STANDARD OPERATION PROCEDURE
Faculty of Biosciences, NMBU

Method name: Various minerals
BIOVIT No.: Arb1078

1. Introduction / purpose

This method can be used to determine the mineral composition and the total amount of various minerals in organic and most inorganic samples, both in solid and liquid state.
Different minerals can be used as a marker in feeding and digestibility studies of fish (Yttrium, Y Arb1073) or in metabolic experiments on ruminants (Chromium, Cr and Ytterbium (Yb) Arb1071)). LabTek has per 022020 **standards with control** for the following minerals: **Macro: Ca, K, Mg, Na, P and Micro: Zn, Y, Fe, Mn, Yb Cu, Se and Cr. We can identify: As, Cd, Co, Mo, Ni, and V.**

Sample decomposition during digestion is the most critical part of the analysis as incomplete digestion can have a great influence on the result. The loss of analyte during sample preparation step must also be eliminated. An effective method is to use microwave digestion with acid as everything takes place in a closed system.

The pre-digested samples are analyzed spectrophotometrically with MP-AES (Microwave Plasma Atomic Emission Spectrometer) from Agilent.

2. Reagents

- Concentrated HNO_3 - (microwave decomposition)
- Hydrogen peroxide H_2O_2 - (microwave decomposition)
- 2% HNO_3 - (washing solution for injector); 20 mL HNO_3 + 980 mL Milli Q water
- 16% HNO_3 - (for dilutions / blank); 160 mL HNO_3 + 840 mL Milli Q water
- A calibration curve is created by first preparing a set of standard solutions with known concentrations of the analyte, at least 5 concentrations of standards are needed. The instrument response is measured for each and plotted vs. concentration of the standard solution. The linear portion of this plot can then be used to predict the concentration of a

sample of the analyte, by correlating its response to concentration. Have the same acid concentration in the standards and samples.

➢ Control test: div. ring test samples for some of the minerals.

3. Risk assessment

- Concentrated HNO_3 - Harmful in contact with skin and eyes, as well as if swallowed.
  - ➢ Wear gloves and work in the fume hood.
  - ➢ In case of skin contact - rinse with water, remove contaminated clothing, call a doctor.
  - ➢ In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

- Hydrogen peroxide (30%) - Harmful if swallowed and in contact with eyes.
  - Harmful to aquatic life with long lasting effects.
  - ➢ Wear gloves and work in the fume hood.
  - ➢ If swallowed - rinse mouth, call a doctor in case of discomfort.
  - ➢ In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

Formation of nitrous gases:

Nitrous gases are formed by the decomposition of nitric acid and can cause irritation in the upper and lower respiratory tracts - can be critical. All work with decomposed samples is done in the same fume hood until the samples are diluted. Leave the diluted samples in the fume hood about 30 min with an open cork. Use autosampler with cover.

4. Equipment

- ➢ MP-AES 4200 (Agilent Technologies)
- ➢ Start D Microwave digestion system (Milestone Srl)

5. Sample material

Grain products / fertilizers / rumen and intestinal contents, etc.
Degree of grinding: 0.5 mm.

6. Work procedure

Sample preparation:
Digestion in microwave oven (rotor = max 24 samples)

1. Weigh out approx. 0.1 gram of material (0,2 grams for microelement analysis)
2. Reagents; 8 mL HNO_3 and 2 mL H_2O_2 (5: 1)
3. REMEMBER; MINIMUM 10 mL REAGENTS / TUBES!
4. Use Lab Dancer after adding reagent - avoid lumps of dry material.
5. REMEMBER; put the protector on the temperature sensor!
6. Retrieve existing method.
7. Enter time / power / temperature.
8. 100 W / sample - up to 1200 W.
9. Remember 10 minutes of ventilation after driving.
10. Do not open tubes until the temperature is below 50 °C.
11. When opening tubes; make sure that the pressure relief valve is facing away from you!
12. Transfer the sample to 50 mL plastic tubes and dilute to the mark with Milli Q water.
    Provides a matrix of 16% HNO_3.
13. Put a cork on the tube, and then mix well.
14. Leave the tube so that any particles sink to the bottom.
15. The plastic tube can be inserted directly into the autosampler.

Start-up of MP-AES:

16. Tight tubing's for washing solution (on autosampler).
17. Add washing solution if necessary.
18. Open **MPExpert** (icon - desktop).
19. Open the **PUMP** tab - press *«normal».*
20. Tighten tubings on the instrument itself (easier when the pump is running).

21. **Plasma -** *"plasma on"* (sounds start up, check in window that plasma is on).
22. **Autosampler -** double click on position for water (Milli Q water) (NB: unscrew the cap).
23. **Pump -** *«fast».*
24. **Instrument status -** overview of the instrument (here you can see if plasma is not turned on due to air in the system or see error messages).
25. Look in the spray chamber- when it has become foggy; Pump - «normal».

