ISSN : 0974-7419 Volume 15 Issue 6

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 15(6) 2015 [213-218]

Development and validation of an analytical method for atomic absorption spectrometry analysis of Pb in bivalve molluscs

S.Benhar[1,2], S.Belouafa[1]*, A.Chafik[2], M.Charrouf[1], R.Chfiri[2], A.Semlali[2], M.Maghraoui[2], A.Bennamara[1], A.Abourriche[1]

[1]Laboratoire Biomolécules et Synthèse Organique, Université Hassan II – Mohammedia, Faculté des Sciences Ben M'Sik, Avenue Cdt D. El harti, BP 7955, Casablanca, (MAROC)

[2]Institut National de Recherche Halieutique (INRH), Casablanca, (MAROC)

E-mail : sbelouafa@yahoo.fr

ABSTRACT

A rapid, sensitive, precise and accurate method for the determination of lead (Pb) in bivalve molluscs by graphite furnace atomic absorption spectrometrywas developed in regard to AOAC Official Method 999.10 and ISO/IEC 17025. This approach was investigated so as to minimize the analysis time, taking into consideration the sensitivity. For method validation, precision and accuracy by addition recovery tests have beenevaluated as performance criteria.

KEYWORDS

Bivalve mollusks;
Lead;
Analytical method;
Atomic absorption spectrometry;
Validation.

INTRODUCTION

The growing concern for public health obligates to set maximum permissible levels for major toxic elements in food. Due to the availability and variety of different analytical methods for the determination of many residues in food samples, it is necessary to ensure the quality and comparability of the analytical results generated by laboratories approved for official control. For this purpose, there is an increasing worldwide interest in performing analytical methods which can provide accurate and precise measurements with low detection limits and short analysis times. This should be carried out by using quality assurance systems and particularly by applying methods validated according to common procedures and performance criteria[1-5].

Several studies have been performed on metal accumulation in various seafood and fish feed samples[6-10]. In these studies diverse analytical techniques, among other inductively coupled plasma-atomic emission spectrometry (ICP-AES)[9], inductively coupled plasma mass spectrometry (ICP-MS)[6], flame (FAAS) and graphite furnace atomic absorption spectrometry (GFAAS)[8] were used for the determination of trace elements after matrix destruction based on oxidation with concentrated acids. However, no official method is available for the estimation of lead (Pb) in bivalve molluscs.

In this study, a rapid method for the determination of Pb in bivalve molluscs by Electrothermal Atomic Absorption Spectrometry was developed according to AOAC Official Method 999.10[11] and ISO/IEC 17025[12]. The developed method was validated and it is now available for the official control of Pb in bivalve molluscs.

MATERIALS AND METHODS

Apparatus

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The following equipments have been used during the proposed validation study:

A Shimadzu AA 6800 atomic absorption spectrometer equipped with an ASC-6100 heated graphite and a GFA-EX7 autosampler.

A Shimadzu AA 6300 atomic absorption spectrometer equipped with an ASC-6100 heated graphite and a GFA-EX7i autosampler.

A CEM MARS microwave oven for the mineralization of samples.

Materials and chemical reagents

Analytical grade chemicals were used as received.

Nitric acid 65% for analysis (Merck).

Magnesium nitrate $Mg(NO_3)_2.6\ H_2O$ for analysis (Merck).

Ultra-pure water.

Reference material (SRM-2976) metal concentrations are certified from the National Institute of Standards and Technology (NIST).

Procedure

Before used, the materials was cleaned and dried separately as follows:

- Stay overnight (12 h) in a 20% special detergent (Micro 90),
- Rinsing with tap water and then with distilled water,
- Stay of 24 h in 10% nitric acid,
- Rinsing with distilled water then with ultra-pure water,
- Drying under a laminar flow hood.
- In reactors of mineralization were added 5 ml of concentrated HNO_3 and 2 ml of H_2O_2 on 0.25 g dry weight of mollusc samples.

The reactors are sealed at room temperature for 1 h, then they are heated by microwave oven according to the following manufacturer's recommended program:

The reactors cooling are obtained by circulation of a cold air flow in the microwave for 1 h. The mineralized samples are then transferred into conical tubes of polypropylene and gauged to 50 ml with the ultra-pure water.

The ammonium dihydrogen phosphate and the magnesium nitrate are used as chemical modifiers.

