Talanta 86 (2011) 442–446

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Short communication

Ultrasonic energy as a tool to overcome some drawbacks in the determination of lead in brain tissue and urine of rats

D. Guimarães[a], J.P. Santos[a], M.L. Carvalho[b], G. Vale[c,d], H.M. Santos[c,e,f], V. Geraldes[g], I. Rocha[g], J.L. Capelo[c,h,*]

[a] Centro de Física Atómica, CFA, Departamento de Física, Faculdade de Ciências e Tecnologia, FCT, Universidade Nova de Lisboa, 2829-516 Monte da Caparica, Portugal
[b] Centro de Física Atómica, CFA, Departamento de Física, Faculdade de Ciências, FCUL, Universidade de Lisboa, Av. Prof. Gama Pinto 2, 1649-003, Lisboa, Portugal
[c] REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Monte da Caparica, Portugal
[d] Centro de Química Estrutural, Instituto Superior Técnico de Lisboa, Torre Sul, Lisboa, Portugal
[e] Institute for Biotechnology and Bioengineering. Centre of Genomics and Biotechnology – University of Trás-os-Montes and Alto Douro, 5001-801 Vila Real, Portugal
[f] Intituto de Biotecnología y Biomedicina, Universidad Autónoma de Barcelona, 08193 Bellaterra, Barcelona, Spain
[g] Instituto de Fisiologia, Faculdade de Medicina de Lisboa and Instituto de Medicina Molecular, Lisboa, Av. Prof. Egas Moniz, 1649-028 Lisboa, Portugal
[h] Physical Chemistry Department, Bioscope Group, University of Vigo at Ourense Campus, Faculty of Science at Ourense Campus, E-32004 Ourense, Spain

ARTICLE INFO

Article history:
Received 8 May 2011
Received in revised form 26 August 2011
Accepted 1 September 2011
Available online 17 September 2011

Keywords:
Rats
Brain
Lead
Pb
Ultrasonic
Electrothermal atomization
Urine

ABSTRACT

An ultrasonic assisted solid–liquid extraction method was developed to determine the level of lead in the brain and urine of rats. Lead was determined by electrothermal atomic absorption spectrometry with longitudinal-Zeeman background correction. Several analytical drawbacks were addressed and overcome, namely small brain sample mass and the formation of precipitate in the urine samples. Ultrasonication provided by an ultrasonic probe succeeded in extracting lead from brain samples. Furthermore, it was demonstrated that the formation of a precipitate lowered the lead content in the liquid phase of the urine. Lead was back extracted from the precipitate to the liquid phase with the aid of ultrasonic energy and acidifying the urine with 10% v/v nitric acid. A microwave-assisted acid digestion protocol was used to check the completeness of the lead extraction. The within bath and between bath precision was 5% ($n=9$) and 7% ($n=3$) respectively. The limit of quantification was 1.05 $\mu g\,g^{-1}$ for brain samples and 2.1 $\mu g\,L^{-1}$ for urine samples. A total of 6 samples of urine and 12 samples of brain from control rats and another 6 samples of urine and 12 samples of brain from rats fed with tap water rich in lead acetate were used in this research. Lead levels in brain and urine from exposed rats ranged from $1.9 \pm 0.2\ \mu g\,g^{-1}$ to $3.5 \pm 0.2\ \mu g\,g^{-1}$ and from $752 \pm 56\ \mu g\,L^{-1}$ to $60.9 \pm 1.2\ mg\,L^{-1}$ respectively. Statistically significant differences of levels of lead in brain and urine were found between exposed and non exposed rats.

1. Introduction

The presence of lead in brain has been linked to neurotoxicity, mild mental retardation and low IQ scores in children [1–4]. Therefore to determine the level of lead in the brain is a key feature in the study of these health problems. Many studies regarding brain issues are first investigated using rats. To determine the level of lead in a rat's brain is a difficult process, especially if the target is formed of specific brain areas. This happens for two main reasons. One is the low sample mass available, typically 10–20 mg of wet tissue, which makes analytical replicates of the same sample extremely difficult to be obtained and the second reason is the low lead concentration usually found in rat brain. Lead is generally extracted from soft tissues, such as brain, by solubilising them using microwave pressurized acid digestion [5,6].

