Determination of Iron in Cereal Grains and Seed Oils by Flame AA

WORKING INSTRUCTIONS

United States Department of Agriculture
Grain Inspection, Packers and Stockyards Administration
Technical Services Division
Quality Systems & Services Unit

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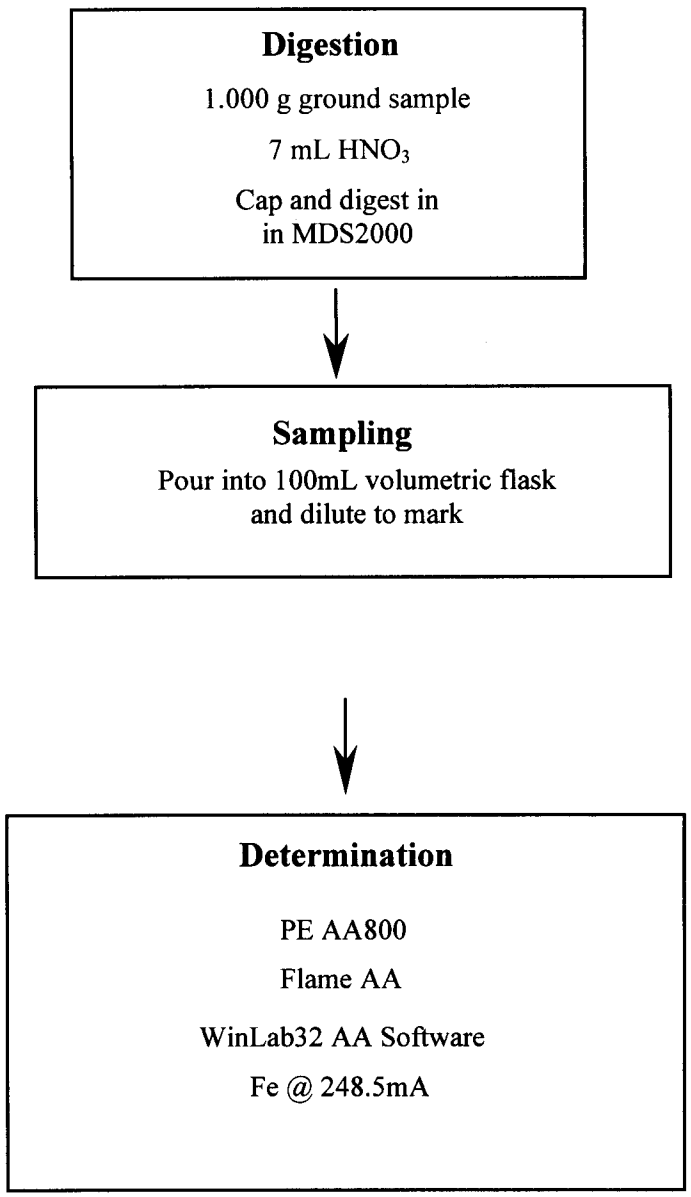
Method Flowchart



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1. Purpose and Scope of Application

The purpose and scope of this Working Instruction (WI) is to establish the operational parameters, methodology and requirements for the quality assurance and acceptability of data in the determination of Iron (Fe) in cereal grains and oil seeds.

2. Analyst Qualifications and Responsibilities

The analyst(s) will receive proper training in the conduct of this WI and will follow the WI as written. The Supervisory Chemist (or Project Leader) is responsible for ensuring this WI is followed, and modified as necessary or appropriate. The Deputy Director of the Technical Services Division must approve all revisions to this WI prior to implementation.

3. References

- AA WinLab32 Software Guide, Rev. G, Perkin-Elmer Corporation, March 2006.
- Microwave Digestion Applications Manual, CEM Corporation 1994.
- Gawalko, E.J. et al. (1997), J.AOAC Int. 80, 379-387

4. Safety and Hazardous Waste

a. Use latex/nitrile gloves, eye protection, and perform operations in a hood. An exhaust fan is required during: (1) adding of the nitric acid to the sample before the digestion, (2) tightening the lids of the digestion cups and placing the cups in the microwave digestion oven, and (3) releasing the pressure and opening of the sample cups after digestion.

b. Grinding Samples: All samples should be ground so that 95% of the material passes through a 20-mesh sieve.

c. Toxic wastes: This procedure will generate small amounts of liquid waste containing nitric acid and Iron. The nitric acid waste **(after neutralization)** can be flushed down the drain to the sewer. The iron waste from the instrument will be collected in a 10L plastic jug located on the bottom shelf of the instrument table. When full, it must be neutralized before dumping down the drain.

