.1 Alternatively, the individual solutions can be prepared as described below.[5]

7.4.1.1 *Reducing Agent*—Hydroxylamine hydrochloride, 10 percent by weight solution in hydrochloric acid: Dissolve 10 g of reagent grade hydroxylamine hydrochloride ($NH_2OH.HCL$) in 30 g of deionized water in a plastic bottle; add 50 mL of reagent grade concentrated hydrochloric acid and mix well. Prepare this solution fresh daily.

7.4.1.2 *Color Reagent*—FerroZine, 0.514 weight percent solution: Dissolve 0.514 g of FerroZine reagent in 100 g of deionized water in a plastic bottle, and mix well. Discard the reagent after seven days.

7.4.1.3 *Buffer Reagent-pH 10.0 Buffer*—Dissolve 200 g of reagent grade ammonium acetate in a minimum of deionized water, add 175 mL of concentrated ammonium hydroxide and dilute to 500 mL in a volumetric flask. Mix well. Check the pH of the buffer to verify that it is pH 10 ± 0.5. If it is not in the required pH range, remake the buffer. Store the buffer in a plastic bottle. Discard after four weeks.

8. Sampling

8.1 Because this is a general test method for the final steps in determining iron, specific procedures for sample preparation are not included (see 4.1 and 4.2).

9. Calibration

9.1 *FerroZine Reagent Solution Method (7.4):*

9.1.1 By means of suitable pipets or a buret, transfer 0 (reagent blank), 2.0, 4.0, 6.0, 8.0, and 10.0 mL, respectively, of the standard iron solution and approximately 20 mL of water to each of six clean, dry, 50-mL, glass-stoppered volumetric flasks. These flasks represent 0, 2.0, 4.0, 6.0, 8.0, and 10.0 µg of iron. Add 2.0 mL of FerroZine reagent solution to each flask, dilute the contents of each flask to volume with water, stopper, and mix well by inverting the flasks several times. Let the solutions stand for a minimum of 5 min but not more than 10 min to develop the magenta color. Measure the absorbance of each calibration standard in accordance with 10.3.

9.2 *Individual Solution Method (7.4.1):*

9.2.1 By means of suitable pipets or a buret, transfer 0 (reagent blank), 2.0, 4.0, 6.0, 8.0, and 10.0 mL, respectively, of the standard iron solution and approximately 40 mL of water to each of six clean, dry, 100-mL, glass-stoppered volumetric flasks. These flasks represent 0, 2.0, 4.0, 6.0, 8.0, and 10.0 µg of iron. Add the individual reagents (reducing reagent, color reagent and buffer reagent) as described in 10.2 and 10.3 to each flask, dilute the contents of each flask to volume with water, stopper, and mix well by inverting the flasks several times. Let the solutions stand for a minimum of 5 min but not more than 20 min to develop the magenta color. Measure the absorbance of each calibration standard in accordance with 10.3.

9.3 Construct a calibration graph by plotting the absorbances against the corresponding micrograms of iron present in the calibration solutions, including the blank. Obtain the best straight line through the points (calibration function) by applying simple linear regression. Determine the slope (S) of the linear calibration function.

9.3.1 Evaluate and verify the obtained calibration graph and function by checking for a random scatter of the y-residuals around an average of zero, and by preparing and analyzing a control solution.

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[7] *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmacopeial Convention, Inc. (USP), Rockville, MD.

NOTE 4—Many spectrophotometers have the ability to calculate a calibration graph automatically after measuring the calibration solutions and subsequently to show the concentration of the component being measured directly on a display. In such cases no manual calibration graphs need to be constructed. It is, however, recommended to verify the calculation procedure of the instrument and to establish the characteristics of the calibration graph according to suitable regression analysis software.

NOTE 5—As the calibration function has been derived from a single prepared calibration standard, verify the accuracy of the calibration function by preparing and analyzing a control solution containing an accurately known amount of approximately 5 µg of iron. If reasonably possible this control solution should be completely independent, that is, prepared by a different operator, different batches of chemicals etc. The difference between the known value and the measured value should be within the confidence limits for the control solution, as derived from the confidence limits of intercept and slope of the calibration function.