This methodology involves a complicate and time consuming sample handling, rendering a low throughput. In addition, the volumes of the solutions used in the treatment tend increase the limit of detection. The use of ultrasonic energy, UE, overcomes the above mentioned problems. UE allows high sample throughput as the sample treatment is typically completed in 1 min. Furthermore, the amounts of sample required are regularly of tens of milligrams. In addition, the final sample volume is formed of some hundreds

* Corresponding author at: Physical Chemistry Department, Bioscope Group, University of Vigo at Ourense Campus, Faculty of Science at Ourense Campus, E-32004 Ourense, Spain. Tel.: +34 610 835 903.
E-mail address: jlcapelom@uvigo.es (J.L. Capelo).
URL: http://www.bioscopegroup.org (J.L. Capelo).

0039-9140/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2011.09.001

Table 1
Instrumental parameters and heating program for Pb determination in urine.

		Spectrometer setup
Line wavelength (nm)		217
Lamp type		HCL
Spectral bandpass (nm)		0.5
Lamp current (mA)		4.0
Stage	Temperature/time (°C s^{-1})	Argon flow rate (L min^{-1})
Dry 1	90/35	2
Dry 2	105/25	2
Dry 3	110/13	2
Pyrolysis	900/13	2
AZ*	900/4	0
Atomize	1800/4	0
Cleanout	2300/5	2

HCL, hollow cathode lamp; AZ*, autozero for the graphite tube.

of microliters, typically 500. This renders low limits of detection. On the overall UE match many of the concepts of analytical minimalism as outlined by Halls [7]: low sample mass and low reagent volume consumption, low waste generation, easy to handle, high throughput and short treatment time.

A precipitate tends to be formed in non preserved, bad preserved or long-term preserved urine [8]. As consequence, the concentration of lead in urine lowers in time as it co-precipitates with the solid. As result, accurate lead quantification in urine is achieved only if the solid phase and the liquid phase are both investigated for lead content. This problem makes the analysis time consuming and expensive. UE has been described as a powerful tool to extract lead from solids [9–15]. The technique is effective when the lead is softly bounded to the solid, as in the case of a co-precipitation.

This work describes an ultrasonic based methodology that helps determine the levels of lead in brain and urine of rats, overcoming the drawbacks above described. The lead is determined by electrothermal atomic absorption spectrometry with longitudinal-Zeeman background correction. The method proposed is fast and cheap, easy to handle, it does not waste time and it has high sample throughput.

2. Experimental

2.1. Apparatus

An ultrasonic probe, UP200 s, from dr. Hielscher (Teltow, Germany, 200 W, 24 kHz) was used to perform the ultrasonic-assisted sample treatments. 1 mm and 14 mm diameter probe tips were used for the treatment of brain and urine samples, respectively. Ultrasonic energy irradiation was fixed at a power setting of 50%. A Sky Line minicentrifuge-vortex (ELMI, Riga, Latvia), a centrifuge Tehtnica Centric 150 (Železniki, Slovenia) and an oven from JP Selecta, Sa were used throughout the sample treatment when necessary. Acid digestions were done using 45 mL capacity Parr reactors and a De Longui microwave oven model Perfecto Easy Microwave MW314 (700 W, 2450 MHz). Two PTFE capped digestion vials were used with the respective microwave pressure digestion bombs. Lead absorbance was measured with an Analytik Jena AG atomic absorption spectrometer model AASZenit 650 equipped with a transversely heated graphite furnace, a MPE 60 autosampler and a Zeeman-effect background correction system. The atomic signals were measured in the peak area mode. High purity argon was used as purge gas. 20 μL of sample and 5 μL of matrix modifier were introduced in the graphite furnace. The instrumental parameters and thermal programs are listed in Table 1. The graphite tube used was an AAS Z-standard tube.

