STANDARD OPERATION PROCEDURE
Faculty of Biosciences, NMBU

METHOD NAME: Chromium and ytterbium
BIOVIT-No.: Arb1071

1. Introduction

Chromium (Cr) and ytterbium (Yb) are used as markers in metabolic experiments in ruminants. The marker substances are dissolved in water and injected via a peristaltic pump through a plastic tube directly into the rumen of the animal. The concentration of Cr and Yb is determined spectrophotometrically with MP-AES after dilution or decomposition of the samples. The concentration of the injection solution will vary from 1000-1800µg / ml.

Sample decomposition during digestion is the most critical part of the analysis as incomplete decomposition can have a great influence on the result. In the microwave-assisted closed system, complete digestion is performed by using concentrated nitric acid (HNO_3) and hydrogen peroxide (H_2O_2).

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 – (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
- Cr/Yb standards (0,01-0,05-0,1-0,25-0,5-0,75-1,0-2-4-6 mg/L). Have the same acid concentration in the standards and samples.

- Control test: cow manure added Cr/Yb

3. Risk assessment

- Concentrated HNO_3 – Harmful in contact with skin and eyes, as well as swallowed.
  - Wear gloves, and work in the fume hoods.
  - In the case of skin contact- rinse with water, remove contaminated clothing, call a doctor/physician.
  - In case of eye contact – 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 hoods.
- If swallowed- rinse mouth, call a doctor in case of discomfort.
- In case of eye contact – 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. Equipement

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

5. Sample material

Feed, faeces etc samples 0.5 degree of grinding.

6. Work procedure

Sample preparation:

For fluid samples:

Digestion is not necessary for injection fluid samples.

1. Centrifuge the tubes at 3000 rpm for 10 minutes.
2. Mix 0,25 ml sample with 49,75 ml Milli-Q water (1:200 dilution)

Urine and rumen fluid is digested in the same manner as solid samples.

1. Spin the sample to get particles in suspension.
2. Pipette 2 ml of sample to the tubes. After digestion transfer the sample to 50 ml tubes and dilute with Milli-Q water to the 50 ml mark, this is a 1:25 dilution.

For solid samples:

The microwave acid digestion method is applied to the decomposition of solid samples. (rotor = max 24 samples)

1. Weigh out approx. 0,1-grams of sample.
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 to ventilate for 10 minutes after the digestion process.
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 to 50 mL plastic tubes and dilute to the mark with Milli Q water. Provides matrix of 16% HNO_3.
13. Put the lid on the plastic tube and turn several times to mix.
14. Particles in a matrix will settle down when left undisturbed.
15. The plastic tube can be inserted directly into autosampler.

Start up MP AES:

16. Tighten the tubing for washing solution (on autosampler).
17. Add 2 % HNO3 washing solution if necessary.
18. Open **MPExpert** (icon – desktop)
19. Open the **PUMP** tab – press «*normal*»
20. Tighten the tubing on the instrument itself (easier when the pump is running)
21. **Plasma** – «*plasma on*» (start signal sound, check in window that plasma is on)
22. **Autosampler** – doble click on the position for water (milli Q) (***NB: take the lid off***)
23. **Pump** – «*fast*»
24. **Instrument- Status** – (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 «Calibration overdue»-perform a wavelength calibration point pkt 52 (once per month).

Check sensitivity:

26. Autosampler- double click on the position for sensitivity (remember to take off the lid)
27. Pump - fast
28. Instrument: Quick read – press «Y» in the 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 (press read 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 the injector in the sample
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. Double click: ex. Yttrium_180323
39. Insert the blank + the standards in rack, 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 the sample codes, NB correct positions. If necessary, rename the samples. If the samples are running overnight; adjust «*turn plasma and pump off*»
43. Autosampler- Check that the standards and samples are in the same positions as shown on the screen.
44. Press «**Run»** (upper tab).
45. Question about storage – save under ÅÅMMDD_RekvXX_Name (should be mpws after)
46. Check Autosampler racks – press "OK"
47. Analysis – The results of the sequence run are displayed on the monitor during the run.
48. The analysis is complete: *Worksheet run has been completed* **-** press "OK"
49. Save the data: **Analysis**-left click on the blue triangle next to the rack tube to highlight the runs: Right click «*Export selected solutions*»; stored desktop under: «Results MP AES».
50. Enter the excel file and copy the results under «*concentration*» (mg/L); Put the results in desirable requisition.

Turn off the instrument:

48. Pump- *off*
49. Plasma- *off*

50. Loose the tubing on the instrument.
51. Loose the tubing for washing solution (autosampler).

Wavelength calibration (once per month)

52. Put the injector in the calibration solution.
53. Instrument – Instrument calibration -Wavelength Calibrate and Check
54. Check
55. Zero order check
56. Run-When done: "*last successful calibration*" will show up with date.

7. How to calculate the results:

Results taken from MP-AES are in mg/L (put them in a Excel worksheet)
All formulas are inside the Excel worksheet (requisition sheet), as follows:
mg/L x final volume (0,05 L) / weighed amount (g) = mg/g or g/kg

If final volume is scaled down (small samples) this should be adjusted in the formula.
Remember to pay attention to any dilutions.

8. Different notices:

- Try to prevent accidental contact with the probe arm, if yes- restart it (on/off button) on the instrument.
- If any drops in the spray chamber, wash in 50% aqua regia.
- Standards: If the calibration curve has low linearity "rational" can be selected and error can be set up (by multicomponent method).
- Rack 1 should be used for standards (defaults if there are different size of racks, so be careful when creating a new template).
- Check if the optical window is dirty, wash it with soap, rinse and wipe. It can get cloudy. In «del katalog» (desktop) for ordering: Pre-optic window: G800-64112.
- The torch can be washed in 10 % HNO_3 or 50 % aqua regia.
- The spray chamber can be washed if it gets dirty and drops form on the inside. Wash in 10 % s10 % HNO_3, and dry lightly. G800-70007.
- Other parts that are nice to have:
- One Neb-nebulizer: 2010126900.
- Tubing: orange/green with flared ends. 371006800.
- Blue/blue (going from the spray chamber).
- Autosampler: s 26 (atom abs) SPS 3:
- Probe: 9910111900 (replace if chipped, cracked or distorted).