

STANDARD OPERATION PROCEDURE
Faculty of Biosciences, NMBU

Method name: Yttrium

BIOVIT No.: Arb1073

1. Introduction

Yttrium (Y_2O_3) 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 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
- Yttrium standards (0.05-0.1-0.2-0.5-1.0 mg / L)- Remember to have the same acid concentration in the standards and samples used.
- Control test: pig feed supplemented with 0.01% Yttrium.

3. Risk assessment

- Concentrated HNO_3 - 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.

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- 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.

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.

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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:

16. Tighten the tubing 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 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** - double click on position for water (milli Q water) (**NB: unscrew the cap**).
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 52 (Once per month).

Check sensitivity

26. Autosampler – double click on the position for the sensitivity test (remember to take off the lid).

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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 (press read 3 times).
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. **MPE**expert - “*New From*”.
38. Double-click: ex. Yttrium_180323
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 the samples codes, NB correct positions. If necessary, rename the samples. If 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).

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45. Questions about storage - save under ÅÅMMDD_RekvXX_Navn (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 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 the calibration solution.
56. Instrument - Instrument calibration-Wavelength Calibrate and Check.
57. Check.
58. Zero order check.
59. 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:
 $\text{mg / L} \times \text{final volume (0.05 L)} / \text{weighed amount (g)} = \text{mg / g or g / kg}$

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:

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- 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.

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