IRON (Colorimetric)

PRINCIPLE

Organic matter in the sample is destroyed by ignition in the presence of a small amount of sulfuric acid. Iron in the residue is reduced to iron (II) and reacted with orthophenanthroline. The absorbance of the orange-red complex is determined spectrophotometrically at 508 nm.

SCOPE

This method is applicable to syrups and starch hydrolyzates, starches, sugars, and to most other products of the corn wet milling process.

SPECIAL APPARATUS

1. Spectrophotometer: A suitable instrument capable of accurate measurements in the visible spectrum equipped with 1 cm absorption cells is recommended (Note 1).

2. Muffle Furnace: Equipped with pyrometer and capable of operating at controlled temperatures up to 600 °C

3. Platinum or Silica Dishes: 100-200 mL capacity

REAGENTS (Note 2)

1. Orthophenanthroline Reagent: Dissolve 3.0 g of 1,10-phenanthroline monohydrate (orthophenanthroline) in 700 mL of purified water, warming if necessary to hasten solution. Add 40.0 g of anhydrous sodium acetate ($NaC_2H_3O_2$) and 20.0 g of hydroxylamine hydrochloride ($NH_2OH \bullet HCl$); cool to room temperature and dilute to 1 L. The mixed reagent is stable for about 30 days; discard when precipitation occurs, or when color is excessive.

2. Hydrochloric Acid Solution, (7.5% by weight): Add 172 mL of concentrated hydrochloric acid (37% HCl, sp g 1.19) to 500 mL of purified water and dilute to 1 L.

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3. Sulfuric Acid Solution (1+3 by volume): Cautiously pour 100 mL of C.P. sulfuric acid (96% H_2SO_4, sp g 1.84) into 300 mL of purified water and mix.

4. Ammonium Hydroxide Solution (14% by weight) (1+1 by volume): Add 100 mL of ammonium hydroxide solution (28-30% NH_3 sp g 0.90) to 100 mL of purified water.

5. Potassium Permanganate Solution, 0.1 *N*: Dissolve 0.32 g of potassium permanganate ($KmnO_4$) crystals in purified water and dilute to 100 mL. Filter through sintered glass before using. Make fresh as required. Standardization is not necessary.

6. Standard Iron Solution:

   Stock Solution: Dissolve 0.7022 g of reagent grade ferrous ammonium sulfate hexahydrate [$Fe(NH_4)_2(SO_4)_2 \bullet 6H_2O$] in 100 mL of purified water (Note 3). Add 10 mL of sulfuric acid solution and heat the solution to 60-65 °C. Add 0.1 *N* potassium permanganate from a buret, with constant stirring, to the first permanent pink color (Note 4). Cool to room temperature, transfer quantitatively to a 1 L volumetric flask, and dilute to volume with purified water.

   Standard Solution, 10 µg/mL Iron: Pipet 50.0 mL of stock solution into a 500 mL volumetric flask and dilute to volume with purified water.

PROCEDURE

A. Standardization: Pipet 1.0, 2.0, 5.0, 10.0 and 15.0 mL aliquots of standard iron solution (10, 20, 50, 100 and 150 µg iron, respectively) into separate 50 mL volumetric flasks. Add purified water to each flask to make a total volume of about 20 mL, and add 20 mL of water to another flask to serve as a blank.

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To each flask, add 2 mL of hydrochloric acid solution and 10 mL of orthophenanthroline reagent. Dilute to volume with purified water. Mix thoroughly and allow to stand for 30 mins. (Note 5).

Measure absorbance of each solution at 508 nm. Subtract the absorbance of the reagent blank from each calibration standard.

Plot absorbance vs. iron content (µg per 50 mL) on rectilinear graph paper (Note 6).

B. Analysis: Weigh accurately a sample containing 20 to 150 µg of iron (Note 7) into a platinum or silica dish; add 5 mL of sulfuric acid, distributing the acid uniformly in the sample. Place the dish on a steam bath or low-temperature hot plate to evaporate the water; increase heat to carbonize the sample and expel most of the sulfuric acid. Place the dish in a muffle furnace at 525-600 °C until the ash is carbon free.

Cool to room temperature; wash down the sides of the dish with 3-5 mL of purified water and add 5 mL of 7.5% hydrochloric acid. Place the dish on a steam bath and evaporate to dryness. Dissolve the ash in 2 mL of hydrochloric acid solution, heating briefly on a steam bath if necessary to effect solution (Note 8). Filter quantitatively to remove any insoluble residue, receiving the filtrate to a 50 mL volumetric flask. Add 10 mL of orthophenanthroline reagent. Dilute to volume with purified water, mix thoroughly, and allow to stand for 30 mins. (Note 5). Measure the absorbance in a 1 cm cell at 508 nm using the reagent blank as reference.

Read µg of iron from the standardization curve.

CALCULATIONS

$$\text{Iron, ppm (as is)} = \frac{\mu g \text{ of Iron (From Graph)}}{\text{Sample Wt., g}}$$

$$\text{Iron, ppm (dry basis)} = \frac{(\text{Iron, ppm (as is)})(100)}{(\text{Sample Dry Substance, g})(100)}$$

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NOTES AND PRECAUTIONS

1. As absorbance is proportional to absorption cell length, iron contents of sample and standard solutions should be reduced when absorption cell length is increased.

2. As in all analyses for trace materials, purity of reagents is essential. This is most easily checked by running a reagent blank as recommended under "Procedure A. Standardization." If the blank shows more than 0.005 A at 508 nm against a water reference, prepare fresh solutions using iron-free reagent.

3. Ferrous ammonium sulfate is used as a standard because it is readily available in high purity. It is oxidized to iron (III) to provide a check on efficiency of the reduction procedure.

4. Oxidation of 0.7 g of ferrous ammonium sulfate requires about 17 mL of 0.1 *N* potassium permanganate solution.

5. Color development can be hastened by heating the solution on the steam bath for 5 mins. after adding reagents. The solution must then be cooled to room temperature before dilution to volume. The color is stable for many days.

6. The standardization curve is reproducible. It should be repeated when new reagents are prepared.

7. A 5 or 10 g syrup sample usually provides an amount of iron in the range stated. Smaller samples should be taken when iron contents are unusually high.

8. If it is necessary to use more than the stated quantity of acid, solution pH must be adjusted so that a final pH of 3-4 is obtained. Add a 0.25 in. strip of congo red paper to the filtrate in the flask; add ammonium hydroxide solution dropwise with vigorous swirling until the congo red paper turns pink. Add 2 mL of hydrochloric acid solution and 10 mL orthophenanthroline reagent and proceed in the usual fashion.

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METHOD HISTORY

Combined the Iron (Colorimetric) methods for Corn Starch (Unmodified) (B-30), Corn Syrup (E-32) and Corn Syrup (E-33) on 4-15-2010.

Corn Starch (Unmodified), Iron (Colorimetric) (B-30), Date of Acceptance 11-13-1961, Revised 3-05-2004.

Corn Syrup, Iron (Colorimetric) (E-32), Date of Acceptance 9-21-1971,Revised 3-01-1995.

Corn Syrup, Iron (Colorimetric) (E-33), Date of Acceptance 9-22-1970, Revised 7-09-1993.