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TECHNICAL NOTE

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Determination of aluminium in the edible part of fish by GFAAS after sample pretreatment with microwave activated oxygen plasma

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Abstract An analytical method for the determination of aluminium in the edible part of fish was developed using a new pre-treatment method in a microwave activated oxygen plasma and graphite furnace atomic absorption spectrometry. The linearity of the calibration line as well as of different standard addition lines were very good within the measurement area of 0–60 µg Al/L. The recovery with spiked ocean perch fillet was good. The unsatisfactory recovery of aluminium with the reference-material (mussel tissue) may be due to high quantities of insoluble aluminium-silicates. An installed quality-control-card indicated that the method showed no significant fluctuation as well as contamination over the complete analysis time. The limit of detection calculated as 3 times the standard deviation of the blank absorbance divided by the slope of the calibration line was 1 µg Al/L.

Introduction

Although aluminium is the most abundant metal in the earth's crust, it shows a low bioavailability and it is still questionable if aluminium has biological functions. Acid rains cause partial dissolution of soil aluminium leading to an increase in the aluminium concentration in natural waters and biological systems. Recent reports associated aluminium with several skeletal [osteomalacia: 1–3, 31, 32] and neurological disorders [encephalopathy: 4–7, Alzheimer's disease: 8–13] in humans. It was suggested that exposure to aluminium might present a hazard to health. Therefore the interest in aluminium related to human health increased in the last years. Many possible sources of aluminium intake by the human body, e.g. drugs, foods, drinking water, industrial exposure, exist. Food is one of the routes by which the normal intake of aluminium occurs, therefore the examination of aluminium-contents in different foodstuff is necessary. An analytical method for the determination of aluminium in fish tissue was developed using graphite furnace atomic absorption spectrometry (GFAAS). The methods usually employed for the determination of aluminium at part per billion levels (µg/kg) are GFAAS [14–20], and, more recently, inductively coupled plasma atomic emission spectrometry [21–22]. Analysis of solid food usually requires a pre-treatment step to make AAS determination possible. For biological materials different sample preparation methods have been proposed, e.g. aluminium-extraction with ethylenediaminetetraacetic acid [23], dry digestion and wet acid digestion techniques [16], and the high-pressure closed-vessel acid digestion [17, 20–21]. The here used digestion in an activated oxygen plasma is a new pre-treatment for the aluminium determination in food. The application of this digestion under vacuum, assisted by low temperature microwave heating, offers a promising alternative. The decomposition with plasma asher has some benefits, as: no use of chemicals, no contamination by chemicals, simultaneous mineralisation of up to 40 samples, complete destruction of the organic matrix, simple handling and cleaning [24–25]. However, the plasma asher cannot decompose all inorganic aluminium-components completely, and the ashing time is long, mainly depending on the fat content of the sample. It is generally difficult to measure traces of aluminium in biological and food samples. Several authors [21, 26] reported difficulties in the digestion of insoluble aluminium compounds. Even the mineralisation with nitric acid and hydrogen peroxide (HNO_3/H_2O_2) in a microwave-based closed system resulted in low recoveries of aluminium because of incomplete digestion. The content of insoluble and inorganic aluminium components in the edible part of fish is very low. Therefore the proposed method is usable for the aluminium determination in the edible part of fish. The method was validated

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by establishing adequate requirements for performance criteria, such as linearity, accuracy, precision, and the detection limit.

Method and experimental

Procedure

The frozen samples were freeze dried in a FINN-AQUA LYOVAC GT 2 freeze-dryer (parameters: ambient temperature 15–25 °C, vacuum 5–10 Pa, duration at least 48 h). The freeze dried samples were finely ground and homogenised with an agate-ball-mill (Fritsch, Idar-Oberstein, Germany) and kept in high-density polyethylene bags at room-temperature in an exsiccator until analysis. Approximately 1 g (weight dependent on aluminium concentration) of lyophilised sample was weighted into petri-dishes. The petri-dishes were put in a plasma asher chamber for mineralisation (power supply 400–420 W, vacuum 60–90 Pa, oxygen partial pressure 2.0–2.5 · 10^5 Pa). After the adequate mineralisation the remaining ash was dissolved with nitric acid (0.2%, w/w) and transferred quantitatively into a polypropylene volumetric flask (volume depended on aluminium concentration). For the measurement of the aluminium concentration 20 µL aliquots of diluted ash-solutions were injected into the graphite tubes of the electrothermal graphite furnace atomic absorption spectrometer.

