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Cd and Pb determination in some Romanian south eastern region cereals

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Abstract The purpose of this paper is to present original studies about the internal validation of trace Cd and Pb determination in cereals using flame atomic absorption spectrometry (FAAS) technique before the metal occurrence in real samples investigation. The following criteria have been assessed: selectivity, repeatability, reproducibility, limits of detection (LOD), limits of determination (LOQ) and the uncertainties of the assigned values. Certified reference materials ZC 73009 wheat flour and ZC 73010 maize, a GBC Avanta FAAS spectrometer and high purity reagents have been used to perform the proposed investigations. Samples have been mineralized by wet digestion with HCl, HNO_3 and hydrogen peroxide in several steps. The obtained results for method validation demonstrated that the studied method corresponds to determine low concentrations of Cd and Pb in different cereals matrix. 108 samples of wheat, maize, rice, barley, two-row barley and mixed cereals grains from the Romanian south eastern region collected in 2007 and 2008 have been analyzed using FAAS technique and the results show that Cd and Pb levels in all samples are below the imposed limits.

Keywords: cadmium, lead, cereals, FAAS, internal validation, uncertainty

1. Introduction

Heavy metals normally occurring in nature are not harmful, because they are only present in very small amounts. However, if the levels of these metals are elevated, then they can show negative effects [1-3]. The use of vegetables as indicators to the environment pollution represents an interesting feature [4]. Lastly, the public awareness of the impact of environmental pollution on nutrition and with environmental issues in general provoked a major dietary conversion of a sizeable part of the population towards organically produced food, in the hope of a healthier and environmentally friendly alternative [5].

The interest in the determination of Cd and Pb in the environment, food and biological samples are increasingly growing due to the knowledge their extremely toxicity for human even at very low levels. It is known that lead is health-endangering metal for human and its effects include blood enzyme changes, anemia, hyperactivity, and neurological disorders [6].

In food safety and environmental studies it is imperious necessary to have the appropriate analytical method which will deliver a valid result; this leads to demonstrate properly the validity of the method [7].

The aim of the paper is to present original studies about the internal validation of trace Cd and Pb determination in cereals using flame atomic absorption spectrometry (FAAS) technique. The following criteria have been assessed: selectivity, repeatability, reproducibility, limits of detection (LOD), limits of determination (LOQ) and the uncertainties of the assigned values. The assessed method was applied to analyze Cd and Pb in 108 samples of different cereals harvested in 2007 and 2008 from the Romanian south eastern region.

2. Experimental

2.1. Materials and reagents

Analytical grade chemicals (HCl 37%, HNO_3>69%, hydrogen peroxide 30%, Cd, Fe and Pb certified analytical standard solutions 1000mg/L purchased from Merck and Fluka) and certified reference materials ZC 73009 wheat flour, and ZC 73010 maize have been used.

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2.2. Sampling

For Cd an Pb determination, 10 – 20 grams of dried cereal samples (wheat, maize, rice, barley, two-row barley and mixed cereals grains or certified reference materials) have been processed in calcination furnace using the next temperature program: 2 hour at 100^0C, slowly increase to 350^0C (50^0/hour), increase to 450^0C and 16 hours keep at 450^0C. The ashes have been dissolved in 1 mL HNO_3 1:10 (v/v) and 1-2 mL hydrogen peroxide, the resulted solutions have been evaporated to dryness; the deposit was kept 1-2 hours in the furnace at 450^0C, the white ashes have been treated with 5 mL HCl 1:1, evaporated to dryness, treated with 1mL HNO_3 1:1, evaporated to dryness, treated with 5mL HCl 1:1, heated 15 minutes until the dissolution, dilution with 10 mL deionized water, filtration and the solutions made up to V (50-100mL) with deionised water in calibrated flasks.

To obtain fortified samples, certified materials have been enriched with different Cd or Pb standard solutions before sample dissolution, and the same procedure has been applied for sample dissolution.

