**STANDARD OPERATION PROCEDURE**

**Faculty of Biosciences, NMBU**

**METHOD NAME: Chromium and ytterbium**

BIOVIT-No.: Arb1071

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**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 (HNO3) and hydrogen peroxide (H2O2).

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

**2. Reagents**

* Concentrated HNO3 – (microwave decomposition)
* Hydrogen peroxide – (microwave decomposition)
* 2 % HNO3 – (washing solution for injector); 20 mL HNO3 + 980 mL milli Q-water
* 16 % HNO3 – (for dilutions/ blank); 160 mL HNO3 + 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)
* Control test: cow manure added Cr/Yb

**3. Risk assessment**

* Concentrated HNO3 – 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.

**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 HNO3 and 2 mL H2O2 (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% HNO3.
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:

1. Tighten the tubing for washing solution (on autosampler).
   1. Add 2 % HNO3 washing solution if necessary.
   2. Open **MPExpert** (icon – desktop)
   3. Open the **PUMP** tab – press «*normal*»
   4. Tighten the tubing on the instrument itself (easier when the pump is running)
   5. **Plasma** – «*plasma on*» (start signal sound, check in window that plasma is on)
   6. **Autosampler** – doble click on the position for water (milli Q) (***NB: take the lid off***)
   7. **Pump** – «*fast*»
   8. **Instrument- Status** – (here you can see if plasma is not turned on due to air in the system, or see error messages)
   9. 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:

* 1. Autosampler- double click on the position for sensitivity (remember to take off the lid)
  2. Pump - fast
  3. Instrument: Quick read – press «Y» in the periodic table.
  4. Check that the line for 371.029 nm is highlighted.
  5. Pump – normal (when the sample has reached the spray chamber)
  6. Read
  7. 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)
  8. Autosampler – rinse

Quick read

* 1. Put the injector in the sample
  2. Instrument-quick read
  3. 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:

* 1. **MPExpert- “***New From***”.**
  2. Double click: ex. Yttrium\_180323
  3. Insert the blank + the standards in rack, from left- blank - standard 1- standard 2 etc.

***NB:*** remove caps.

* 1. Put samples in the next rack (position 1= right corner)
  2. **Standards-** can add/remove standards. Set expected calibration error % (0,999 or 0,990).
  3. **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*»
  4. **Autosampler-** Check that the standards and samples are in the same positions as shown on the screen.
  5. Press «**Run»** (upper tab).
  6. Question about storage – save under ÅÅMMDD\_RekvXX\_Name (should be mpws after)
  7. Check Autosampler racks – press “OK”
  8. **Analysis** – The results of the sequence run are displayed on the monitor during the run.
  9. The analysis is complete: *Worksheet run has been completed* **-** press “OK”
  10. 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».
  11. Enter the excel file and copy the results under «*concentration*» (mg/L); Put the results in desirable requisition.

Turn off the instrument:

1. **Pump**- *off*
2. **Plasma**- *off*
3. Loose the tubing on the instrument.
4. Loose the tubing for washing solution (autosampler).

Wavelength calibration (once per month)

1. Put the injector in the calibration solution.
2. Instrument – Instrument calibration -Wavelength Calibrate and Check
3. Check
4. Zero order check
5. Run-When done: “*last successful calibration*” will show up with date.
6. **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.

1. **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 % HNO3 or 50 % aqua regia.
* The spray chamber can be washed if it gets dirty and drops form on the inside. Wash in 10 % s10 % HNO3, 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).