APPLICATION NOTE

Atomic Absorption

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Analysis of Fish and Seafoods with AAnalyst 800 Atomic Absorption Spectrophotometer for Trace Metal Contamination, in Accordance with AOAC Methods 999.10 and 999.11

Introduction

Increased knowledge about the nutrient content of biological organisms is essential for a thorough understanding of ecological stoichiometry and nutrient transport in and among ecosystems. As a result of water pollution in coastal area, many problems in food safety like heavy metal accumulation have been recognized in farmed fish, which is one of the important fishery food resources. The heavy metals accumulated in fish not only have a bad influence on fish but they also affect the health of human beings through the food chain. It is pointed out that remarkable heavy metals were contained in fish meals that are used as major raw materials for aquaculture feeds. The Itai-itai disease of the Toyama Jintsu River area in Japan was the documented case of mass cadmium poisoning. Itai-itai disease is known as one of the Four Big Pollution Diseases of Japan.

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In the present work, we demonstrate the ability of AAnalyst™ 800 atomic absorption spectrophotometer in analyzing a variety of fish samples. Sample preparation has been done in two different ways i.e. by AOAC Method 999.10, which is the official method for the sample preparation of fish samples with microwave digestion and AOAC Method 999.11, which is the preparation of fish samples with conventional dry ashing using a muffle furnace.

Experimental

The measurements were performed using the PerkinElmer® AAnalyst 800 atomic absorption spectrophotometer (PerkinElmer, Inc. Shelton, CT, USA) (See Figure1) equipped with WinLab32™ for AA Version 6.5 software, which features all the tools needed to analyze samples, report and archive data and ensure regulatory compliance. PerkinElmer high efficiency double beam optical system and solid-state detector provide outstanding signal-to-noise ratios and Deuterium background correction eliminates most interferences. A PerkinElmer corrosion-resistant nebulizer, which can be used for solutions containing hydrofluoric acid, was used for all the flame absorption measurements. A single slot air-acetylene 10 cm burner head was used for all air-acetylene experiments.

The AAnalyst 800 features longitudinal Zeeman-effect background correction and a solid-state detector which is highly efficient at low wavelengths. The AAnalyst 800 uses a transversely heated graphite atomizer (THGA) which provides uniform temperature distribution across the entire length of the graphite tube. The THGA features an integrated L'vov platform which is useful in overcoming potential chemical interference effects common in the GFAAS technique.

Figure 1. PerkinElmer AAnalyst 800 Atomic Absorption Spectrophotometer.

A PerkinElmer/Anton-Paar Multiwave™ 3000 Microwave Oven was used for the microwave-assisted digestion of fish and seafood samples. This is an industrial-type oven which can be equipped with various accessories to optimize the sample digestion. In this case, the samples were digested in the Rotor 8XF100 comprising eight 100 mL high pressure vessels made of PTFE-TFM in their respective protective ceramic jackets. TFM is chemically modified PTFE that has enhanced mechanical properties at high temperatures compared to conventional PTFE. This vessel has a "working" pressure of 60 bar (870 psi) and can operate at temperatures up to 260 ˚C. A Pressure/Temperature (P/T) Sensor Accessory was also used for this work. The P/T sensor simultaneously measures temperature and pressure for one vessel. All vessels' temperatures were monitored with the IR Temperature Sensor Accessory. This device gives thermal (over-temperature) protection to the reactions in all of the vessels by measuring the temperature remotely at the bottom surface of each vessel during the digestion process.

Figure 2. PerkinElmer/Anton-Paar Multiwave 3000 Microwave Digestion System.

A laboratory grade muffle furnace was used for ashing purpose.

PerkinElmer, NIST® traceable calibration standards in acid for atomic spectroscopy was used as the stock standards for preparing working standards. All these working standards were prepared daily with ASTM® type I water acidified in Suprapur® nitric acid, in polypropylene vials (Sarstedt®) on volume-by-volume dilution. Micropipettes with disposable tips (Eppendorf®, Germany) were used for pippetting solutions. Certified Reference Standard for trace metals in fish from High Purity Standards (Lot # 0801404) was used for validating the developed method. Multielement ICP standard for trace metal ions in 5% HNO_3, from Spex Certiprep® (New Jersey, USA), prepared at midpoint of the calibration curve for all elements was used as quality control check standard. The acids used (nitric acid and hydrofluoric acid) were of Suprapur® grade, from Merck® in Germany.

Sample Preparation

Three different dry fish and seafood samples were brought directly from the local fish market and were kept sealed in resealable polypropylene bags. The samples were kept at room temperature and before sampling was dried at 65 ˚C in a laboratory oven until they attained constant weight.