2.3. Apparatus

For the development and evaluation of the method, a GBC Avanta flame atomic absorption spectrometer has been used. The main characteristics of the equipment for Cd and Pb determination are presented in table 1.

Table 1. Technical characteristics for Cd and Pb determination using FAAS

El.	λ (nm)	Optimum conc. range (μg/L)	Gases flow L/min	
			Air	Acety-lene
Cd	228.8	0 – 1.8	10	1
Pb	217.0	0.2 – 2	10.2	1.1

2.4. Measurements

Appropriate quality assurance procedures and precautions were carried out to ensure reliability of the results. Samples were generally carefully handled to avoid contamination. To assure the results quality and to control relevant errors sources, duplicate samples, control samples (reference materials and laboratory fortified samples) have been used and new calibration curves have been determined for each set of samples. The recovery percent must be in the range of 80-120%.

For each set of samples it was verified a point on the curve and the concentration, in terms of recovery, must be in the range of 80-120%.

The metal concentrations to plot calibration curves were (in μg/mL): for Cd: 0.01; 0.05; 0.08; 0.1; 0.5; and for Pb: 0.2; 0.5; 1.0; 1.5; 2.0.

To determine metal concentration, it was prepared also a witness solution containing all reagents used in sample preparation step.

The metal concentration in solid samples has been calculated using the formula:

$$E,\ mg/kg = (C_{sample} - C_{witness}) \times V \times dilution / m$$

where:

- C_{sample} and $C_{witness}$ – metal concentration, mg/L from calibration curve in the sample solution respectively in the witness (all reagents except sample)
- V – total volume of the sample' solution (50 or 100 mL
- m – solid sample's weight (g)

2.5. Internal validation procedure

The following criteria have been assessed: selectivity, liniarity, repeatability, reproducibility, limits of detection (LOD), limits of determination (LOQ) and the uncertainties of the assigned values.

To assess the method selectivity, there was studied the influence of iron at 100 mg/L concentration on the lead different concentrations determination. The other parameters have been verified using the general procedure recommended by actual regulations [7-9].

To be effective, a proposed method must permit a LOQ under the maximum residue limit (MRL) minus three times standard deviation of repetability/ reproductibility for a fortified sample to the MRL.

The samples enrichments have been done to reach 1.0 and 1.5 times the MRL which are 0.2 mg/kg for Pb in all cereals and for Cd in wheat and rice and 0.1mg/kg for all other cereals [10].

3. Results and Discussions

The study of the iron concentration (100 mg/L) influence on two Pb standard solutions shows that

there are no significant differences between measurements (Table 2), that proves the method selectivity.

Table 2. Iron interference study on the two lead standard solutions

Sample	Pb, mg/L	
	no Fe	100 mg/L Fe
1	0.603	0.603
2	0.605	0.604
3	0.598	0.601
mean	**0.602**	**0.602667**
1	1.028	1.011
2	1.01	1.028
3	1.03	1.041
mean	**1.022667**	**1.026667**

Three superimposed calibration curves for Cd and Pb (Fig. 1 and Fig. 2) demonstrate the linearity of the method, with excellent values of correlation coefficients (0.9993 for Cd and 0.9973 for Pb).

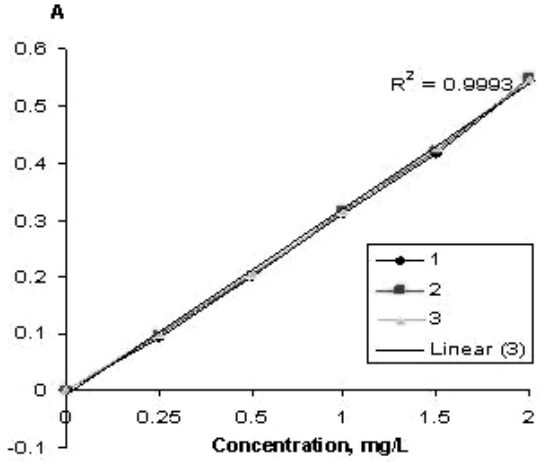

Fig.1. Cd superimposed calibration curves

The linearity for trace metals determination using FAAS is respected in the range of 0.01-0.05 mg/L for Cd in 0.2-2 mg/L for Pb.