2.2. Reagents

All chemicals used were of analytical-reagent grade. Deionized water (18.3 MΩ) from a Milli-Q purification system (Millipore, Molsheim, France) was used throughout the experiments. The stock standard solution of lead (1000 μg mL^{-1}) was obtained from Fluka (Buchs, Switzerland). Lead calibration standard solutions were prepared using an appropriate dilution of the stock standard solution. The 0.2% w/v lead acetate solution used as drinking water was prepared by dissolving the appropriate amount of lead (II) acetate 3-hydrate (Sigma-Aldrich Quimica SA, Madrid, Spain) in deionized water. Ten percent (v/v) formaldehyde (Panreac, Barcelona, Spain) solution (pH 7.4) was used to preserve brain samples. Nitric acid 65% m/m (Merk, Darmstadt, Germany) and hydrogen peroxide 30% m/V (Panreac, Barcelona, Spain) were used in the ultrasonic-assisted extraction of lead and in the microwave digestions. Palladium nitrate 10 μg mL^{-1} (Fluka, Buchs, Switzerland) was used as a chemical modifier for Pb determination by Zeeman-ETAAS.

2.3. Calibrator material

Analytical validation was done using the ClinCal Pb urine calibrator from RECIPE (Munich, Germany). It was supplied in lyophilized form and it was reconstituted by dissolving the total content with high purity distilled water. The calibrator originally closed was stored at 4 °C and after its reconstitution at −20 °C. The reference value was 64.2 ± 3.7 μg L^{-1}.

2.4. Rats urine and brain samples

Brain tissue and urine from rats with different periods of lead-exposition were collected and analysed by ETAAS. Rats aging between 1 and 11 months were divided in two groups. One group was fed with 0.2% w/v lead acetate in tap water, while a second group was used a control group. Animals were confined in cages under laboratory conditions in agreement with the directives of EU legislation on animal experimentation. Six urine from control (test) rats and six urines from exposed rats were studied. To collect these samples, rats were placed in metabolic cages during 24 h.

Concerning the brain samples, two different areas of the brain were studied: the Hypothalamic Defence Area (HDA) and the Nucleus Tractus Solitarius (NTS). A total of twelve tissues from control (test) rats (were analysed), six HDA and six NTS, and twelve tissues from exposed rats, six HDA and six NTS, were analysed. To obtain the soft tissues, the rats from both groups were killed with an overdose of sodium pentobarbitone (100 mg mL^{-1} per kg of body weight). The brain samples were placed in four different vessels, depending on type of tissue and exposure regime, preserved in formaldehyde and stored in the refrigerator at 2 °C. Urine samples were also placed in plastic vessels with no reagents addition and stored at 2 °C.

2.5. Sample treatment

2.5.1. Formaldehyde analysis

The lead content of the formaldehyde (n = 4 different bottles) used to preserve brain samples was investigated for Pb content. Samples were centrifuged at 2500 rpm for 10 min to avoid the brains' solid particles. From each sample a 0.5 mL aliquot was withdrawn and its content in Pb was determined in triplicate by ET-AAS.

2.5.2. Urine analysis

The urine samples were taken and left with no further treatment in closed vessels. After a few weeks, the formation of a precipitate was observed. Afterwards, samples were acidified with nitric acid

up to 10% v/v, shaking them for 10 min and then left to stand for at least 48 h before analysis. Then, samples were analysed before and after ultrasonic treatment. Samples (volume 5–25 mL) were sonicated in steps of 2 min (×3, 50% ultrasonic amplitude, tip of 14 mm). Samples were allowed to cool in an ice bath for 1 min between the sonication steps.

