Cadmium Contamination Levels in Seafood Determined by Electrothermal Atomic Absorption Spectrometry after Microwave Dissolution

Carmen Cabrera,*,† María L. Lorenzo,† Carmen Gallego,† María C. López,† and Enrique Lillo‡

Department of Nutrition and Bromatology, School of Pharmacy, University of Granada, E-18071 Granada, Spain, and Laboratorio Agroalimentario de Granada, Junta de Andalucía, E-18014 Atarfe, Spain

A method is presented for the determination of cadmium in seafood species; the method is based on electrothermal atomic absorption spectrometry of tissues previously treated in a microwave acid digestion bomb. The limit of detection was 0.16 pg. Sample recoveries, precision studies, and analyses of BCR-CEC reference material demonstrated the reliability and accuracy of the technique. In samples of seafoods frequently consumed in Mediterranean coastal regions of Spain, Cd concentrations ranged from undetectable to 45.75 ng/g, the highest concentrations appearing in crustaceans and cephalopods.

INTRODUCTION

Cadmium is a toxic element present in low concentrations in nature. High levels of Cd are often associated with human activity and are found in urban and industrial waste disposal areas. Cadmium contamination has increased sharply in recent years, and the element is now commonly found in aquatic and terrestrial environments (Concon, 1988; Robards and Worsfold, 1991).

Marine Cd contamination can severely damage the ecosystem, affecting different levels of the food chain, especially populations of frequently consumed seafoods. In these species, Cd leads to teratogenesis and death in the early stages of development, at concentrations that may be sublethal to adult animals. Reproduction, growth, and food quality are also diminished (Zook et al., 1976; Del Prete and Bolleta, 1984). The monitoring of marine organisms can serve as an indicator of variations in marine pollution (Martin and Coughtrey, 1982; Alikhan et al., 1990; Rule and Alden, 1990).

As an analytical sample, seafoods are complex matrices, most methods of analysis requiring prior mineralization (AOAC, 1990). Several decomposition procedures have been recommended for fish samples, but many of these techniques are time-consuming (Zook et al., 1976; Dabeka and Makenzie, 1986; AOAC, 1990). Recently, there has been great interest in using microwave ovens to speed up the digestion of a variety of samples (Kingston and Jassie, 1986; Stripp and Bogen, 1989).

Because of their specificity and versatility, atomic absorption spectrometry (AAS) and inductively coupled plasma–atomic emission spectroscopy (ICP–AES) are considered the leading techniques for the quantification of trace elements in biological samples and foods (White, 1989; Schuhmacher et al., 1990; Tahvonen and Kumpulainen, 1991). Electrothermal atomization (ETA) enhances the sensitivity, precision, and accuracy of the analytical technique used, considerably reducing matrix interferences (Slavin and Manning, 1980; Vidal, 1990). In this study we determined Cd in seafoods frequently consumed in regions along the Mediterranean coast of Spain, where fish, crustaceans, and mollusks form a basic part of the diet and are of considerable commercial interest. Moreover, the levels of Cd in these species can serve as an indicator of contamination of the marine environment.

* Author to whom correspondence should be addressed (fax +34-58-243869).

† University of Granada.

‡ Junta de Andalucía.

Table I. Furnace Conditions for the Determination of Cadmium in Seafood by Electrothermal Atomic Absorption Spectrometry

step	temp, °C	ramp time, s	hold time, s	gas flow rate, mL of Ar min^{-1}
dry	130	40	10	100
char	450	60	30	100
atomize	2100	1	4	stop
clean	2650	1	2	100
	20	1	2	100

Table II. Accuracy and Precision of the Method against a Standard Reference Material of BCR-CEC (RM 278)

ref material	Cd values, μg/g of dry wt		accuracy, %	precision CV, %
	certified[a]	determined[a]		
mussel tissue (*M. edulis*)	0.34 ± 0.02	0.33 ± 0.04	97.06	18.2

[a] Mean ± SD, at 95% confidence interval about the mean (n = 10).

EXPERIMENTAL PROCEDURES

Apparatus. A Perkin-Elmer 2380 double-beam atomic absorption spectrometer equipped with a deuterium arc background corrector was used with a 6-mA hollow cathode lamp, at a slit width of 0.7 nm. The samples were atomized under the conditions shown in Table I using a Perkin-Elmer HGA-400 furnace and a pyrolytic graphite tube with a L'vov platform at 228.8 nm. The samples were injected manually with a Pipetman micropipet. Signals were recorded in integrated absorbance mode on a Perkin-Elmer 024 potentiometric recorder.

Samples were digested with a microwave acid digestion bomb (Parr, Model 4782) heated in a Moulinex FM-460 microwave oven at 15–100% full power (600 W) in 25% increments.

