D-JRP15-FED-AMR-WP4.1

Protocol for Elements Quantification

Version 1
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A. Preliminary considerations

Sample collection for further quantification of elements in **WP4** is described in the corresponding sampling protocols of each analysed matrix and/or compartment (see **annex of D-JRP15-FED-AMR-WP2.1).** Likewise, the sample identifiers should be consulted in the corresponding sampling protocols.

B. Sample preparation

1. Digestion procedure for solid samples

This procedure can be applied to solid samples with high content of organic matter, including vegetation, fodder/feed for animal consumption, food for human composition (raw or "as-consumed"), hair/fur, and organic waste. Same procedure must be followed for "method blanks" and certified reference materials.

Step 1 Weight 3 aliquots (replicates) of 0.2500 – 0.5000 ± 0.0001 g for each of the samples directly in acid-washed ceramic crucibles.

Step 2 Dry ash the samples using a muffle furnace (Carbolite AAF1100) at 500oC for 12 hours.

Step 3 After calcination, dissolve the resulting homogenised ash with 1 mL of concentrated nitric acid (HNO3 for trace metal analysis 68% (v/v), Fisher Scientific). This operation must be carried out in fume-hood.

Step 4 Transfer digest to a pre-weighed 25 mL Sterilin® tube. Wash the crucible with dionised water (DIW, 18 MΩ) multiple times with small aliquots and transfer to the Sterilin® tube, to ensure complete transfer of all digested solution. Make up to a final volume of 25 mL before weighting and recording final mass to calculate dilution factor (analytical balance, ±0.0001g).

Step 5 Filter samples with 0.45-µm syringe-top filters (Millex®-GP, Millipore, Hertfordshire, UK) and transfer into 15-mL polypropylene tubes, ready for analysis.

2. Digestion procedure for geological samples

This procedure can be applied to solid samples of geological origin and/or with high content of silicates. This may include soils, sediments, road dust, atmospheric particulate matter, cement and cement clinkers, ash, slag or pulverised rock. Same procedure must be followed for "method blanks" and certified reference materials.

Step 1 Weight 3 aliquots (replicates) of 0.2500 ± 0.0001 g for each sample, directly into acid-washed and dried polypropylene digestion squat beakers.

Step 2 Place glass beakers (one for each replicate) filled with water and anti-bubbling granules on top of a hot plate installed in a fume hood designated for HF digestion. Set the temperature to 120□C.

Step 3 Add 12 mL of aqua regia (HNO3:HCl, 1:3, trace metal analysis reagent grade) to each replicate samples and place the squat beakers into the water-filled digesters.

Step 4 Leave the samples to heat up, and allow any effervescence to subside before adding 10 mL of conc. HF.

Step 5 Reduce the acid mixture to near dryness and allow to cool in the digesters. Top up the water in the digester as necessary during the process.

Step 6 Remove the squat beakers with the samples from the digesters and dissolve the residues were with 1 mL of conc. HNO3 (for trace metal analysis 68% (v/v), Fisher Scientific)

Step 7 Transfer digest to a pre-weighed 25 mL Sterilin® tube. Wash the crucible with dionised water (DIW, 18 MΩ) multiple times with small aliquots and transfer to the Sterilin® tube, to ensure complete transfer of all digested solution. Make up to a final volume of 25 mL before weighting and recording final mass to calculate dilution factor (analytical balance, ±0.0001g).

Step 8 Filter samples with 0.45-µm syringe-top filters (Millex®-GP, Millipore, Hertfordshire, UK) and transfer into 15-mL polypropylene tubes, ready for analysis.

3. Preparation of biological liquid samples for analysis

This procedure is recommended for liquid samples of biological origin. These may include, whole blood, blood serum, milk and urine.

Step 1 Prepare alkaline diluent of composition: 1% v/v ammonium hydroxide (Fisher Scientific Ltd, Leicestershire, UK), 0.05% v/v Triton X-100 (Fisher Scientific Ltd, Leicestershire, UK), 0.05% v/v ethylenediaminetetraacetic acid (EDTA disodium salt, dissolved in DI water 18.2 MΩ) (Fisher Scientific Ltd, Leicestershire, UK) and 2% v/v methanol (Sigma-Aldrich, Dorset, UK).

Step 2 Dilute samples as required to fit in the linear calibration range for instrumentation (typically 1000 µg/L for ICP-MS, 10-100 mg/L for MP-AES). Each sample dilution must be carried out in triplicate and dilution factors must be determined by mass on analytical balance (±0.0001 g) to ensure accuracy.