Equipment

Plasma asher (Plasma Prozessor 200-G, Technics Plasma GmbH, München, Germany). The mineralisation of the samples was performed in a closed low temperature microwave oxygen plasma processor system equipped with a high performance pump. The parameters were: analyst-selectable power supply 400–420 W, vacuum 60–90 Pa, oxygen partial pressure 2.0–2.5 · 10^5 Pa and duration of decomposition 96–120 h.

Table 1 Recommended instrument settings and furnace programme

AAS-Instrument	Perkin Elmer AAS 4100ZL, THGA, AS 70
Al-hollow cathode lamp	current 25 mA
Wavelenght	309.6 nm
Bandwidth	0.7 nm
Sample volume	20 µL
Measurement area	0–60 µg Al/L
Matrix modifier	1. 5 µg $Pd(NO_3)_2$ 2. 3 µg $Mg(NO_3)_2$
Detection limit (3σ)	1 µg Al/kg
Characteristic mass	15 pg/0.0044 A s
Sensitivity	30 µg Al/L ≅ A s 0.180
Signal	Peak area
Background compensation	with inverse longitudinal Zeeman-effect

Temperature programme

Step	T [°C]	Ramp [s]	Hold [s]	Gas flow [mL/min]
1a. Dry	110	1	20	250
1b. Dry	130	5	30	250
2a. Ash	800	5	10	250
2b. Ash	1500	5	15	250
3a. cool down	1010	1	10	250
3b. cool down	20	1	10	250
4. Atomise	2300	0	5	0 (read)
5. Cleaning	2400	1	3	250
Purge gas		Argon		

Atomic absorption spectrometry

A Perkin Elmer Model 4100ZL atomic absorption spectrometer with Zeeman-background correction equipped with transversely heated graphite atomiser (THGA) was used for the aluminium determination. Pyrolytically coated graphite tubes and pyrolytic graphite platform were used. The instrument settings and furnace programmes for analysis of aluminium have been described in Table 1. The two drying steps and extended drying times ensured the complete drying prior to the ashing steps. Further the conditions of the time-temperature program ensured a long lifetime of the graphite tube (about 800 firings) and the program resulted in an optimal peak area signal (Fig. 1A, B). Samples and standards were diluted with two modifiers (Table 1) using the AS-70 autosampler. The use of the modifiers and the two ashing steps ensured complete removal of interfering compounds and stabilised the aluminium-ions (Pd-Al). The light source was a single element hollow cathode lamp whose operating parameters (current and spectral bandwidth) were those recommended by the manufacturer. Argon was employed in the graphite furnace as external and internal gas, and the flow of the latter was interrupted during atomisation.

Reagents/Chemicals

Standard. Aluminium solutions in 0.2% (w/w) nitric acid containing 0 (blank), 5, 10, 20, 30, 40, and 60 µgAl/L were prepared from a Titrisol concentrate containing 1000 mgAl/L (Merck, Darmstadt,

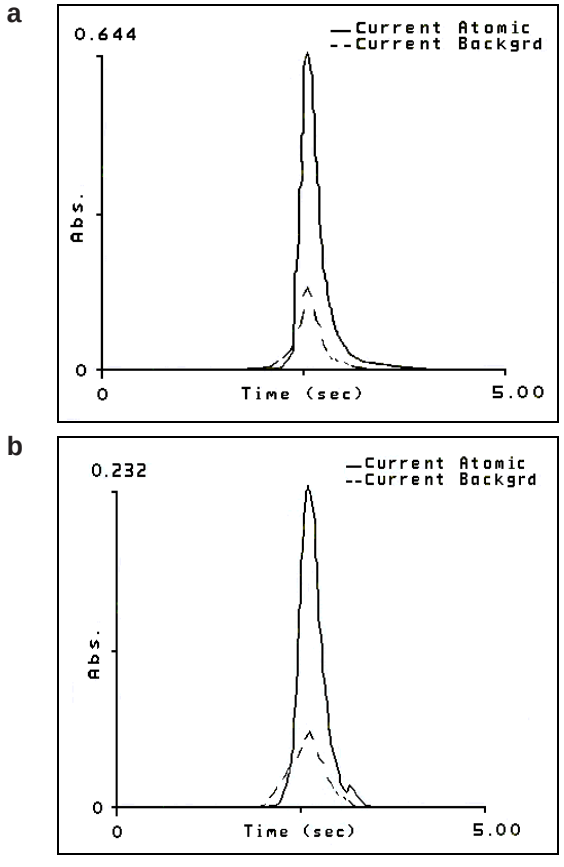

Fig. 1 Peak signal from **a** a standard solution 30 µg Al/L (20 µL), **b** from canned herring fillet (20 µL)