5. Equipment

This section is meant to specify the equipment being used in the laboratory during the method validation process. Other equipment can be substituted for these WI as long as the supervising chemist/technician approves. In some cases, side-by-side testing will be necessary to verify equivalency.

a) Perkin-Elmer AA800 Atomic Absorption spectrometer with transversely heated graphite atomizer and Flame atomizer: Complete with Electrodeless Discharge Lamps (EDL), Hollow Cathode Lamps (HCL), Automatic Sampler AS-800, computer, AA WinLab32 software, printer and high purity Argon gas regulated at 60 psi, high purity Air regulated at 80 psi, and high purity Acetylene gas regulated at 60 psi.

b) Perkin-Elmer S-10 Auto-sampler.

c) CEM Microwave Digestion System, MDS-2000 or equivalent. Equipped with closed cup digestion assemblies Teflon vessels, lids, fittings, 200 psi diaphragms, etc. The digestion vessels should be made of high quality Teflon and is capable of digesting whole kernel grain samples to clear solution or homogenous suspension in approximately a half-hour.

d) Falling Number Grinder 3300, or equivalent.

e) Analytical balance capable of weighing 0.0001 g.

f) Adjustable pipettor: 1-5 mL adjustable with 5 mL tips.

g) MilliQ RO Plus/MillQ UV Water Purification System (Millipore, Corp., Marlborough, MA).

6. Materials

a) Water: High-purity Milli-Q water.

b) Nitric acid: Ultra pure, prepared by sub-boiling distillation, available from Fisher catalog # A467-1, or equivalent.

c) Argon gas: UHP/Zero grade (ultra high purity).

d) Certified Fe Standard: Available from Perkin-Elmer Inc. Norwalk, CT.

e) Corn Soy Blended: Blended in-house for Quality Control Check Sample.

f) Blue Falcon Tube: 15mL, or equivalent (Fisher Scientific 352096).

7. Quality Control Procedures

7.1 *Definitions*

Furnace/Flame Blank: The instrument reading taken, but no sample present to check the alignment of the system.

Reagent Blank: The instrument reading taken from a digested sample of acid only.

Characteristic Mass: The mass of analyte that gives 1% absorption or 0.0044-absorbance unit. This is instrument dependent and should be within 20% of the manufacturer's "Cook Book" value.

Standard Check Sample: Sample produced by properly mixed batch of Corn Soy Blend using any appropriate mixer to establish an in-house reference standard.

7.2 ***Preparation of Standards***

7.2.a Instrument Calibration Standard: 1000 µg/mL Iron Calibration Standard (Perkin-Elmer, Corp.)

7.2.b Preparation of 2% nitric solution:

1. Add 28.6 mL of 70% Nitric Acid into a 1000 mL volumetric flask.
2. Dilute to volume with high-purity Milli-Q water.
3. Cap and then slowly invert several times to mix.

7.2.c Preparation of Iron Standard Solution (500 µg/mL):

1. Add 50.0 mL of the Iron Calibration Standard (1000 µg/mL) to a 100 mL volumetric flask.
2. Dilute to volume with 2% nitric acid solution.

7.2.d Preparation of 20 µg/mL Working Standard:

1. Add 4.0 mL of the Iron Standard Solution (500 µg/mL)to a 100 mL volumetric flask using an adjustable pipettor and disposable 5 mL pipette tip.
2. Dilute to volume with 2% nitric acid solution.

7.2.e Preparation of 15 µg/mL Working Standard:

1. Add 3.0 mL of the Iron Standard Solution (500 µg/mL)to a 100 mL volumetric flask using an adjustable pipettor and disposable 5 mL pipette tip.
2. Dilute to volume with 2% nitric acid solution.

