Standard Operation Procedure

Analysis of Major, Minor and Trace Elements in Animal Tissue Samples with ICP-OES and ICP-MS

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1. Application

This method covers the digestion of animal tissue samples and the analysis of major, minor and trace elements (total minerals, heavy metals and micro-nutrients) in these samples by ICP-OES (Thermo Jarrell Ash IRIS Advantage Inductively Coupled Plasma Optical Emission Spectrometry) and ICP-MS (VG PlasmaQuad PQ2 Turbo Plus Inductively Coupled Plasma Mass Spectrometry).

1.1 Animal tissue samples mainly consist of carbon, hydrogen, oxygen and nitrogen. Other abundant components are Na, K, Mg, Ca, Fe, Cu, Zn, P and S. These nine elements account for about 5% of a sample on a dry weight basis. Bone samples are extremely high in Ca and P. Hair samples are extremely high in S. An open-vessel acid digestion with HNO_3 + H_2O_2 (or HNO_3 + $HClO_4$ in some applications) is almost complete for most kinds of samples except some fatty materials.

1.2 When a sample is digested at a dilution factor of 100 (e.g. 0.5 g to 50 mL digestion and dilution), the amount of total dissolved solids is close to or less than 0.05% (500 mg/liter or ppm). The sample solution is directly analyzed by ICP-OES, but may be further diluted (e.g. 1 + 1 dilution) for ICP-MS.

1.3 A flexible digestion and dilution procedure can be used since the amount of available sample is frequently limited or since the concentrations of targeted elements are either too high or too low in samples. Because the digestion of animal tissue samples is complete in most cases, a change in digestion conditions (e.g. ratio of sample to acid, digestion temperature, or dilution factor) would not significantly affect the analytical consistency. Sample types (meat, bone, liver, hair, blood, urine, etc.) and target elements are the two primary parameters for setting analytical priorities.

2. Summary of method

2.1 Half a gram of dried sample (or equivalent) and five mL of concentrated nitric acid are added to a 50-mL Folin digestion tube. The mixture is heated to 120-130 ºC for 14-16 hours and is then treated with

hydrogen peroxide. After digestion, the sample is diluted to 50 mL. This solution is analyzed by ICP-OES for major and minor components, and further 1:1 (or 1+1) diluted and analyzed by ICP-MS for minor and trace components.

2.2 Alternatively, wet samples may be directly digested without having been dried. The water content is obtained from sub-samples or is assumed to be 78 – 80%.

2.3 After solid samples are converted into solutions samples, the procedures of "Elemental analysis of solution samples with ICP-OES" and "Elemental analysis of solution samples with ICP-MS" are followed.

3. Safety

3.1 All chemicals should be considered as potential health hazard. All relevant laboratory safety procedures are followed.

3.2 The use of perchloric acid for a sample digestion must be conducted in a hood designed specifically for perchloric acid. The user must be aware of the dangers involved using perchloric acid, such as the explosive nature of anhydrated perchloric acid and its extreme corrosive nature.

3.3 All animal samples could be a biomedical health hazard. Extreme caution should be exercised when animal samples are handled.

4. Interference

4.1 This method covers the analysis of over 30 elements in different kinds of samples by ICP-OES and ICP-MS. A general discussion of interference is lengthy but not necessarily relevant to a specific element, which is especially true if the sample matrix is not specifically defined. An enormous amount of literature is available to the analysis of metals and non-metals by ICP-OES and by ICP-MS. Reading the published articles is recommended.

4.2 In this method, the solution contains less than 500 ppm of dissolved solids for ICP-OES and ICP-MS analysis. The major components are Na, K, Ca, Mg, P and S. These components either do not pose significant interferences with other elements/isotopes or the potential interferences are well understood and controlled. Significant interferences are not expected, although some specific elements and or isotopes may be interfered.