Reagents. All solutions were prepared with ultrapure water with a specific resistivity of 18 MΩ·cm, obtained by filtering double-distilled water through a Milli-Q purifier (Millipore) immediately before use. As the Cd standard solution (1.00 ± 0.002 g), Titrisol (Merck) was used. Nitric acid (65%) (Merck, Suprapure), vanadium pentaoxide, and ammonium molybdate (Merck, analytical grade) were also used.

As a standard reference material we used mussel tissue (*Mytilus edulis*) RM 278 (Community Bureau of Reference, Commission of the European Communities), with a certified Cd content of 340 ± 20 ng/g.

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Table III. Statistical Analysis of the Results of Determinations of Cadmium in Seafood by Electrothermal Atomic Absorption Spectrometry

sample	$\bar{x}^a$	$S_{n-1}{}^b$	$S_m{}^c$	$\bar{x} + S_m t$	$S_m t/\bar{x}$, %
Belone belone L.	0.065	3.375×10^{-3}	1.067×10^{-3}	$0.065 \pm 2.414 \times 10^{-3}$	3.71
Galeorhinus galeus L.	0.086	3.026×10^{-3}	9.568×10^{-4}	$0.086 \pm 2.164 \times 10^{-3}$	2.52

[a] $\bar{x}$, mean value of 10 determinations. [b] S_{n-1}, standard deviation for 10 replicate determinations. [c] S_m, standard error of the mean. The value of Student's t was 2.262 for both samples.

Table IV. Concentrations of Cadmium in Seafood Determined by Electrothermal Atomic Absorption Spectrometry

sample	Cd,[a] ng/g	sample	Cd,[a] ng/g
Seppia officianalis L.[b]	45.75	*Belone belone* L.	3.15
Urophycis bleunioides	35.23	*Boops salpa*	3.08
Squilla mantis	31.39	*Boops boops* L.	2.74
Sarda sarda Bloch.	22.10	*Loligo vulgaris*	2.05
Allotenthys media L.	18.98	*Helicolenus dactylopterus*	2.00
Parepenaeus longirostris	17.15	*Lophius piscatorius*	1.90
Brama raii Bloch.	7.92	*Octopus vulgaris*	1.88
Luvarus imperialis	6.18	*Engraulis encrasicholus*	1.70
Cepola rubescens	5.75	*Mullus surmuletus*	1.15
Leander serratus	4.93	*Scomber scombrus*	0.80
Trachinus araneus L.	4.90	*Aspitrigla cuculus*	0.75
Crangon vulgaris	4.45	*Pagellus cantabricus*	0.62
Sardina pilchardus V.	4.13	*Merluccius merluccius*	0.47
Galeorhinus galeus L.	3.62	*Mugil* sp.	0.45
Illex coindetti Ver.	3.44	*Trachurus trachurus* L.	0.45
Aphya minuta Risso	3.36	*Gadus poutassou* L.	nd[c]

[a] Fresh weight of the edible fraction. [b] Scientific names taken from Ministerio de Sanidad y Consumo (1988). [c] nd, not detectable.

Samples. Thirty-two different species consumed as seafood in Mediterranean coastal regions of Spain were analyzed. The edible portions were obtained from samples weighing 500–1000 g (fresh weight), sliced thinly, and desiccated in a microwave oven at low setting. Parallel analyses were run with freeze-dried samples; there were no significant differences between the results obtained with the two procedures. All determinations were done in triplicate.

Procedure. Amounts of 200 mg of dried and homogenized sample were treated with 2.5 mL of nitric acid and a few micrograms of vanadium pentaoxide as a catalyst and then digested in a microwave acid digestion bomb. Mineralization was complete in 90 s with the oven at its highest setting. The digestion bomb was cooled by freezing at −18 °C for 30–40 min, and the solution was then diluted with 25 mL of ultrapure water. An aliquot of 20 μL was injected into the tube, which had been treated with saturated ammonium molybdate to avoid the formation of refractory carbides, and run under the optimized conditions shown in Table I. The conditions were optimized on the basis of time–temperature studies using certified standards.

Graphite Furnace Program Optimization. Mineralization of the matrix was completed after heating at 450 °C for 30 s. The atomization temperature that yielded maximum signals was 2100 °C for 4 s, with an integration time of 5 s. Stopping the flow of the argon purge gas at this point increased sensitivity without altering the lifetime of the tube. The furnace was cleaned by raising the temperature to 2650 °C, and the graphite tube was allowed to cool to 20 °C between each analysis. The use of a L'vov platform gave more reproducible results.