For microwave digestion, about 0.50 g of homogenized and dried samples of fish was accurately weighed directly into the PTFE-TFM digestion vessels. To each sample, 5.0 mL of concentrated nitric acid, 2.0 mL of hydrogen peroxide and 1.0 mL water were added. The analytical reagent blanks were also prepared and these contained only the acids. The vessels were sealed and placed into the Rotor 8 for the microwave digestion. After the digestion process, the digestate liquids were transferred to the 50.0 mL auto-sampler polypropylene vials and laboratory ASTM® Type I water was added to a final total volume of 20.0 mL.

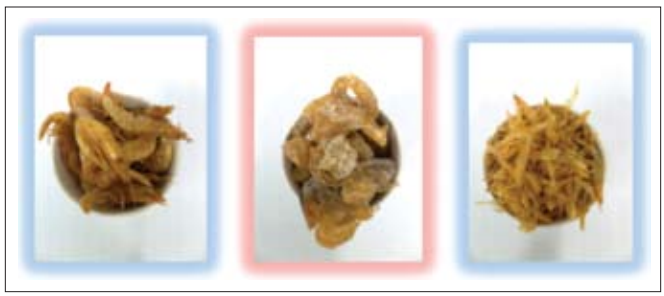

Figure 3. Samples.

For dry ashing, quartz crucibles used were rinsed with 20% nitric acid and then dried. Samples were homogenized using an agate mortar and pestle and then 10.0 g sample was taken in the crucibles. The samples were dried at 100 ˚C in a laboratory oven. These crucibles were then placed in the muffle furnace at ambient temperature. The temperature was then raised at the rate of 50 ˚/hour to 450 ˚C and let the dish stand for 8 hours or overnight. Then it was wet-ashed with 1.0-3.0 mL of water and evaporated on a hot plate. This procedure is repeated until the product is completely ashed. The ash should be white/grey or slightly colored. 5.0 mL of 6.0 M HCl was added to the crucible to completely cover the ash. The ash was evaporated on the hot plate and the residue was redissolved in 10.0-30.0 mL of 0.10 M nitric acid. The crucible was swirled with care so that all ash comes in contact with the acid. The crucible was covered with a watch glass and left standing for 1-2 hours. The solution in the crucible was stirred thoroughly with a stirring rod and then transferred into the plastic bottle. The sample reagent blank was also prepared in the same way.

Results and Discussion

The official AOAC methods were compared for their performance in digesting the fish samples.

Microwave digestion offers complete dissolution of the samples with less possibility of contamination from the environment, in less than 60 minutes including the cooling step. The conventional muffle furnace ashing method normally results in the loss of some more volatile analytes like cadmium and lead, and it is extremely tedious usually lasting more than 24 hours.

The analysis results shows close agreement between the values obtained for sample duplicates with microwave digestion. A four point calibration which includes three standards and one blank were created for both Flame and GFAA measurements. With both Flame and GFAA techniques, excellent correlation coefficients better than 0.999 were obtained. The quality control check standard recoveries (Prepared at midpoint of calibrations) were excellent and were within 95-105%. The recovery of various metal ions from the high purity standard certified reference material for fish were excellent.

The AAnalyst 800 uses a transversely heated stabilized temperature platform system to ensure the minimum influence of matrix interferences possible. The longitudinal Zeeman background correction combined with other STPF conditions further ensured interference-free analysis of lead and cadmium in various fish and seafood samples. The big difference between the concentration values obtained by microwave digestion and muffle furnace ashing shows that analytes are lost during ashing.

Conclusions

The patented THGA tube used in the AAnalyst 800 system provides a uniform temperature distribution along its entire length. This eliminates cooler temperatures at the tube ends and removes most interferences. With the THGA tube design, accuracy and sample throughput are improved by reducing the need for the time-consuming standard additions technique. With the longitudinal Zeeman-effect background correction, the amount of light throughput is doubled by eliminating the need for a polarizer in the optical system. All other commercial Zeeman designs incorporate inefficient polarizers that reduce light throughput and diminish performance. With this unique design, the AAnalyst 800 provides the lowest detection limits available.

In conventional furnace systems, the heating rate during atomization depends on the input-line voltage. As voltage varies from day to day, season to season or among laboratory locations, so does the heating rate. The AAnalyst high-performance systems use enhanced power control circuitry to maintain a uniform heating rate, so no matter where a system is located, one can be sure that it provides outstanding, consistent performance.

The AAnalyst 800 produces highly accurate, fast and reproducible results with difficult matrices such as fish and seafoods. The developed method has been validated by using reference material and the method has been successfully applied for the analysis of different fish and seafood samples.