5. Sample Collection, Preservation and Handling

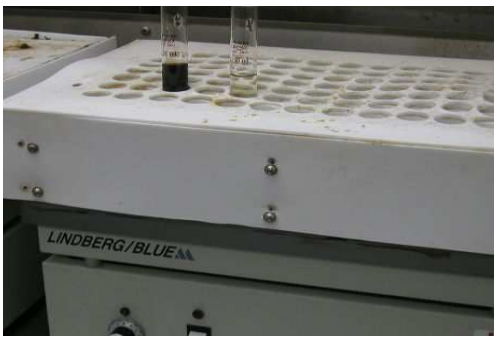
A representative sample of animal tissue is dried and ground. A 5-gram vial or equivalent is used to hold a sub sample in airtight storage. Wet samples may be kept frozen.

6. Apparatus and Device

6.1 Analytical balance (accurate to one milligram with a custom-made weighing pan for easier sample handling). The balance is interfaced to a computer via an RS-232 cable.

6.2 Borosilicate digestion tubes or equivalent (25 mm o.d. × 200 mm length) with graduations of 12.5, 25, 35 and 50 mL (e.g. KIMAX Borosilicate 47125-50 for use in Folin-Wu non-protein nitrogen determinations). The tubes are cleaned by soaking in 10% nitric acid bath overnight and rinsed with de-ionized water several times. The cleaned tubes are placed in tube racks upside down and let air-dried.

6.3 Insulated aluminum block with holes drilled to it to accommodate the Folin-Wu digestion tubes. Half of the tube (about 100 mm) is still exposed to air. The aluminum block is stacked on the top of a hot plate (e.g. Lindberg/Blue Hot Plate. Model: HP 53014C).



6.4 Ten universal pipette for dispensing concentrated nitric acid (e.g. Fisher Cat #136-8720).

6.5 ICP-OES: TJA Iris Advantage ICP-OES.

6.6 Eight-mL polystyrene test tubes (13 mm × 100 mm. e.g. Cat # 2110 by Perfector Scientific) for the ICP-OES autosampler are used "as is."

6.7 ICP-MS: VG PlasmaQuad PQ2 Turbo Plus ICP-MS (quadrupole ICP-MS).

6.8 Fourteen-mL polystyrene test tubes (17 mm × 100 mm. e.g. Falcon plastic tubes. Cat # 14-959-8 by Fisher Scientific) for the ICP-MS autosampler are cleaned by soaking in 10% nitric acid overnight and rinsed with de-ionized water. The tubes are air-dried before use.

7. Reagents

7.1 Concentrated nitric acid (e.g. TraceMetal grade. Fisher A509-212).

7.2 Hydrogen peroxide (>30%) (e.g. Certified A.C.S. grade. Fisher H325-500). Note: hydrogen peroxide is usually preserved with tin (Sn).

8. Pre-Digestion

8.1 Process fresh animal tissue samples "as is" without drying under certain limitations. The water content is assumed to be 78% or the water content is obtained from a sub-sample. Otherwise, dry samples at 60^{o}C for two days. Grind in a stainless steel Wiley mill or cut dried samples into small pieces. Store in a 5-gram vial or other appropriate container for airtight storage.

Note: In some applications, the final results may be expressed on wet weight basis. In this situation, obtain the water content if a sample is dried. Samples may be dried at 60°C or at 110°C. The water content could be different.

8.2 Weigh 0.50±0.01 g of dry sample, or 1.0±0.02 g of wet sample, or 5 mL of liquid sample (unknown samples, in-house quality control sample, and/or NIST SRMs) into 50-mL cleaned and air-dried Folin digestion tubes. Make one to three digestion blanks.

Note: Depending on sample availability, the sample size can be scaled down. The relative significance of "contamination" from lab-wares and from reagents may increase if the sample size is very "small." TEFLON digestion tubes may be used instead of glass digestion tubes.

8.3 Spike 0.04 mL of 10,000 ppm of Y (yttrium) as an internal reference standard (IRS) for the analysis by ICP-OES. Spike 0.04 mL of 10 ppm of Rh (rhodium) as an internal standard for the analysis by ICP-MS.

