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DETERMINATION OF LEAD AND CADMIUM IN BIOLOGICAL MATERIAL BY GRAPHITE FURNACE ATOMIC ABSORPTION SPECTROMETRY METHOD

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Abstract

A graphite furnace atomic absorption spectrometry method for lead and cadmium determination in biological material was prepared. The samples were digested in muffle furnace at 450ºC. The ash was dissolved in 1N hydrochloric acid and the final solution was diluted in 0.2% nitric acid. The heavy metals were determined using a Perkin-Elmer atomic absorption spectrometer equipped with electrodeless discharge lamps (EDL) at 283.3 nm for Pb and 228.8 nm for Cd. Ammonium dihydrogen phosphate and magnesium nitrate were used as matrix modifiers for both Pb and Cd analyses. The method was validated in terms of basic analytical parameters. The mean recoveries of lead and cadmium were 82.05 and 98.40% and their analytical detection limits were 0.001 and 0.0001 µg/g, respectively. Certified reference materials and participation in national and international proficiency studies were used for analytical quality assurance programme.

Key words: lead, cadmium, atomic absorption spectrometry, biological materials.

Lead and cadmium are industrial pollutants which have strong negative effect on human and animal health. These metals are accumulated in the organism, mainly in the liver and kidneys. The exposure to toxic elements could be minimalised by regular control of food and feed and setting maximum levels for heavy metals in these products. Commission Regulation (EC) No 466/2001 of 8 March 2001, and Polish Regulation of the Ministry of Health of 30 April 2004 established limits for lead and cadmium in foodstuffs. The limits for food of animal origin range from 0.01 to 1.00 mg/kg (3, 14, 19). A fully validated analytical method was prepared to support introduction of legislation to practice and necessity of lead and cadmium control in monitoring programme. The National Veterinary Residue Control Programme which is organized in Poland according to Council Directive 96/23/EC of 29 April, 1966 and Polish Regulation of the Ministry of Agriculture and Rural Development of April 19, 2004 requires controlling the levels of lead and cadmium in animal tissues and food of animal origin.

Material and Methods

Reagents. All chemicals (concentrated nitric acid, hydrochloric acid, matrix modifiers - magnesium nitrate hexahydrate and ammonium phosphate) were of analytical grade. Stock standard solutions 1 000 µg/ml were the reference solution from Beaker.

Working standard solutions were prepared by dilution of stock and intermediate standards. The working standards were as follows: Pb - 10, 20, 30, 40, 50, and 60 µg/l and Cd - 1, 2, 3, 4, 5, and 10 µg/l diluted in 0.2% nitric acid.

Samples. Lead and cadmium were determined in animal tissues, milk, eggs and feedstuffs. Bovine muscle BCR No 184, Pig kidney BCR No 186 and Milk powder CRM 151 were used as reference materials.

Apparatus. An atomic absorption spectrometer (Perkin-Elmer 4110 ZL) equipped with graphite furnace and As-72 autosampler was used for the determination of Pb and Cd. Argon was used as the pure gas. Mechanical oven and muffle furnace was equipped with temperature controller. All containers (quartz crucibles, plastic tubes) were cleaned with detergent and treated successively by the hydrochloric acid and rinsed with de-ionized water.

Ashing procedure. Weigh 2-10 g of homogenized sample into 50 ml quartz crucible. Dry in an oven (120 ± 20ºC) overnight until the sample is thoroughly dry. Place the sample into cool muffle furnace and raise the temperature of the oven to 450 ± 20ºC (50ºC/h). Next day, remove the samples from the oven and cool to room temperature. Add 1 ml concentrated nitric acid and put ash on a hot plate to get dry. Return the sample to the muffle furnace and raise the temperature to 450ºC. Keep the sample at this

temperature about 1 h. Repeat that step if needed. The ash must be carbon free. Remove the sample from the muffle furnace and cool to room temperature. Dissolve the ash sample in 5-10 ml of 1N HCl (1 g sample in 1ml of HCl). Transfer the solution from the crucible to a clean tube. The final solutions of the samples are diluted in 0.2% nitric acid. Each batch should include reagent blank and control sample containing all the reagents in the same volumes.

Instrument conditions. Lead - wavelength 283.3 nm, slit 0.7 nm, atomization 2000ºC, read time 3 s, sample volume 10 µl, modifiers volume 20 µl. Cadmium - wavelength 228.8 nm, slit 0.7 nm, atomization 1 550ºC, read time 3 s, sample volume 10 µl, modifiers volume 20 µl.

Instrument calibration. The calibration curve for the determination of lead was prepared using a blank and working standards solution (10 – 60 µg/l). The calibration curve for the determination of cadmium was prepared using a blank and working standards solution (1 – 10 µg/l).

Metals determination by Graphite Furnace Atomic Absorption Spectrofotometer (GF AAS). Detailed instructions on the operation of the Perkin-Elmer model 4110 ZL are described in the operator's manual.

The sample (calibration blank, standards, reagent blank, control sample**)** and matrix modifiers were introduced to the furnace by an autosampler. After the atomisation steps, concentrations of the lead and cadmium were reported in the computer in µg of metals/g, wet weight of sample.

The calibration was periodically verified by analysing the standard at the frequency of 20 readings. If the recovery was outside the limits, the analysis was stopped. The problem was corrected and the system was recalibrated.

Statistical analysis. The data obtained from the analysis were evaluated in based statistical parameters using computer program Excel.