The capability of PerkinElmer/Anton-Paar Multiwave 3000 microwave digestion system to digest the fish and seafood samples in accordance with AOAC Method 999.10 was demonstrated. The samples prepared with microwave digestion were compared with samples prepared by conventional dry ashing in accordance with AOAC Method 999.11. The capability of AAnalyst 800 to perform the analysis of fish samples for trace metal contamination with both flame and graphite furnace AAs was demonstrated. Microwave digestion proved to be very effective, time saving and an accurate way of preparing samples of fish and seafood for analysis of trace metal ions. As analytes are lost during the conventional ashing process, microwave digestion is the preferred technique for sample preparation for fish and seafood samples.

Table 1. **Instrumental Conditions for the Flame Analysis on the AAnalyst 800.**

Element	Cu	Fe	Zn
Wavelength (nm)	324.8	248.3	213.9
Slit (nm)	0.7	0.2	0.7
Mode	AA	AA	AA-BG
Flame	Air-Acetylene	Nitrous Oxide-Acetylene	Air-Acetylene
Burner	10 cm Universal	10 cm Universal	10 cm Universal
Calibration	Linear Through Zero	Linear Through Zero	Linear Through Zero
Lamp	HCL	HCL	HCL
Lamp Current (mA)	15	30	10
Standards (mg/L)	1.0, 2.5, 5.0	1.0, 2.5, 5.0	0.05, 0.10, 0.25
Spike Conc. (mg/L)	2.5	2.5	0.10
Read Time (sec)	3.0	3.0	3.0
Replicates	3	3	3
Air Flow (L/min)	17.0	7.0 (Nitrous Oxide)	17.0
Acetylene Flow (L/min)	2.0	16.0	2.0

Table 2. **Instrumental Conditions for the Furnace Analysis on the AAnalyst 800.**

Element	Pb	Cd
Wavelength (nm)	283.3	228.8
Slit (nm)	0.7	0.7
Mode	AA-BG	AA-BG
Processing	Peak Area	Peak Area
Read Time (sec)	5.0	5.0
Replicates	3	3
Lamp	EDL	EDL
Lamp Current (mA)	440	230
Injection Temperature °C	90	90
Sample Volume, µL	20	20
Matrix Modifier Volume, µL	5	5
Calibration Equation	Linear with Calculated Intercept	Linear with Calculated Intercept
Standards (µg/L)	10.0, 25.0, 50.0	0.50, 1.0, 2.0
Spiked Concentration (µg/L)	25.0	1.0

Table 3. **Graphite Furnace Temperature Programs.**

Element	Step	Temp °C	Ramp Time (sec)	Hold Time (sec)	Internal Gas Flow (mL/min)	Gas Type
Pb	1	110	1	30	250	Argon
	2	130	15	30	250	Argon
	3	850	10	20	250	Argon
	4	1600	0	5	0	Argon
	5	2450	1	3	250	Argon
Cd	1	110	1	30	250	Argon
	2	130	15	30	250	Argon
	3	500	10	20	250	Argon
	4	1500	0	5	0	Argon
	5	2450	1	3	250	Argon

Table 4. **Analysis of Certified Reference Material for Fish (Lot # 0801404).**

Metal	Certified Value (µg/mL)	% Recovery from Diluted Solution
Cu	50.0 ±0.5%	100
Zn	1000.0 ±0.5%	97
Fe	100.0 ±0.5%	101
Pb	10.0 ±0.5%	103
Cd	5.0 ±0.5%	102

Table 5. **Spike and QC Recovery Studies.**

Metal	QC 1 (%)	QC 2 (%)	Spike Recovery (%)
Cu	106	108	99
Fe	100	100	107
Zn	101	95	102
Pb	100	102	92
Cd	104	107	105

Table 6. **Method Detection Limits (MDLs).**

Metal	MDL (mg/kg)
Cu	0.2
Fe	0.8
Zn	0.1
Pb	1.6 (µg/kg)
Cd	0.1 (µg/kg)

Table 7. **Results of Analysis of Fish Samples (In mg/kg).**

Metal	Fish I (MDS)	Fish I (Muffle) Furnace)	Fish II (MDS)	Fish II (Muffle Furnace)	Fish III (MDS)	Fish III (Muffle Furnace)
Cu	17	6.7	39	12	86	20
Fe	215	33	153	48	360	57
Zn	53	21	61	19	95	31
Pb	0.13	0.04	0.14	0.08	0.14	0.06
Cd	0.11	0.04	0.20	0.07	0.33	0.11

References

1. AOAC Method 999.10.

2. AOAC Method 999.11.

3. Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory-Preparation Procedure for Aquatic Biological Material Determined for Trace Metals, by Gerald L. Hoffman U.S. Geological Survey Open-File Report 96-362.

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