4. Digestions

4.1 Microwave Digestions

4.1.3 Nitric acid: Plant and animal tissue

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Suitability

Nitric acid digestion is especially suited for digestion of herbaceous, subsurface plant biomass but also for animal tissue and mounting resin. Elements that are often measured are e.g. Fe, Mn, Al, Na, Ca, K, Mg and P. For simultaneous trace element analytics, e.g. Cd, Cu, Pb, Zn, all vessels have to be cleaned with acid.

Nitric acid cannot be used for digestion, if it is planned to measure phosphorus colourmetrically by molybdenum blue (Hansen & Koroleff 1999, chapters 4.1.2 and 5.2.3).

Concentration range

The concentration range and limit of detection for P strongly depends on selection of the detection method. Generally, the ICP-OES is the method with the highest (that means worst) limit of detection and quantification (chapter 5.1). However, it is possible to increase material weigh-in and amount of extraction agent to adjust the concentration in the measurement solution to achieve the measurement range of the instrument.

Generally, the ICP-OES can detect all P compounds in the measurement solution and not only “free” phosphate. However, this might not cause any differences because strong digestion conditions converted almost all P compounds to phosphate. An advantage of the ICP-OES is the wider measurement range than those of photometrical methods (chapter 9), which can decrease potential errors by dilution.

Detection of P in nitric acid containing solutions is possible by vanado-molybdate-yellow (chapter 5.2.5). However, the sample weigh-in has to be relatively high to exceed the limit of quantification (0.3 mg P l^{-1} or 9.7 µmol l^{-1}) in the extract.

Mounting resin is necessary for the visualisation of P in for example biological soil crusts and bone char particles, if they are measured by e.g. XAS-methods. The background of P in the mounting resin is an important baseline for such an element mapping.

Protocol for plant material

Day 1: Preparation

- Put on your protective clothing (gloves, coat, glasses).
- weigh in ca. 0.1 to 0.5 g fine milled plant material into Teflon vessels of microwave (note precise mass)
- Place standards (chapter 6.6) and 2 blanks (10 ml conc. HNO_3) per run in the microwave.
- Add 10 ml conc. HNO_3 under the fume hood (clean inner vessel wall from sample material, if necessary).
- Set vessels with soil samples with acids open under the running fume hood overnight.

Day 2: Digestion

- Close vessels, mark blanks and dissolved standards as "empty place" (if possible, in the microwave), operate microwave according to the instructions (see below), cool down for around 1 h.
- Transfer extraction solution via (plastic) funnel in 50 or 100 ml (plastic) volumetric flask (Fig. 4.1.2-3). The solution has to be clear but may be green or yellow coloured.
- Rinse microwave vessel and funnel with ultra-pure water into the volumetric flask and fill flask with ultra-pure water to 50 or 100 ml. This volume has to be exact because from this volume the element concentration is calculated.
- Filter (e.g. Macherey-Nagel™ folded filter papers MN 612 retention 5-8 µm or phosphorus-poor MN 616 G retention 4-12 µm) the extraction solution into (acid-rinsed) polyethylene bottles (reference sample).
- Fill around 20 ml of solution into “ICP-vessels”.

Table 4.1.3-1 Digestion program for microwave MarsXpress for plant dry matter (modified according to CEM recommendations Plant tissue 1)

Level	Max. Power (W)	Power (%)	Ramp (min)	Temperature (°C)	Holding (min)
1	1600	100*	15:00	200	15:00

* Settings for "Power" depend on numbers of sample-filled vessels: 8-12 vessels (50 %), 13-20 vessels (75 %) and > 20 vessels (100 %).

Day 3: Measurement

- Determination of P at ICP-OES (wavelengths for P 214,914 or 213,617 nm, chapter 5.1)
- or with vanado-molybdate-yellow at the photometer (chapter 5.2.5).