To assess the fidelity of the method, certified reference materials have been analyzed and the obtained results are presented in Table 3. The other calculated parameters of the method performances have respected the recommended criteria and allow demonstrating that the method is adequate to the purpose (Table 4).

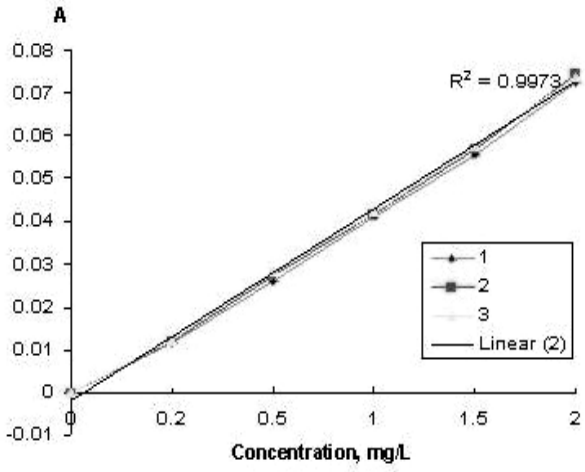

Fig.1. Pb superimposed calibration curves

Table 3. Pb and Cd concentration validation in reference materials

Sample	Pb, mg/kg $\pm u_{ref}$*.		Fidelity, %
	Certified	Found	
Maize ZC 73010	0.07±0.04	0.075±0.015	107.14
Wheat flour ZC 73009	0.065±0.024	0.060±0.012	92.30

* standard uncertainty [11]

Table 4. Method performance parameters

Parameter	Value	
	Cd	Pb
Detection limit, mg/Kg	0.01	0.07
Quantification limit mg/kg	0.02	0.14
Recovery %	87.54	98.45
Recovery, RSD (%)	6.94	9.02
Repeatability, RSD (%)	7.9	7.41
Reproducibility, RSD (%)	8.92	9.58
Standard uncertainty, mg/L	0.0037	0.026134
Measurement uncertainty, mg/L	0.00923	0.00394
Estimated expanded uncertainty mg/kg	0.015	0.037

The real samples analyses (Table 5) show results comparable with other reported values [12, 13].

Table 5. Cd and Pb occurrence in the Romanian South Eastern region' cereals in 2007-2008

County	Samples (Number of)	Concentration. mg/kg	
		Pb (**0.20**)	Cd (**0.2 wheat, rice; 0.1 other**)
Constanța (36)	Maize (10)	ND (9) 0.07	ND (3); 0.011- 0.046
	Two-row barley(1)	0.11	ND
	Wheat (25)	ND (24)	ND (7); 0.010 – 0.052
Ialomița (26)	Maize (3)	ND	ND (2); 0.03
	Barley(1)	ND	ND
	Wheat (20)	ND (15); 0.10-0.19	ND (7); 0.010 – 0.052
	Rice(2)	ND	0.027 – 0.028
Tulcea (34)	Maize (4)	ND	ND
	Barley (6)	ND (2) 0.09 – 0.11	ND (4)
	Wheat (20)	ND (14); 0.07 – 0.15	ND (9); 0.049
	Mixed cereals (4)	ND	0.011 – 0.012
Teleorman (12)	Wheat(6)	ND	ND (3); 0.018 – 0.050
	Mixed cereals (6)	ND	ND (4); 0.010 – 0.018

ND – not detectable

Pb was undetectable in 71 samples and Cd in 45 samples.

4. Conclusions

The obtained results for method validation demonstrated that the studied method corresponds to determine low concentrations of Cd and Pb in different cereals matrix.

108 samples of wheat, maize, rice, barley, two-row barley and mixed cereals grains from the Romanian south eastern region collected in 2007 and 2008 have been analyzed using FAAS technique and the results show that Cd and Pb levels in all samples are below the imposed limits.

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