10. Procedure

10.1 *FerroZine Reagent Solution Method (7.4):*

10.1.1 Weigh to three significant figures a sample containing 0.5 to 10 µg of iron into a clean, dry 50-mL, glass-stoppered, volumetric flask (see Note 6). Add sufficient water to dissolve the sample but do not exceed 40 mL total volume.

NOTE 6—Preliminary tests must be made to determine if the sample or any impurities in the sample interfere in any way with the analysis.

10.1.2 To prepare a reagent blank, add about 20 mL of water to a second clean, dry, 50-mL, glass-stoppered, volumetric flask.

NOTE 7—When running a number of samples, only one reagent blank is needed.

10.1.3 Add 2.0 mL of the FerroZine reagent solution to each volumetric flask, stopper, and swirl to mix the contents. Dilute each volumetric flask to volume with water, stopper, and mix well by inverting the flask several times. The pH range of the final solution should be 4 to 9 to give the best test results.[5,6] Allow the sample solution and reagent blank to equilibrate at room temperature for a minimum of 5 min for color development.

10.2 *Individual Solution Method (7.4.1):*

10.2.1 Weigh 80.0 g of sample to the nearest 0.01 g into a 100 mL glass-stoppered volumetric flask. Add 80 mL of deionized water to a second volumetric flask and reserve as a reagent blank.

10.2.2 Pipet 2 mL of the hydroxylamine hydrochloride solution and 2 mL of the FerroZine solution into each of the flasks, stopper, and mix well by inverting several times. Do not shake.

10.2.3 After 5 min, pipet 2 mL of the buffer into each flask, stopper, and mix well by inverting several times. Do not shake. Make up to 100 mL with deionized water. Mix well by inverting several times; do not shake. The pH of the solution should be in the range of 4 to 9. Color development will not be complete outside of this pH range.

10.2.4 Allow color development for at least 5 min. Measure the absorbance of the sample relative to the reagent blank at the maximum absorbance (560 nm) as in 10.3. Absorbance measurement should be completed within 20 min of adding the buffer.

10.2.5 If the absorbance of the sample is greater than the highest standard (10 µg of iron, (9.2.1)) in the calibration curve, reduce the sample size so that the absorbance of the sample is within the calibration curve. Repeat the procedures in 10.2 using the reduced sample size.

NOTE 8—When using a 10-cm cell, the sample size for samples containing from 0.1 to 1 µg/g iron should be 8 g and for samples containing from 1 to 10 µg/g iron the sample size should be 1 g.

NOTE 9—The 80 g of sample in 100 mLs is proportionally the same as the maximum of 40 g of sample in 50 mLs in 10.1.

10.3 Measure the absorbance of each sample solution at 560 nm in a 5-cm or 10-cm cell using a suitable photometer. Use a matched cell filled with the reagent blank to set the instrument at zero absorbance or 100 % transmittance.

10.4 Refer to the previously prepared calibration curve (9.3) to determine the micrograms of iron found.

10.5 *Quality Control*—It is recommended that a control chart for an aqueous quality control sample of well established iron concentration be established and maintained according to generally accepted guidelines.[8] Measure the control sample each time a (series of) test sample(s) is tested. If the measured value exceeds the action limit of the control chart, take appropriate action before proceeding with sample tests.

11. Calculation

11.1 Calculate the micrograms of iron, B, in the sample solution as follows:

$$B = A \times (S/L) \tag{1}$$

where:

A = absorbance of the sample solution,
B = iron, µg,
S = slope of calibration line (9.3), and
L = cell path length, cm.

11.2 Calculate the iron content of the sample, µg/g, as follows (see 11.1):

$$\text{iron} = B/W \tag{2}$$

where:

B = iron found, µg, and
W = sample, g.