7.2.f Preparation of 10 µg/mL Working Standard:

1. Add 2.0 mL of the Iron Standard Solution (500 µg/mL)to a 100 mL volumetric flask using an adjustable pipettor and disposable 5 mL pipette tip.
2. Dilute to volume with 2% nitric acid solution.

7.2.g Preparation of 5 µg/mL Working Standard:

1. Add 1.0 mL of the Iron Standard Solution (500 µg/mL)to a 100 mL volumetric flask using an adjustable pipettor and disposable 5 mL pipette tip.
2. Dilute to volume with 2% nitric acid solution.

8. Instrument Parameters:

8.1 AtomicAbsorption Spectphotometer

Iron Method	Parameter	Setting
	Wavelength (nm)	248.5
	Slit Width (nm)	0.70
	Signal Measurement	Peak Area
	BOC (sec)	2
	Read Delay (sec)	0
	Read Time (sec)	5
	Calibration Type	Non-Linear through Zero

8.2 Control Parameters:

8.2.a The zero intercept non-linear correlation coefficient of the calibration curve should be 0.999 to 1.000 for Fe. If out, check the furnace/flame head alignment, and then recalibrate the curve.

8.2.b The characteristic mass of Fe should be approximately, 12.0 pg/0.0044 A-s, and at 20 ppb the absorbance should be 0.15 A-s. At 200-ppb the absorbance should be between 1.2 to 1.8 A-s.

8.2.c Each sample is run in triplicate. If the percent relative standard deviation (%RSD) is greater than 20%, rerun the sample.

9. Sample Preparation: (Microwave Digestion Method)

9.1 *Digestion:*

9.1.a Weigh ≈1.0 gram of the representative, ground sample accurately (to the third decimal), record the weight, and place in an MDS-2000 digestion liner. Repeat weighing all samples.

9.1.b Pipette 7 mL of nitric acid into the cup and attach a gray vent cap. Tighten cap finger tight. Repeat for all samples.

9.1.c Put one (1), 200 psi rupture diaphragm in the vent hole of each cap and tighten with a hollow plastic screw.

9.1.d Connect the pressure sensing line to cup #1, which has a special cover for fitting the temperature and pressure sensors.

9.1.e Place the carrousel in the microwave digester and adjust the carrousel so that the least amount of strain is exerted on the temp/press sensor cable when the carrousel is rotating back and forth in half circle intervals.

9.1.f Turn on the power switch located in right rear of the instrument, and press on the top front keypad (Yellow Keys), **F3**, to disable the "Temperature Unit".

9.1.g Press **F3** again to "Recall Method/Data".

9.1.h Press **F1** to "Recall Stored Method".

9.1.i Ensure the method **"Grain"** is highlighted and then press the **Blue "Enter"** button.

9.1.j Press the **Yellow F1** to "Load Program".

9.1.k Press **Yellow F4** to "Start", and then press the **Yellow F1**, to answer "Yes" for the "Advanced Composite Vessels".

9.1.l The unit will then start running the digestion now. It will beep when Stage 1 is over and when it is done.

Stage	1	2	3	4	5
Power (%)	50	100	0	0	0
Pressure (psi) 180	200	500	0	0	0
Time (min)	5	5	0	0	0
Time at Pressure	10	10	0	0	0

9.1.m After the digestion, wearing gloves and using extreme care, disconnect the pressure sensor line inside the MSD2000 so that the toxic vapor can be exhausted through the vent.

9.1.n Transfer the carrousel and the sample liners to a fume hood.

9.1.o Holding the liners away from you, slowly loosen the vent screw on each digestion liner to release the gas.

9.1.p Open the sample liner when no noise of escaping gas is observed.

9.1.q Transfer sample to a properly marked 15-mL Blue Falcon tube, rinse liner with Milli-Q water and transfer to sample tube.

9.1.r Dilute to volume using 2% nitric acid stock solution, and mix.

9.1.s Repeat steps 9.1.q and 9.1.r for all samples.