8.4 Add 5 mL of concentrated nitric acid. Soak the samples at room temperature for 2-3 hours.

Note: Perchloric acid may be used in some special applications but should be avoided as much as possible for safety concerns. Samples digested with $HClO_4$ are not good for the analysis of V, Cr, As, ^{77}Se, Rb and several other isotopes by using quadrupole ICP-MS.

9. Hot Plate Digestion

9.1 Place all of the Folin tubes in the block heater. Cover the tubes with plastic film to retard the water evaporation. Contamination from the plastic film is not considered. Alternatively, use small glass funnels.

Note: Samples should not be charred during digestion. If charred, add nitric acid to re-dissolve. However, this could cause higher digestion blank concentrations for several elements.

9.2 Set the block heater at 130ºC (Block Heater Lindberg Blue: t = 115ºC at mark 2.5, t = 130ºC at mark 3.0, t = >170ºC at mark 7).

9.3 The temperature ramps up to 120-130ºC after 1.5 hours. Keep heating at 120-130ºC for 14-16 hours.

9.4 Remove the film cover and properly dispose it. Take the tubes off the block heater. Let cool for several minutes (This is important).

9.5 Add 30% hydrogen peroxide at a ratio of 1 mL per sample. Place all of the tubes back onto the block heater. Heat for 20-30 minutes.

Note: Samples digested with H_2O_2 are not good for Sn analysis if the H_2O_2 is preserved with tin.

9.6 Take the tubes off the block heater and let them cool. Add H_2O_2 (as indicated in step 9.5 above) and digest for another 20-30 minutes.

9.7 Take all of the tubes off the block heater. Add water to the 50 mL mark. Let sit for 30 minutes or more.

9.8 Mix the samples. Nominal dilution factor = 100. Y = 8 ppm. Rh = 8 ppb.

10. Measurement by ICP-OES

10.1 Sample preparation for ICP-OES

10.1.1 Set 8-mL autosampler tubes in ICP-OES sample racks.

10.1.2 Transfer sample solutions from 50-mL tubes to 8-mL tubes.

10.1.3 For samples with extremely high analytes, the samples may be further diluted. Add 3 mL of sample solution and 3 mL of 2% nitric acid to the 8-mL autosampler tube (2^{nd} dilution. Nominal dilution factor = 200. Y = 4 ppm). Mix

Note: It might be labor intensive if a lot of samples need to be diluted before analysis. In-line dilution might an option. In SPAL, digested solutions are poured to the 8-mL autosampler tubes. The volume is adjusted to 3 mL by inserting a tubing into the autosampler tube to a prefixed depth and sucking any extra solution out (The tubing is connected to a vacuum device). Dispense 3 mL of 2% nitric acid to the autosampler tubes by using a re-pipette. Cover a rack of samples with plastic film and the whole rack of samples are mixed by pushing the film tightly against the tubes and using up-side down actions.

Note: Since an internal reference standard is used, the volume inaccuracy during dilution is irrelevant. A sample solution may be analyzed with other dilution ratios (i.e. 2:8, or 5:5 dilutions). During the data processing in later stage, the nominal dilution factor is always 100, whether the dilution is 1:5, 2:3, or 4:1 (See Appendix 1 in "Elemental analysis of solution samples with ICP-OES").

10.2 Measurement by ICP-OES

10.2.1 A detailed procedure is given in "Elemental analysis of solution samples with ICP-OES."

10.2.2 Digestion blanks are also measured with other samples.

10.3 Reporting after ICP-OES

10.3.1 The details are given in "Elemental analysis of solution samples with ICP-OES."

10.3.2 After the concentration of Y is normalized to 8 ppm, the dilution factor is 100 either for the digested solution (1^{st} dilution, actual DF = 100, Y = 8 ppm) or for the further diluted solution (2^{nd} dilution, actual DF = 200, Y = 4 ppm), if accurately 0.5 gram of soil is spiked with 0.04 mL of 10,000 ppm of yttrium as the internal reference standard.