Calibration and Analytical Characteristics. The calibration plot was obtained from a standard solution of 1 mg/mL and successive dilutions with 10% nitric acid, ranging from 0.1 to 1.0 ng/mL and prepared from a working solution of 10 ng/mL. These solutions, along with the blank (a few micrograms of vanadium pentaoxide, 2.5 mL of nitric acid, and ultrapure water to a total volume of 25 mL), were subjected to the same acid digestion bomb treatment as the samples, although this treatment has been shown to be unnecessary.

To detect possible interferences, the standard additions method was used with five arbitrarily chosen samples of different species. Amounts ranging from 2.5 to 5 ng of Cd were added to 200-mg fractions of dried samples and to blanks, and then all samples and blanks were mineralized and diluted with the same procedure. The slopes of the calibration lines for spiked samples were similar to the slope of the calibration line for the standards in acid medium; thus, matrix effects were considered to be negligible.

The equation for the calibration plot was absorbance = 0.015 + 0.250[Cd, ng/mL], with $r = 0.9989$ and $\alpha = 1\%$ (α, level of significance). The equation was linear to 2 pg of Cd. For the instrumental conditions used, the analytical detection limit (Long and Winefordner, 1983) was 0.16 pg of Cd.

The accuracy and precision of the method were checked with recovery assays. Known amounts of analyte were added to five different samples, which were then processed in triplicate for acid digestion and dilution as described above. Percentage recoveries ranged from 97.67% to 99.33%. The results obtained in ten replicate assays of BCR-CEC-certified reference material are shown in Table II. Absorbance in 10 successive determinations of two different samples were statistically analyzed as recommended by Stiel (1982). The results of the precision test are summarized in Table III.

RESULTS AND DISCUSSION

The dissolution of samples was achieved with a rapid straightforward method using a microwave acid digestion bomb. Mineralization with the microwave acid digestion bomb technique was complete within 90 s, which represents a significant savings in time when compared with other methods that require hours or even days. The use of a small volume of acid and the simplicity of the entire procedure reduce the risk of contamination, an important factor in the determination of trace elements. This procedure makes it possible to analyze several elements at once and may thus be applicable to other food samples (Cabrera et al., 1991, 1992; Navarro et al., 1992).

Cadmium was quantified by ETA-AAS, with an optimized time–temperature program for the drying, charring, and atomization phases in a graphite furnace. Factors with the potential to cause interferences were eliminated, and the final method was found to be highly accurate and precise. The optimized assay conditions obviate most matrix interferences and other sources of unspecific absorption. In addition, the use of standard additions is circumvented, making the analysis more straightforward. The detection limit and sensitivity are suitable for the range of Cd concentrations we encountered and are compatible with estimates given by other authors. Moreover, the analytical precision and accuracy of our method are acceptable (Horwitz, 1982).

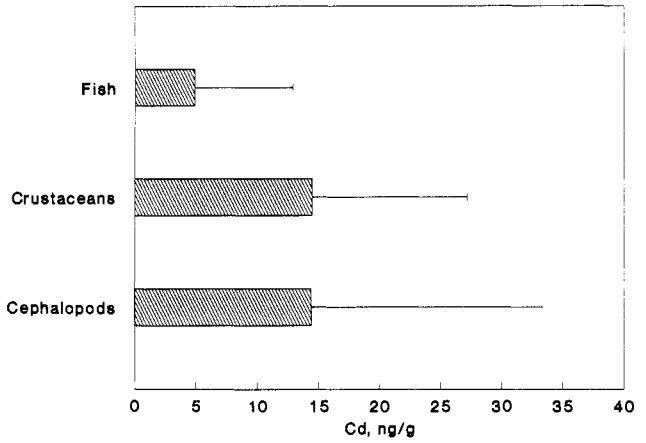

Figure 1. Mean levels of cadmium (nanograms per gram of fresh weight) in samples of fish, crustaceans, and cephalopods.

The advantages of the technique described here make our analytical method useful for the quality control of seafood and for estimating the degree of marine pollution and possible middle- and long-term toxicological risks.

We tested 32 species consumed as seafood, including the most commonly commercialized fish, crustaceans, and cephalopods, in Mediterranean coastal regions of Spain. Cadmium concentrations ranged from undetectable to 45.75 ng/g and are given for each species in Table IV. The concentrations of Cd in our samples were similar to those reported by other authors for species from other regions of the Mediterranean coast (Medina et al., 1986; Rincon et al., 1988; Schuhmacher et al., 1990). Notably, Cd concentrations were higher in crustaceans and cephalopods than in fish (Figure 1). Because of their tendency to concentrate Cd in their tissues even when levels of this element in seawater are not excessive, shrimp and squid may be considered biological indicators of marine Cd contamination.

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