9.1.t Put liner and lid into a container of deionized water to soak overnight.

9.1.u After soaking overnight, put the liners and lids in an oven to dry overnight.

9.1.v Neutralize remaining samples before disposal (flushed down drain) and throw sample tubes into trash.

10. Determination

a) Open the valves on the acetylene and air tanks.

b) Turn the power on to the AA-800 and the computer.

c) Loosen the screw behind the plastic bottle on the AS-80 autosampler, this holds the 2% nitric acid waste rinse solution.

d) Carefully swing open the Automatic Sampler AS-80 assembly, and swing it all the way to the right.

e) Open **"File"** menu and scroll down to **"Change Technique"**, and select **"Flame"**.

f) A message will appear warning you the technique will be changed. Click **"Ok"**.

g) A warning message will appear saying the atomizer will be moved. Click **"Ok"**.

h) Place the large waste stream bottle on the floor and put the sample tray in place beneath the burner. **(Large slots on bottom of the unit.)**

i) On the computer, open **"Workspace"** and select **Flame**.

j) Open the **Lamp** window and turn on the Fe HCL lamp. Allow the lamp to warm up for 30 minutes, and record energy level.

k) Click on the toggle switch icon located in the **"Flame"** window, to turn on the burner.

l) In the **Flame** window, click on **"Align Burner"**. Select "auto align burner" and click next.

m) Aspirating a blank water sample, click **"Adjust"**, and burner will automatically adjust to the correct height (vertical position). Record the vertical location in the log book.

n) Click next, and while aspirating the 20 µg/mL standard, click **"Adjust"** to get the proper flame horizontal position. When found record in log book and click **"Finish"**.

o) In the **"Auto Analysis Control Window"**, on the **"Setup"** tab, double click "**Untitled**" on line 1, to open the methods files.

p) Select **(Iron)**, from the methods menu. The name will appear in the dialog box, and then click **"OK"**.

q) In the **"Sample Info Window"** start entering the sample ID's.

r) In the auto sampler location column start with 9 and end with the last location number for the last sample analyzed.

s) Using the tab button, or mouse enter the weight, and sample conversion units for each sample (i.e. 1.000, 14 and mg/g for the In House Check Sample and the regular samples).

t) At the top enter the Batch Name (i.e. FE082209), volume units (mL), weight units (g), and then type in your name in **Analyst**.

u) When done, click on **File**, scroll down to **Save As** and click on **"Sample Info file"**. Enter the name of the file (i.e. FE082209) and then press enter.

v) In the **"Auto Analysis Window"**, the sample info file name will appear in the Set Up tab window.

w) For the **"Data Set"**, click on the **Browse** button and enter the data set name (i.e. FE082209). The exit window.

x) Click on the **"Analyze Tab"**, and then click the "Rebuild List" button to update analysis list, and then click "Print List" for file copy.

y) Click on **"Analyze Cal"** and wait until finished.

z) Analyze cal curve to insure it meets standard.

aa) If cal curve passes, then click **"Analyze Sample"** to run the batch samples and any QC's.

bb) Check all RSD% to insure compliance and that all QC's meet requirements. If RSD% is out of compliance, repeat as necessary

cc) Generate a control chart for the iron of the RM8436 (NIST) standard or other control charting standard.

dd) Enter sample levels into certificate, stored in Trace Lab file, and give to supervisor for signature.

ee) When analysis is complete, reset unit to furnace.

ff) When technique has finished changing, shutdown the software, turn off the gases, the AA-800, the computer, monitor, printer, and vent fan. (In that order.)

11. Data Validation and Reporting

a) Check the correlation coefficient of the zero- intercept nonlinearity of the standard curve. If its correlation coefficient is less than 0.995, re-run the standard curve.

b) Check the characteristic mass of the analyte. If it is outside the +/- 20% range of the value given by the instrument manufacturer, check the purity of the standards and matrix modifiers.

c) Determine if the in-house standard falls into the appropriate range as established by control charting.

d) Determine the relative standard deviation (RSD) of the duplicate analysis of each sample. If this RSD exceeds 20%, repeat the analysis.