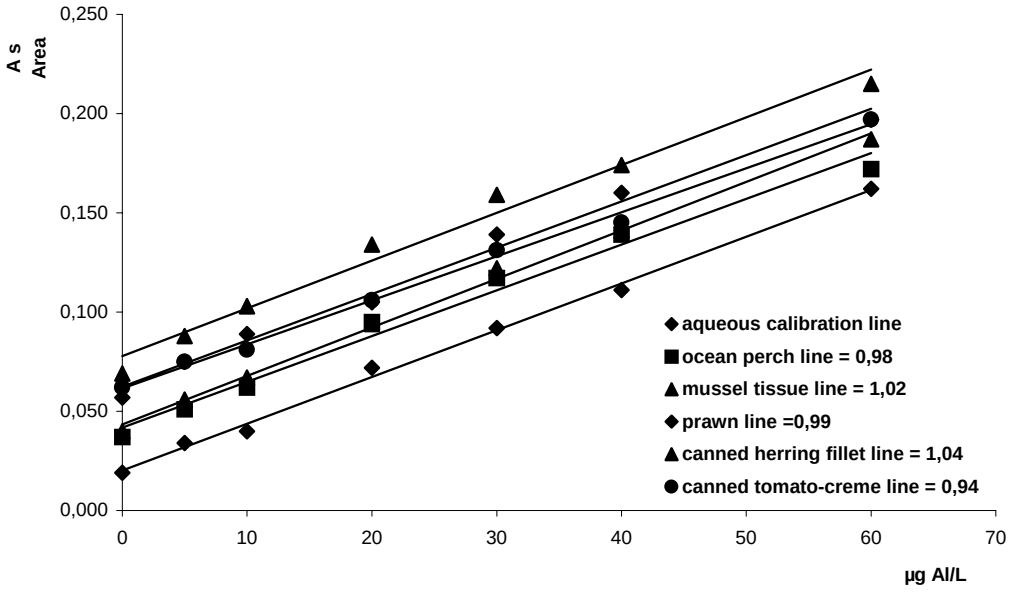

Fig. 2 Comparison and the slope ratios of different standard addition lines over the aqueous calibration line

Germany). Nitric acid (65% w/w, suprapur, Merck) of highest purity was used for the preparation of the standards and for the digestion, while for labware cleaning, analytical reagent grade nitric acid (65% w/w, pro analysi, Merck) was used. De-ionized water ($\geq$ 18 MΩ cm^{-1} resistance) used for the preparation of all solutions was obtained from a NANOpure II water purification system (Sybron/Barnstead, Boston, Massachusetts, USA) and contained no detectable Al. The matrix modifiers were 1. Palladium nitrate solution (c(Pd) = 10.0 ± 0.2 g/L $Pd(NO_3)_2$ in nitric acid (15% w/w), Merck) and 2. Magnesium nitrate solution (c(Mg) = 10.0 g/L in nitric acid, Perkin Elmer) prepared by dissolving in de-ionized water (1:10) and stored in pre-cleaned polypropylene container.

Cleaning

To avoid contamination from the containers, polypropylene vessels (flask, volumetric flask), high density polyethylene (HDPE) bottles and further plastic equipment (beakers, autosampler cups, spoon, removable tips, tweezers) were used. They were cleaned by soaking into 2% (w/w) nitric acid (pro analysis grade) for > 24 h followed by soaking in de-ionized water for > 24 h. After this cleaning procedure all cleaned vessels and equipment were dried and kept in aluminium-free containers until use. This avoided an aluminium contamination through room dust.