Principle of analysis

TABLE 1: Microwave's program

Power	Percentage ofpower	Ascent times	Pressure	Temperature	Holding time
800 W	75 %	20 mn	300 PSI	200 °C	10 mn

TABLE 2: Limit of detection, limit of quantification and ratio of conformity

LOD (µg/l)	LOQ (µg/l)	LOD (mg/kg)	LOQ (mg/kg)	R
0.557	1.856	0.022	0.074	5.819

The principle of analysis is to determine lead in bivalve molluscs using graphite furnace atomic absorption spectrometry as describes by the Association Official Agricultural Chemists (AOAC)[12]. Sample preparations are briefly described above.

The measurements are performed in triplicate at 324.7 nm (specific wavelength measurement of Pb).

The signal is quantified in height and the background correction is carried out using a deuterium lamp. The temperature is programmed at 200°C (TABLE 1). Samples are diluted at 1/10 in polypropylene cups (100 µl of serum + 900 µl of distilled water), then a third dilution is carried out by the automate.

The quantification is performed using a range of external calibration from a synthetic range prepared in distilled water. Indeed, the matrix effect is minimal since the sample is diluted with the water at 1/30.

Validation of spectrometry method

The analytical method was validated by various parameters as recommended in protocol specifications of the DR-12-VMC[13] and in accordance with the design of parameter/matrix specified in Directive DR-12 VAL[14] about validation of analytical methods in chemistry.

The following parameters were considered: limit of detection (LOD), limit of quantification (LOQ), linearity, precision, sensitivity, specificity and accuracy.

Limit of detection (LOD)

LOD is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. It corresponds to 3 times the standard deviation of 10 replicates of samples. The result has been shown in TABLE 2.

Limit of quantification (LOQ)