Results

A series of experiments was conducted to establish optimum of analytical parameters. Optimalization of digestion condition for different biological matrices and selection of instrumental programmes for the determination of lead and cadmium were done. Dry ashing procedure at 450ºC with additional treatment of concentrated nitric acid produced satisfactory digestion of all biological samples (animal tissues, milk, eggs, feedstuffs).

The method was tested by studying the certified reference materials (Bovine muscle BCR No 184, Pig kidney BCR No 186, Milk powder CRM 151) with the certified values and was regularly evaluated by participation in proficiency programmes organised by Food Analysis Performance Assessment Scheme (FAPAS) and European Community Reference Laboratories. The results of proficiency tests were within 2 Z-scores for both metals. The results of the determination of lead and cadmium in certified reference materials are presented in Table 1.

Discussion

Different methodologies based on atomic absorption spectrometry have been reported for the determination of trace elements in various matrices (1, 2, 7, 8, 10-13, 15).

Sample digestion and temperature of atomisation are the most important stages in lead and cadmium analysis (4, 9, 16-18, 20, 21). Described dry ashing sample digestion procedure is very useful for lead and cadmium determination in biological materials. No significant loses of the metals (volatility) were observed during ashing procedure at 450ºC. All recovery results above 80% confirmed this observation.

There are two major advantages of dry ashing procedure: possibility of ashing more than 30 samples in one set and lack of samples contaminations from reagents.

The method was validated in terms of the linearity, limit of detection (LOD), limit of quantitation (LOQ), precision, recovery and uncertainty (5, 6). Validation reports are presented in Tables 2 and 3.

Atomisation at 2 000°C for lead and at 1 550ºC for cadmium was satisfactory to run all kinds of biological samples (animals tissues, milk, egg, feedstuffs). Magnesium nitrate hexahydrate and ammonium phosphate were necessary to use as matrix modifiers when lead and cadmium were determined in biological material.

Table 1

Comparison of the found and certified values of lead and cadmium in the certified reference materials (accuracy), n = 6

Certified materials	Lead			Cadmium		
	Certified value µg/g	Found µg/g x ± S	Recovery (%)	Certified value µg/g	Found µg/g x ± S	Recovery (%)
Bovine muscle BCR No 184	0.239	0.285 ± 0.051	119.25	0.013	0.014 ± 0.001	107.69
Pig kidney BCR No 186	0.306	0.316 ± 0.042	103.27	2.71	2.59 ± 0.198	95.57
Milk powder CRM 151	2.002	1.894 ± 0.301	94.61	0.101	0.099 ± 0.006	98.02

Table 2
Validation report – Cd determination by GF AAS

Parameters	Results					
Linearity (working range), mg/kg	0.001 – 0.010					
Limit of detection (LOD), mg/kg	0.0001					
Limit of quantitation (LOQ), mg/kg	0.0002					
Matrix	Liver			Eggs		
Levels of spiked samples, mg/kg	0.200	0.300	0.400	0.010	0.040	0.070
Repeatability						
x, mg/kg	0.203	0.284	0.362	0.011	0.039	0.075
SD, mg/kg	0.011	0.031	0.019	0.001	0.002	0.002
RSD, %	5.41	10.91	5.24	9.09	5.12	2.66
Intralaboratory reproducibility						
x, mg/kg	0.212	0.273	0.394	0.012	0.036	0.072
SD, mg/kg	0.012	0.027	0.018	0.001	0.001	0.002
RSD, %	5.66	9.89	4.56	8.33	2.77	2.77
Recovery, %	105.80	90.80	98.60	107.50	91.10	102.50
Uncertainty						
combined (uc)	0.043			0.004		
expanded (U)	0.400 ± 0.086 mg/kg			0.040 ± 0.008 mg/kg		
coverage factor (k)	2			2		

Table 3
Validation report – Pb determination by GF AAS

Parameters	Results					
Linearity (working range), mg/kg	0.010 – 0.060					
Limit of detection (LOD), mg/kg	0.001					
Limit of quantitation (LOQ), mg/kg	0.002					
Matrix	Liver			Eggs		
Levels of spiked samples, mg/kg	0.200	0.300	0.450	0.050	0.200	0.400
Repeatability						
x, mg/kg	0.172	0.243	0.395	0.049	0.175	0.374
SD, mg/kg	0.022	0.041	0.054	0.005	0.011	0.017
RSD, %	12.79	16.87	13.67	10.20	6.28	4.54
Intralaboratory reproducibility						
x, mg/kg	0.161	0.247	0.376	0.044	0.170	0.346
SD, mg/kg	0.016	0.027	0.035	0.003	0.007	0.018
RSD, %	9.93	10.93	9.30	6.81	4.11	2.20
Recovery, %	80.30	82.20	83.60	87.70	84.90	86.50
Uncertainty						
combined (uc)	0.058			0.0074		
expanded (U)	0.450 ± 0.116 mg/kg			0.050 ± 0.015 mg/kg		
coverage factor (k)	2			2		

Value and suitability of the developed method was fully supported by validation results. The validation procedure is in agreement with the Commission Decision of 12 August 2002 (2002/657/EC) implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. All validation data confirm that the method could be used as routine procedure for the determination of lead and cadmium levels in food and feed in official monitoring control programme. LOD and LOQ values are much below the maximum levels which were settled for lead and cadmium in foodstuffs. Generally, good results of precision (RSD below 10%), recoveries (above 80%) and reasonable value of

uncertainty additionally support the described method as a routine procedure for lead and cadmium determination in biological materials.

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