11. Measurement by ICP-MS

11.1 Sample preparation for ICP-MS

11.1.1 Set 14-mL Falcon tubes in the ICP-MS autosampler racks. Transfer the sample solutions to the Falcon tubes.

11.1.2 Adjust the volume to 5 mL. Add 5 mL of 2% nitric acid. Mix well. The nominal dilution factor is 200 and the IRS is 4 ppb of Rh.

11.1.3 Since an internal reference standard is used, the volume inaccuracy during dilution is irrelevant. If the concentrations of target elements are expected to be relatively high, the

samples are further diluted, either by 2+8 dilution or 1+9 dilution. Otherwise, a sample solution may be directly analyzed without any further dilution (i.e. 10+0 dilution). During the data processing in later stage, the nominal dilution factor is always 200, whether the dilution is 1+9, 2+8, 5+5 or 10+0.

11.2 Measurement by ICP-MS

11.2.1 A detailed procedure is given in "Elemental analysis of solution samples with ICP-MS."

11.2.2 Edit the menu depending on specific samples or analytical requests.

Note: The analysis by ICP-MS is flexible and is easily expanded to other elements. In combination with the working standard, both of the working standard and the acquisition menu can be changed accordingly for additional elements.

11.3 Data processing

11.3.1 The details are given in "Elemental analysis of solution samples with ICP-MS."

11.3.2 The overall DF is 200, after this procedure is followed exactly, although the actual dilution could be variable as presented above in 11.1.3. Otherwise, adjust the DF accordingly.

Scenario one: 10 ppm (or 10,000 ppb) of element X in 0.5 gram of solid sample with 0.04 mL of 10 ppm Rh is digested and diluted to 50 mL (1^{st} DF = 100). This 1^{st} solution (X = 100 ppb, and Rh = 8 ppb) is further diluted by 5:5 (2^{nd} DF = 2) to contain 50 ppb of X and 4 ppb of Rh in a 2^{nd} solution (overall DF = 200). This 2^{nd} solution is measured against a standard containing 10 ppb of X and 4 ppb of Rh and the measured result is 50 ppb. After applying the overall dilution factor of 200, the concentration of X in the solid material is 50 ppb × DF 200 = 10,000 ppb = 10 ppm.

Scenario two: Element X in the 2^{nd} solution (X = 50 ppb and Rh = 4 ppb) is still much higher than the standard (X = 10 ppb and Rh = 4 ppb). This 2^{nd} solution is diluted by 5 times (3^{rd} DF = 5, total DF = 100 × 2 × 5 = 1000) to contain 10 ppb of X and 0.8 ppb of Rh and this 3^{rd} solution is measured. There are two ways to process here. Option 1: ignore the third dilution factor. The signal ratio of 3^{rd} solution (10 ppb X/0.8 ppb Rh) is compared to the signal ratio of standard (10 ppb X/4 ppb Rh) and the concentration in the 3^{rd} solution is calculated to be 50 ppb of X per 4 ppb of Rh. After applying the dilution factor, X in the solid sample is 50 ppb × 200 = 10 ppm. Option 2: At step 13.1, set the IRS concentration to be 0.8 ppb Rh for this specific sample (3^{rd} solution), X in this 3^{rd} solution will be calculated to be 10 ppb against a standard of 10 ppb X with 4 ppb Rh. Now the total DF is 1000 and X in the solid sample is 10 ppb × 1000 = 10 ppm.

12. Quality assurance (QA) and quality control (QC)

12.1 It should be kept in mind that ICP-OES and ICP-MS, either combined or used alone, have broad applications in unlimited situations. A general discussion about QA/QC practice is not specific to a particular application,

yet detailed discussions about various applications become too lengthy and are beyond the scope of this procedure.

12.2 Some QA/QC practices are presented in “Elemental analysis of solution samples with ICP-OES” and in “Elemental analysis of solution samples with ICP-MS.”

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