**STANDARD OPERATION PROCEDURE**

**Faculty of Biosciences, NMBU**

**Method name: Yttrium**

BIOVIT No.: Arb1073

**1. Introduction**

Yttrium (Y2O3) is often used as a marker in digestive studies of fish as Yttrium should not affect the fish's metabolism and it can be added to the feed in relatively low concentrations (0.1 g/kg).

It is then important to be able to determine Yttrium in the feeds and faeces. 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 and hydrogen peroxide.

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 H2O2 - (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
* Yttrium standards (0.05-0.1-0.2-0.5-1.0 mg / L)
* Control test: pig feed supplemented with 0.01% Yttrium.

**3. Risk assessment**

* Concentrated HNO3 - Harmful in contact with skin and eyes, as well as swallowed.
* Wear gloves, and work in the fume hood.
* 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.

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

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

**4. Equipment**

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

**5. Sample material**

Feed, faeces e.g. samples 0.5 mm degree of grinding.

**6. Work procedure**

Sample preparation:

Support for decomposition in microwave oven (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 Lap 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 of MP-AES:

1. Tighten the tubing for washing solution (on autosampler).
2. Add washing solution if necessary.
3. Open **MPExpert** (icon - desktop).
4. Open the **PUMP** tab - press *«normal».*
5. Tighten the tubing on the instrument itself (easier when the pump is running).
6. **Plasma -** *"plasma on"* (start signal sound, check in window that plasma is on).
7. **Autosampler -** double click on position for water (milli Q water) **(NB: unscrew the cap).**
8. **Pump -** *«fast».*
9. **Instrument -Status** (here you can see if plasma is not turned on due to air in the system, or see error messages).
10. Look in the spray chamber- when it has become foggy; **Pump -** *«normal».*

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

Check sensitivity

1. Autosampler – double click on the position for the sensitivity test (remember to take off the lid).
2. Pump – fast.
3. Instrument: Quick read - press «Y» in 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).
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 blank + standards in rack at the back, from left- blank - standard 1- standard 2 etc. ***NB:*** remove caps.
4. Put samples in the next rack (position 1 = right corner)
5. **Standards -** can add /remove standards. Set expected calibration error % (0.999 or 0.990).
6. **Sequence -** Enter the samples codes, NB correct positions. If necessary, rename the samples. If samples are running overnight; adjust *"turn plasma and pump off".*
7. **Autosampler -** Check that the standards and samples are in the same positions as shown on the screen.
8. Press **"Run"** (upper tab).
9. Questions about storage - save under ÅÅMMDD\_RekvXX\_Navn (should be mpws after)
10. Check Autosampler racks - press “OK”.
11. **Analysis –** The results of the sequence run are displayed on the monitor during the run.
12. The analysis is complete: *Worksheet run has been completed* - press “OK”
13. 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».
14. Enter the excel file and copy the result under *"concentration"* (mg / L); enter in the requisition.

End the instrument:

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

Wavelength calibration (once per month)

1. Put 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"* show up with date.

 **7. Calculation of the analysis result:**

 Results taken from MP-AES are in mg / L (put them in 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 must be adjusted in the f

 formula. Remember to pay attention to any dilutions.

**8. Various tips:**

* Try to prevent accidental contact with the probe arm on the autosampler, 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 “delkatalog” (located on desktop) for ordering: Pre-optic window: G800-64112.
* The torch can be washed in 10% nitric acid or 50% aqua regia.
* The 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 (atom abs) 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.