LOQ is the lowest amount of analyte in a sample

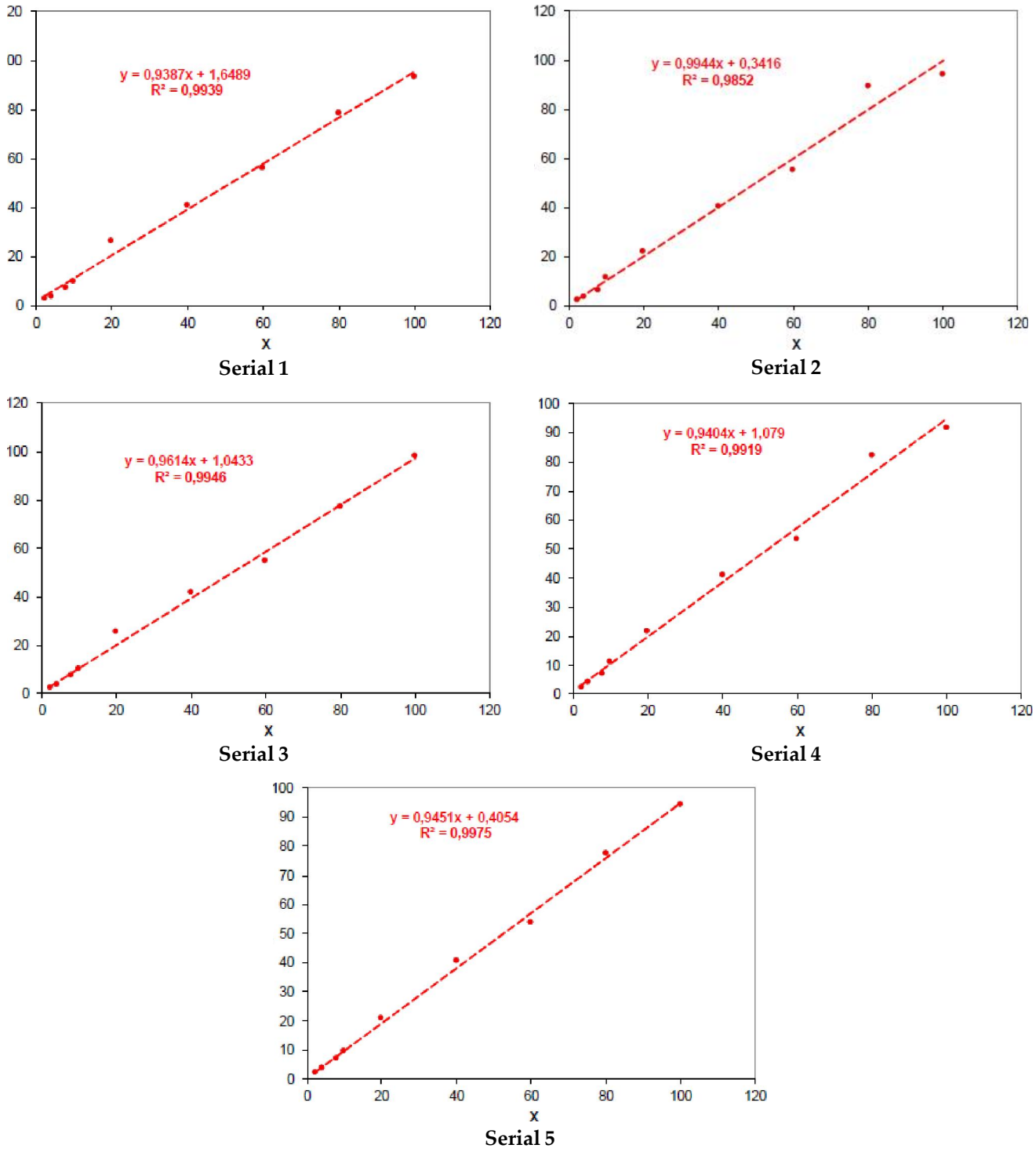

Figure 1 : Calibration curves

which can be quantitatively determined with suitable precision and accuracy. It corresponds to 10 times the standard deviation of 10 replicates of samples. The result has been shown in TABLE 2.

Linearity

The linearity of analytical method was determined by studying standard calibration curves. The range of analytical method was decided from the interval between upper and lower level of calibration curves. Thus, nine concentrations of reference material were analyzed 5 times over the range 2.5-100 mg/kg. The results of

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TABLE 3 : ANOVA table

Source	SS	df	MS	F	$F_{critical}$	p-value	α
Between	15.74	4	3.94	0.003	2.606	1.000	0.05
Within	48305.61	40	1207.64				
Total	48321.35	44					

TABLE 4 : Result of precision study

	Repeatability	Reproducibility	Replicability
Number (n)	10	10	10
Mean (d)	1.198	1.181	0.989
Standard Deviation (Sd)	0.103	0.133	0.072
α		0.050	
t(crit)		2.262	
(tcrit x Sd)√n	0.074	0.095	0.051
Confidence Interval	1.198 ± 0.074	1.181 ± 0.095	0.989± 0.051
$t_{observed}$	36.6599642	28.0837606	43.494
$RSD_{calculated}$	8.626	11.260	
$RSD_{estimated}$	6.736	6.736	
$RSD_{calculated}/RSD_{estimated}$	1.280	1.672	
Limit	0.289	0.266	
Ratio of Repeatability limit/ Reproducibility limit		1.088	

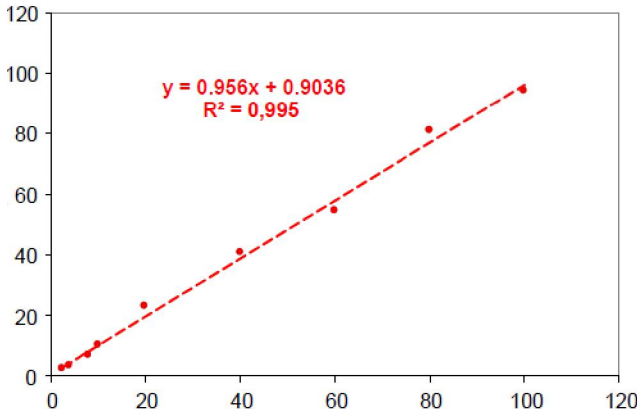

Figure 2 : Linear regression curve

linearity study for each group are shown in the Figure 1 and TABLE 3 respectively.

Precision

The precision of an analytical method was studied by performing Replicability, Repeatability and Reproducibility. They were performed for 10 replicates with simples using reference material (SRN-2976). The results of statistical evaluation are mentioned in TABLE 4.