Step 3 Filter the diluted samples using a 0.45-µm membrane syringe-top filter (Millex®-GP, Millipore, Hertfordshire, UK) and transfer into 15-mL polypropylene centrifuge tubes (Fisher Scientific, Leicestershire, UK) ready for analysis.

4. Instrumental operation

1. Analysis of trace elements by ICP-MS

This procedure can be applied for the quantification of trace elements by ICP-MS (inductively coupled plasma mass spectrometry) in the range from 0 to 1000 µg/L. Sample dilution may be required.
For solid samples prepared according procedures in sections 1.1 and 1.2 of this document, acid digests will be diluted so that the concentrations of acid must be between 1 and 5% v/v (depending on sensitivity and concentration in the sample) and never higher than 5% v/v. Dilution factors should be determined by mass on analytical balance (±0.0001 g) to ensure accuracy.
Steps 1, 2 and 3 described bellow must be carried out on a daily basis, to ensure optimum performance of the instrumentation and accuracy of results.

Step 1 Tuning of ICP-MS (Agilent 7800x): Optimisation of the instrumentation must be carried out using autotuning procedure recommended by manufacturer, to ensure good sensitivity, accuracy and precision This must be completed after warming up of the plasma and using the ICP-MS tuning solution containing Li, Mg, Y, Ce, Tl and Co; 100 mL, 10 mg/L (matrix 2% HNO3) from Agilent.

Step 2 Calibration: A range of multielement standards from 0 to 1000 µg/L must prepared in 1% HNO3 (v/v) from 1000 mg/L standard stock solution (Aristar, Fisher Scientific, UK) for each of the analytes. A 100 µg/L mixture of internal standards (Sc, Ge, Rh and Bi;

Agilent Technologies, UK) should be injected into the plasma simultaneously with the samples and standards to compensate for any drift in the signal intensity during the ICP-MS analyses. Selection of the appropriate internal will be made on the basis of the proximity of the m/z ratios for the internal standards and the analyte, and the composition of the unknown sample. The ratio of the cps (count per second) for the analyte and internal standard will be used for calibration against the concentrations in the standards.

Step 3 Validation: The ICP-MS performance should be assessed on a daily basis using at least two certified reference waters; namely SRM 1640a (National Institute of Standards and Technology, Maryland, USA) and TMDA-54.4 (National Water Research Institute, Ontario, Canada) or equivalent, depending on availability from providers. These will be measured under the same conditions of the standards and samples.

2. Analysis of major elements by MP-AES

This procedure can be applied for the quantification of major elements by MP-AES (microwave induced plasma atomic emission spectroscopy) typically in the range from 0 to 10 mg/L for alkaline and alkaline-earth elements and in the range from 0 to 100 mg/L for other major elements. Sample dilution may be required.

For solid samples prepared according procedures in sections 1.1 and 1.2 of this document, acid digests will be diluted so that the concentrations of acid must be between 1 and 5% v/v (depending on sensitivity and concentration in the sample) and never higher than 20% v/v. Dilution factors should be determined by mass on analytical balance (±0.0001 g) to ensure accuracy.

Step 1 Wavelength calibration of MP-AES (Agilent 4210). Optimisation of wavelength resolution should be carried out on a weekly basis. This must be completed after warming up of the plasma and using the wavelength calibration solution containing 50 mg/L Al, As, Ba, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Se, Sr, Zn and 500 mg/L K (5 % HNO3) from Agilent.

Step 2 Calibration. Calibration must be completed on a daily basis. A range of multielement standards must prepared in 1% HNO3 (v/v) from 1,000 mg/L or 10,000 mg/L standard stock solution (Aristar, Fisher Scientific, UK) for each of the analytes. The standard should cover the calibration range from 0 to 10 mg/L for alkaline and alkaline-earth elements and in the range from 0 to 100 mg/L for other major elements. A 5 mg/L mixture of internal standards (Sc, Y and Be; Aristar, Fisher Scientific, UK) should be injected into the plasma simultaneously with the samples and standards to compensate for any matrix effects and any drifts in the signal intensity during analyses. The used of Sc and Y is recommended for ionic lines, while Be can be used for both atomic and ionic lines. The ratio of the emission intensity (arbitrary units) for the analyte and internal standard will be used for calibration against the concentrations in the standards.

Step 3 Validation. The MP-AES performance should be assessed on a daily basis using at least two certified reference waters; namely SRM 1640a (National Institute of Standards and Technology, Maryland, USA) and TMDA-54.4 (National Water Research Institute, Ontario, Canada) or equivalent, depending on availability from providers. These will be measured under the same conditions of the standards and samples.