Standard reference material

The accuracy and the recovery of the aluminium determination was checked by the reference material CRM No 278 mussel tissue (*Mytilus edulis*) from the Commission of the European Communities, Community Bureau of Reference, Luxembourg and by the analysis of aluminium-spiked muscle tissue from ocean perch (*Sebastes marinus*). The aluminium addition was performed by pipetting different amounts of two different aluminium solutions (A and B) before freeze-drying. The recovery was monitored with these two in-house muscle tissue quality control pools. In regular intervals the aluminium content was determined and the recovery checked. The aluminium concentrations of the two different pools were:

A. aluminium Titrisol solution from Merck (c = 1.000 ± 0.002 gAl/L): 4.2, 8.4, 17.0, 27.8, 35.4, 73.6, 83.6 and 95.8 µg Al/g dry matter (DM)

B. alum ($KAl(SO_4)_2$ $12H_2O$) solution (c = 1.0014gAl/L): 3.1, 6.6, 10.4, 18.2, 26.3, 38.9, 49.8 and 71.1 µg Al/g DM

Results and discussion

To check the linearity of the calibration line within the measurement area of 0–60 µg Al/L and possible matrix interference, the slope of the aqueous calibration line and a standard addition line were compared. The method of additions has been performed with digests of fresh fillet of ocean perch, mussel tissue, prawns and canned herring fillets in tomato-creme. Figure 2 shows the calibration line and the standard addition lines. The injection volumina were: A. aqueous calibration line: 10 µL standard and deionized water +5 µL modifier I+3 µL modifier II; B. standard addition line: 10 µl standard and sample +5 µL modifier I+3 µL modifier II.

The slope ratios of the standard addition line over the aqueous calibration line were 0.98 (ocean perch fillet), 1.02 (mussel tissue), 0.99 (prawns) and 1.04 (canned herring) and 0.94 (canned tomato-creme), respectively. For none of these digests a statistically significant difference in slope between the calibration line and the standard addition line could be found and no significant matrix effects were observed. The linearity within the measurement area of 0–60 µg Al/L was very satisfying (R^2 = 0.99–1.00). Therefore the aluminium determination of all investigated samples was performed by a direct calibration against aqueous standard solutions.

The accuracy was evaluated by recovery experiments and by the analysis of certified standard reference materials. In Table 2, the results obtained for the reference material mussel tissue (Commission of the European community, Community bureau of reference, Luxembourg, No 278) are given, together with the indicative values based on results obtained by laboratory I and II.

Mussels are known to filter and accumulate sediment which can contain high quantities of acid insoluble ingredients, for example aluminosilicates. Therefore it is not possible to mineralise the acid insoluble components of the

Table 2 Comparison of the analytical results obtained for the aluminium content of Standard Reference Material CRM No 278 mussel tissue (*Mytilus edulis*) from the Commission of the European Communities, Community Bureau of Reference. Values given in SRM are only indicative, not certified, based on results obtained by laboratory I and II

Analytical procedure	Found ± St.Dev. µg Al/g dry wt	Replicates
Laboratory I: Digestion HNO_3/ H_2SO_4; ICP	71.9 ± 1.1	4
Laboratory II: Dry ashing; diss. in HF/$HClO_4$; ICP	68.6 ± 1.0	5
Exp. results: plasma ashing; diss. in HNO_3, ETAAS	42.8 ± 0.7	5

mussel tissue with the plasma asher system completely. For aluminium determination the use of hydrofluoric acid in sample preparation has been proposed because incomplete digestion, which might occur in the presence of siliceous material [21, 27, 28, 33]. It can be assumed, that the aluminium ion will be mineralised into the heavy soluble α-aluminium oxide under the condition in the plasma asher (combustion with activated oxygen). The formation of γ-aluminium oxide, however, is more probable by the low mineralisation temperature 300 °C in the activated oxygen stream. The γ-aluminium oxide, however, is soluble in diluted acids. Only above 500 °C the γ-Al_2O_3 changes its structure from the spinell- to the high-temperature-modification, the so-called korund (α-Al_2O_3), respectively. Therefore the plasma asher mineralisation is suitable for the quantitative aluminium determination from organic material without siliceous ingredients.