Replicability

TABLE 5: Result of specificity study

Regressionequation(y = ax + b)	y = 0.956x + 0.9036
Slope (a)	0.9560
Intercept (b)	0.9036
Correlation coefficient (r)	0.9975
Sa	0.0256
Sb	1.2713
Sy/x	2.6219
Degrees of freedom (DF)	7
sum of squared residuals (SSR)	48.1221
(1 - a)/Sa	1.718646
(0 - b)/ $t_{estimated}$	-0.710826
α	0.05
t(crit)	2.36

TABLE 6 : Result of accuracy study

Number (n)	10
Mean (d)	0.989
Standard Deviation (Sd)	0.072
Concentration of Reference Material (C) (µg/g)	1.190
Uncertainty of reference materials	0.180
Relative error (%)	16.879
Accuracy (%)	83.121
Accuracy error	1.036

TABLE 7 : Result of recovery study

C	Cf	Cf - C	Ca	% Recovery
1.5638	2.3260	0.762	1.000	76.22
3.4396	4.6253	1.186	1.500	79.05
3.1946	5.8600	2.665	2.500	106.62
4.2333	6.1344	1.901	2.000	95.06
9.1722	12.151	2.979	3.000	99.30
	Mean			91.248
	Sd			13.136
	α			0.05
	t(crit)			2.78
	t(obs)			15.53208229

C: Concentration before standard additions; Cf: Concentration after standard additions; Ca: Concentration of standard additions

Replicability result indicates the precision under the same operating conditions (same analyst, same apparatus and same day) over a short interval of time.

Repeatability

Repeatability result indicates the precision under the

following operating conditions: same analyst, same apparatus and different days.

Reproducibility

Reproducibility result indicates the precision under the following operating conditions: different analysts, different apparatus and different days.

Sensitivity

The range standards using in linearity study, was used as template for assessing the sensitivity of the developed method. The sensitivity is expressed as the mean slope of the calibration curves. This may also be expressed as the ratio between the signal obtained and the concentration of a standard. The result was reported in Figure 2.

Specificity

The results of sensitivity, were used to assess the specificity of the method by studying the regression, the slope of the line and the intercept. TABLE 5 summarizes the results.

Accuracy

Accuracy of the method was determined by analyzing the experimental technical, at ten replicates, using reference material NIST (SRM-2976). Data of accuracy was presented in TABLE 6.

Furthermore, in order to ensure the suitability and reliability of proposed method, recovery studies were carried out. To five concentrations, a known quantity of standard was added and the contents were re-analyzed by the proposed method. The addition is done directly before the mineralization on the sample and after digestion. The % of recovery was calculated and validated (TABLE 7).

RESULTS AND DISCUSSION

LOD, as required by Regulation (EU) n° 836.2011[16], must be less than or equal to one tenth of the maximum admissible content of Pb in bivalve molluscs (1.5 mg/kg). This shows therefore the validity of the calculated LOD (TABLE 2). Moreover, Ratio of Conformity (5.8) is between 4 and 10[13], thus LOD was validated again.

As regards the limit of quantification LQM, according to Regulation (EU) n° 836.2011[16], must be less than or equal to one fifth of the maximum content of Pb in bivalve molluscs (0.3 mg/kg). This checks the validity of the calculated LOQ (TABLE 2).

The results obtained in Figure 1 show that correlation coefficients were well above 0.99 for all studied serials, which demonstrates the linearity of the method.

To compare the results of five repetitions, Fischer test was used to validate the linearity range (TABLE 3). F ratio is less than the critical value of F corresponding to Fisher variable for á = 5%. Then, the results show that the linear range is validated and the regression model is acceptable.

Student's t test was used to validate the replicability, repeatability and reproducibility (TABLE 4).

For Pb, the usual concentration is less than 1.5 mg/kg that to say a concentration ratio equals 15.10^{-7}[16]. Which permit the application of Horwitz equation with $RSD_{estimated} = 2C^{(-0.15)}$[17]. The ratio of repeatability limit /reproducibility limit are below the limit value of 2[16,18].

All these results suggest that there is a closeness of agreement between the results obtained by applying the method at several replicates under determined conditions.

All regression curves (Figure 1), including that of the mean of the calibration curves (Figure 2) show that an increase of concentration results a gain of the signal. This means that the method is sensitive.

The Student test (TABLE 5) has concluded that the specificity of the method is acceptable with no interference.

The calculated accuracy error (TABLE 6) is less than 2. So, it is considered insignificant[19]. Therefore, the uncertainty associated to accuracy of the method is equal to the uncertainty of the reference material used for testing accuracy study.

Recovery study (TABLE 7) shows that the calculated percentage recovery varies between 76% and 106%. These percentages were validated by Student's t test[20].

CONCLUSION

The analytical results and the statistical evaluation of this validation study conducted to the following conclusions:

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The presented analytical procedure for lead determination in bivalve molluscs it is compliant and may be used in this purpose in imposed conditions.

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