2.5.3. Microwave-assisted digestion of urine precipitates

The precipitate formed in the urine samples was dissolved using microwave-assisted acid digestion. Once the urine samples were acidified and sonicated as explained above, 1 mL was withdrawn and centrifuged at 7000 rpm for 3 min. The supernatant was carefully removed and the precipitate was allowed to dry at 50 °C until constant weight was observed (24 h). Then, the precipitate was transferred to a PTFE digestion bomb and 50 μL of H_2O_2 was added. The mixture was left to stand for 15 min. Afterwards 2 mL of HNO_3 concentration were added and the mixture was left to stand for 15 min. Finally, the microwave-assisted acid digestion was done (350 W, 2.5 min). Once the digestion was finished, the sample was made up to 5 mL with water. Blanks were treated in the same way.

2.5.4. Ultrasonic-assisted solid–liquid extraction of lead from brain samples

Brain samples were removed from the formaldehyde solutions and allowed to dry until constant weigh at 50 °C (24 h). Dry samples (from 2 mg up to 4 mg) were transferred to 1.5 mL cups and 1 mL of 10% v/v HNO_3 was added. Then samples were sonicated in steps of 1 min each (×3, 50% ultrasonic amplitude, tip of 1 mm). Between sonication steps samples were allowed to cool in an ice bath (1 min).

2.5.5. Microwave-assisted acid digestion of brain samples

After the brain samples were sonicated, as explained above, they were removed from the 10% v/v HNO_3 solutions and allowed to dry until constant weigh at 50 °C (24 h). Dry samples (from 2 mg up to 4 mg) were transferred to a PTFE digestion bomb and 50 μL of H_2O_2 were added. Then the mixture was left to stand for 15 min. Then 2 mL of HNO_3 concentration were added and the mixture was left to stand for 15 min. Finally, the microwave-assisted acid digestion was done (350 W, 2.5 min). Once the digestion was finished, the sample was made up to 5 mL with water. Blanks were prepared in the same way.

2.6. Statistical analysis

The results are expressed as mean value ± standard deviation. Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS) software, version 17.0. Earlier studies have shown that concentration levels of trace elements, such as Pb, do not usually follow a normal distribution [16–19]. Accordingly, data were analysed with the non-parametric significance Mann–Whitney test. A value of P lower than 5% was considered statistically significant [20,21].

3. Results and discussion

3.1. Ash/atomization curves

The instrumental parameters and electrothermal program is shown in Table 1. When ET-AAS is used to directly determine the Pb in organic samples such as formaldehyde and urine, some problems must be carefully addressed while using this method of analysis. Thus, volatilization of the organic phase leads to an increase in the actual concentration of the analyte. In addition, the auto sampler dispensing becomes complicated as the formation of the drop is hindered by the superficial tension of the liquid, which makes the drop become adhered to the dispenser's tip. Furthermore, in some cases aqueous standard cannot be used for calibration [22]. To complicate things further Volynsky et al. [23] emphasized that the spreading of organic samples over the graphite furnace surface distorts the atomic absorption profiles, renders the analytical curve non-linear and decreases the sensitivity. Furthermore, according to Tserovsky and Arpadjan [24], the removal of organic liquids after their penetration into the graphite requires long pre-treatment at a high temperature. Hence, the volatile compounds would be lost at this stage. Taking into consideration the above mentioned problems that can hinder the analysis, first we did an ash/atomization study to find out the best temperature for the ashing and atomization stages. The ash/atomization curves are presented in Fig. 1. The matrix modifier used was palladium nitrate, which was selected as it was successfully used in previous studies dealing with lead determination in complex samples [12,25]. As may be seen in Fig. 1, nitrate palladium stabilizes the lead inside the graphite tube up to 1100 °C for both aqueous standards and urine samples. To extend the life of the graphite tubes a lower ashing temperature of 900 °C was selected as optimum. As far as the temperature of atomization is concerned, we selected 1800 °C as optimum.