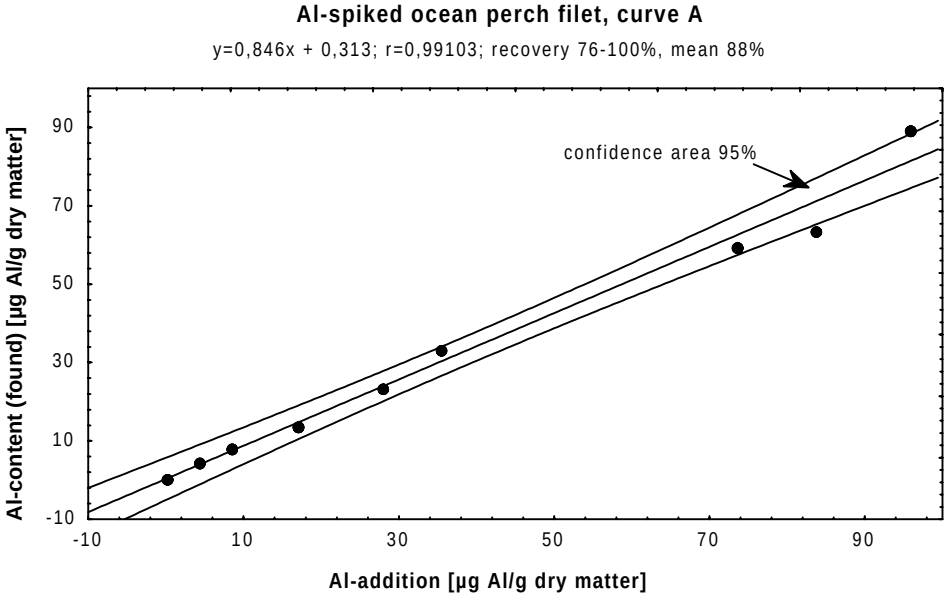

Fig. 3 Al-spiked ocean perch fillet. Al addition with Merck Titrisol solution before freeze drying

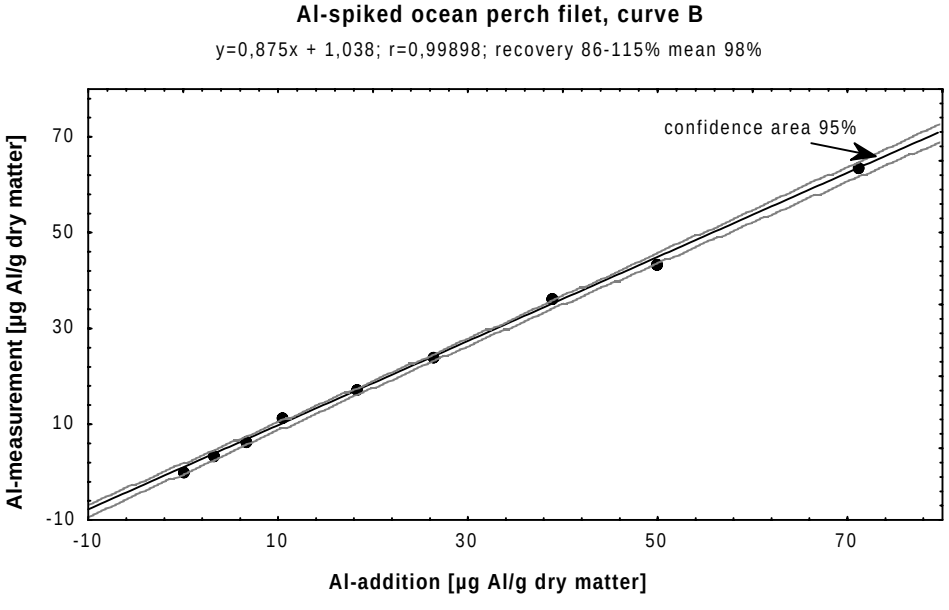

Fig. 4 Al-spiked ocean perch fillet. Al addition with alum ($KAl(SO_4)_2 \times 12H_2O$) solution before freeze drying