    If it says "Calibration overdue" - perform a wavelength calibration point 52 (approx. Once per month).

Check sensitivity

26. Autosampler - double-click on the position for the sensitivity test (remember to take off the lid).
27. Pump – fast.
28. Instrument: Quick read - press «Y» in periodic table.
29. Check that the line for 371,029 nm is highlighted.
30. Pump - normal (when the sample has reached the spray chamber).
31. Read.
32. Read off the intensity x 3 times. Write the result in logbook. Intensity should be around 100 000 (Between 85 000 and 120 000)
33. Autosampler – rinse. Quick read
34. Put a tube with at least 4 mL test solution on certain position of the autosampler.
35. Instrument-quick read.
36. Measure the intensity of the selected mineral, for example press Sodium and then read: scan 588,995: 120,000 intensity. Write in the lab journal. Gives an indication of whether you need to dilute the sample further. Dilute stock solutions if necessary, to the appropriate ranges using a diluent that will match the sample matrix.

Create sequence:

37. **MPExpert -** *"New From"*
38. Analytical calibration can be also carried out using multimineral standard solutions. You may have to run through the sample several times for the different selected minerals because they are in different concentration levels. This needs to be tried out a bit.
39. Insert blank + standards in rack at the back, from left: blank - standard 1- standard 2 etc. **NB: remove caps**
40. Put samples in the next rack (position 1 = right corner).
41. **Standards-** can add / remove standards. Set expected calibration error % (0.999 or 0.990).
42. **Sequence -** Enter samples, NB correct positions. If necessary, rename the samples. If driving overnight; uncheck "turn plasma and pump off".
43. **Autosampler -** Check that standards and samples are in the same positions as shown on the screen.
44. Press **"Run"** (upper tab).
45. Gets up questions about storage - save under ÅÅMMDD_RekvXX_Navn (should be mpws after)
46. Check Autosampler racks - press "OK".
47. **Analysis -** can follow the results while driving.
48. The analysis is complete: *Worksheet run has been completed* - press "OK".
49. Save raw data: **Analysis** left click on the blue triangle next to the Rack tube to highlight the runs; right click *«Export selected solutions»;* stored on desktop under: «Results MP AES».
50. Enter the excel file and copy the result under *"concentration"* (mg / L); enter in the requisition.

End the instrument:

51. Pump-off.

52. Plasma- off.

53. Loose the tubing on the instrument.

54. Loose for tubing for washing solution (autosampler).

Wavelength calibration (once per month)

55. Put injector in calibration solution.

56. Instrument - Instrument calibration-Wavelength Calibrate and Check.

57. Check.

58. Zero order check.

59. Run-When done: *"last successful calibration"* comes up with a date.

7. Calculation of the analysis result:

Results taken from MP-AES come in mg/L (these are transferred in the excel sheet).
All formulas are inside the excel sheet (requisition sheet), but are as follows:
mg/L x final volume (0.05 L) / weighed amount (g) = mg/g or g/kg.
If final volume is scaled down (for small samples) this must be adjusted in the formula.
Remember to pay attention to any dilutions.

8. Various tips:

- If you come across the probe arm on the autosampler, it must be restarted (on / off button) on the instrument.
- If there are a lot of drops in the spray chamber, this must be washed. It can be put in aqua regia.
- Standards: if the calibration curve has low linearity, "rational" can be selected.
  Rational fit is a nonlinear curve fit and allows an extended working range so that sample analysis can be carried out using a single wavelength without further dilutions being required.

- Rack 1 should be used for standards (defaults if there are different size racks, so be careful when creating a new template).
- Check the optical window and wash it with soap, rinse and wipe. It can get cloudy. In sub-catalog (located on desktop) for ordering: Pre-optic window: G800-64112.
- Torch can be washed in 10% nitric acid or 50% aqua regia.
- Spray chamber can be washed if it gets dirty and drops form on the inside. Wash in 10% nitric acid and dry lightly. G800-70007.
- Other parts that are nice to have:
    - ➢ One Neb Nebulizer: 2010126900.
    - ➢ Tubing orange/green tabs with flared ends. 371006800.
    - ➢ Blue / blue (going from the spray chamber).
    - ➢ Autosampler: s 26 (atomabs) SPS 3:
    - ➢ Probe: 9910111900 (Replace if chipped, cracked or distorted.).

9. References:

(1) Austreng, E. Storebakken, T., Thomassen, M. Refstie, S., Tomassen, Y., 2000,Aquaculture, 188, 65-78.

(2) Reis, P., Valente, L., Almeida, M., 2008, Food Chemistry, 108: 3, 1094-1098.