Protocol for animal tissue

This protocol has not been widely tested yet. First experiences exist for fish and mussels. According to the recommendation of Fa. CEM fresh matter can be digested. However, in this case the dry matter has to be determined separately!
The samples have to be dried in each case because element concentrations have to be presented in relation to dry matter. Animal tissues should be lyophilised (instead of air-dried) for less odour.

Day 1: Preparation

- Put on your protective clothing (gloves, coat, glasses).
- Weigh in < 0.1 g dry matter or < 0.5 wet matter of crushed (fish)meat into Teflon vessels of the microwave.
- Place standards (chapter 6.6) and 2 blanks (10 ml conc. HNO_3) per run in the microwave.
- Add 10 ml conc. HNO_3 under the fume hood (clean inner vessel wall from sample material).
- Set vessels with soil samples with acids open under the running fume hood overnight.

Day 2: Digestion

- Transfer extraction solution via (plastic) funnel in 50 or 100 ml (plastic) volumetric flask (Fig. 4.1.2-3). The solution has to be clear and without residues.
- Rinse microwave vessel and funnel with ultra-pure water into the volumetric flask and fill flask with ultra-pure water to 50 or 100 ml. This volume has to be exact because from this volume the element concentration is calculated.
- Filter (e.g. Macherey-Nagel™ folded filter papers MN 612 retention 5-8 µm or phosphorus-poor MN 616 G retention 4-12 µm) the extraction solution into (acid-rinsed) polyethylene bottles (reference sample).
- Fill around 20 ml of solution into “ICP-vessels”.

Table 4.1.3-2 Digestion program for microwave MarsXpress. Insert level 0 if digestion is incomplete

Level	Max. Power (W)	Power (%)	Ramp (min)	Temperature (°C)	Holding (min)
0	1600	100*	20:00	160	5:00
1	1600	100	20:00	200	15:00

* Settings for "Power" depend on numbers of sample-filled vessels: 8-12 vessels (50 %), 13-20 vessels (75 %) and > 20 vessels (100 %).

Day 3: Measurement

- Determination of P at ICP-OES (wavelengths for P 214,914 or 213,617 nm, chapter 5.1)
- or with vanado-molybdate-yellow at the photometer (chapter 5.2.5).

Protocol mounting resin

Mounting resin is decomposed relatively abrupt at around 160 °C. For this reason, the ramp to 200 °C is quite long (information of CEM, table 4.1.3-3). If the decomposition of the resin is incomplete with the first program (table 4.1.3-3), the microwave has to be ramped first to 160 °C, hold for 5 minutes, then ramped to 200 °C (table 4.1.3-4). 200 °C are the maximum temperature, which should never be exceeded. This alternative program can also be used for fish, if the digestion is incomplete.

Day 1: Preparation

- crush resin to increase surface
- sample weigh-in < 0.2 g dry matter
- extraction agent: 10 ml conc. HNO_3

For the following have a look at the descriptions before. Please note: The P concentration in the resin should be zero (usage for element mapping). Therefore, the final volume should be as low as possible.

Table 4.1.3-3 Digestion program for microwave MarsXpress for mounting resin (modified according to CEM recommendations Plant tissue 1)

Level	Max. Power (W)	Power (%)	Ramp (min)	Temperature (°C)	Holding (min)
1	1200	100	20:00	200	15:00

Table 4.1.3-4 Adjustment of digestion program for microwave MarsXpress if digestion is incomplete (modified according to CEM recommendations Plant tissue 2 and personal information CEM)

Level	Max. Power (W)	Power (%)	Ramp (min)	Temperature (°C)	Holding (min)
0	1200	100	20:00	160	5:00
1	1200	100	20:00	200	15:00

References

CEM Recommendations (2004) Microwave Digestion Applications, MARS 6 Application Notes.

Hansen H P, Koroleff F (1999) Determination of nutrients. In: Grasshoff K, Kremling K, Ehrhardt M (Eds.) Methods of seawater analysis. Wiley-VCH, 159-251, DOI: 10.1002/9783527613984.ch10

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