12. Report

12.1 Report the iron content to the nearest 0.001 µg/g.

13. Precision and Bias[9]

13.1 In 2007, Committee E15 on Industrial and Specialty Chemicals conducted and completed Interlaboratory Study #52 to determine precision data for six test methods used in the analysis of glycols. The precision of this test method is based on the interlaboratory study of E1615, Standard Test Method for Iron in Trace Quantities Using the FerroZine Method. Each of fifteen laboratories were asked to test three different materials. Thirteen laboratories tested MEG, eleven laboratories

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[8] *ASTM Manual on Presentation of Data and Control Chart Analysis*; 7th edition, ASTM Manual Series MNL 7A. (Revision of Special Technical Publication (STP) 15D).

[9] Supporting data have been filed at ASTM International Headquarters and may be obtained by requesting Research Report RR:E15-1064.

TABLE 1 ASTM E1615 Iron by FerroZine[2]

Test Result, mcg/g	Sample	Average over all Laboratories	Repeatability, Standard Deviation	Intermediate Standard Deviation	Reproducibility, Standard Deviation	Repeatability Limit	Intermediate Limit	Reproducibility Limit
Iron	MEG	0.0987	0.0037	0.0075	0.0382	0.0104	0.0211	0.1070
Iron	DEG	0.2655	0.0093	0.0230	0.0897	0.0261	0.0644	0.2512
Iron	TEG	0.3535	0.0101	0.0101	0.1075	0.0282	0.0282	0.3009

tested DEG, and ten laboratories tested TEG. Every test result represents an individual determination. Two test results were conducted on each of two days for a total of four test results per assay. Note that in the combined study, 8 labs used a single analyst, 7 labs used two analysts (on different days), and 2 labs did not record this information. In the event that there were missing values for one or more labs, this information was noted in the results. The details of this study are given in ASTM Research Report RR:E15-1064.

13.1.1 *Repeatability*—Two test results obtained within one laboratory shall be judged not equivalent if they differ by more than the "r" value for that material; "r" is the interval representing the critical difference between two test results for the same material, obtained by the same operator using the same equipment on the same day in the same laboratory.

13.1.2 *Reproducibility*—Two test results shall be judged not equivalent if they differ by more than the "R" value for that material; "R" is the interval representing the difference between two test results for the same material, obtained by different operators using different equipment in different laboratories.

13.1.3 *Intermediate Precision*—The day-to-day standard deviation within a laboratory for results produced by the same operator, determined through statistical analysis following Practice E180. Practice E180 was used to conform to this particular study design which required an estimate of intermediate precision. The statistical analysis was conducted using the SAS statistical analysis software, Version 8.0.

13.1.3.1 The Practice E180 analysis considers the two test results from each day as being run under repeatability conditions and estimates the repeatability, intermediate, and reproducibility precision for each assay. The repeatability precision would be estimated from the two sets of duplicate test results within each day, and the intermediate precision would be estimated from the agreement between the two days, all pooled over laboratories. Caveat: Since two days is a short time period, the intermediate precision would probably be underestimated by the Practice E180 analysis.

13.1.4 Any judgment in accordance with these two statements would have an approximate 95 % probability of being correct.

13.2 *Bias*—At the time of the study, there was no accepted reference material suitable for determining the bias for this test method, therefore no statement on bias is being made.

13.3 The precision statement was determined through statistical examination of qualified results, from fifteen laboratories, on three materials. These three materials were described as the following: Fluid 1: Monoethylene Glycol. Fluid 2: Diethylene Glycol. Fluid 3: Triethylene Glycol. To judge the equivalency of two test results, it is recommended to choose the material closest in characteristics to the test material.

14. Keywords

14.1 FerroZine; iron; photometric

SUMMARY OF CHANGES

Committee E15 has identified the location of selected changes to this standard since the last issue (E1615–08) that may impact the use of this standard.

(1) Revisions were made in the following sections: 1.4, 9, 10.2.5, and 11.

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