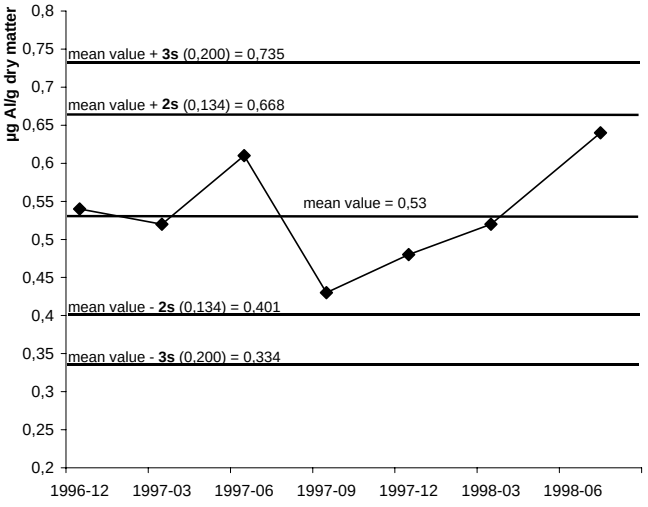

Fig. 5 Quality control card 1: Al concentrations in identical samples processed from ocean perch fillet. 7 Al-determinations in regular intervals within 1½ year

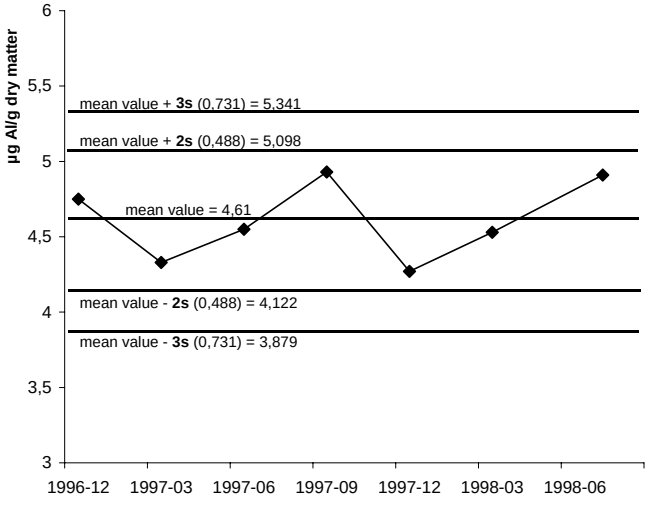

Fig. 6 Quality control card 2: Al concentrations in identical samples of Al-spiked ocean perch fillet. 7 Al-determinations in regular intervals within 1½ year

In Figs. 3 and 4 the results from the two in-house muscle tissue quality control pools are shown.

The two recovery experiments were performed with ocean perch fillet. For both quality control pools, eight different aliquots of an aluminium Titrisol solution and of an alum solution were spiked, digested and prepared as described previously.

The good recoveries of both quality control pools confirmed the previous hypothesis that the organic-bound aluminium mineralises to soluble compounds, in first instance to γ-Al_2O_3. This indicated the effectiveness of the plasma-asher digestion and GFAAS measurement of aluminium in the edible part of fish.

For the control of the contamination two quality-control-cards were applied. For this purpose the aluminium contents of ocean perch fillet and Al-spiked ocean perch fillet were determined in regular intervals and the aluminium content was checked within a defined confidence area (mean value ± 2 s (double standard deviation)). Figures 5 and 6 show the aluminium concentrations of the ocean perch fillet and of the Al-spiked ocean perch fillet. The determinations of the aluminium concentrations were performed seven times within 1½ year.

During 1½ year no significant aluminium contamination was observed. All results of the two ocean perch samples with different aluminium concentrations (normal and spiked) were within the defined confidence area (mean ± 2 s). The relative standard deviation of both aluminium concentrations (low and high) indicated that the precision was better than 5%. Nevertheless, frequent monitoring of the whole procedure is necessary for the aluminium determinations at the microgram per litre levels.

The limit of detection is defined as 3 times the standard deviation of the blank absorbance divided by the slope of the calibration line [29]. It was calculated from the absorbance of each standard solution blank to be < 1 µg Al/L. By taking into account the sample weight (max. 1 g) and the dilution used (25 mL), the detection limit in the dried ocean perch fillet was approximately 0.03 µg Al/g on a dry weight basis.

Conclusion

The proposed method has an important impact in simplifying the analysis of aluminium in tissues of different fish. The analysis handling is practicable by using the plasma asher. Oxygen, nitric acid and de-ionized water are the only reagents used for sample digestion. The results of the recovery experiments indicated that no contamination has occurred during the whole procedure. Therefore, without further treatments, a direct calibration against aqueous solutions was possible. These advantages, combined with a low detection limit, indicated the usefulness of the method for the aluminium determination in a wide variety of fish tissues. The method has been successfully applied for the determination of aluminium in fish tissue.

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