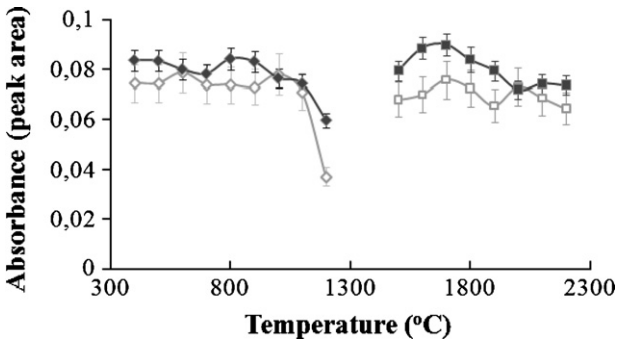

Fig. 1. Ashing and atomization curves for Pb in (i) an aqueous standard solution (♦, ashing curve; ■, atomization curve) (15 μg L^{-1}); and (ii) urine liquid fraction (◊, ashing curve; □, atomization curve) (15 μg L^{-1}). Data was obtained in different days.

To investigate the performance of the thermal program for formaldehyde, a sample was spiked with known amounts of lead up to 6 μg L^{-1}, 15 μg L^{-1} and 24 μg L^{-1}. The samples were analysed against a calibration curve done with aqueous standards. The recoveries obtained were (X ± R.S.D, n = 3) 100 ± 9%, 103 ± 4% and 101 ± 2% respectively. On regarding to the above results, the formation of carbonaceous residues inside the graphite tube was not observed throughout the experiments. Formation of carbonaceous residues is a common problem when samples with high organic content are introduced into the graphite tube, leading to a number of problems that have been described elsewhere [13].

Fig. 2 shows the absorption profiles of the samples studied in this work. Similar atomic absorption profiles are observed for aqueous standard, formaldehyde, urine liquid phase, and brain extract. This similarity facilitates calibration with aqueous standards.

3.2. Validation of the method

To validate the experimental conditions chosen to perform the sample treatment, the lead content in one calibrator urine was determined. The sample was ultrasonically treated in triplicate as explained in Section 2, and then diluted 1:3 with HNO_3 10% V/V. The lead recovery was calculated to be 62 ± 6 μg L^{-1}, with no statistical difference from the reference value (64.2 ± 3.7 μg L^{-1}, Mann–Whitney test, P < 0.05, n = 3).

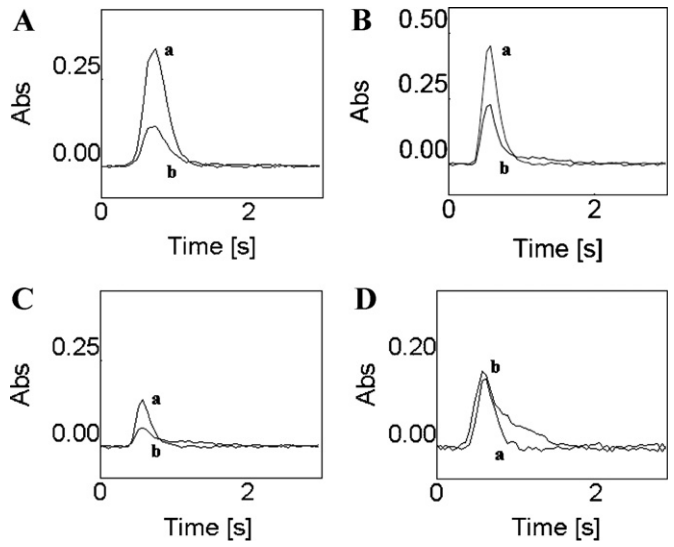

Fig. 2. Pb Absortion profiles for: (A) aqueous calibration Standard (18 μg L^{-1} Pb); (B) formaldehyde containing NTS control brain sample (24 μg L^{-1}, Pb was spiked); (C) ADH exposed rat brain sample after USLE (5.4 μg L^{-1} Pb); (D) Urine of exposed rat after USLE (6.7 μg L^{-1} Pb). (a) Atomic absorbance profile and (b) background absorbance profile.

3.3. Analysis of urine sample from rats: overcoming the formation of precipitate

It is well known that when a solid is formed in a solution, metals can be absorbed on its surface, leading to a variation in the actual content of these metals in the liquid fraction as the solid precipitation takes place [26,27]. To demonstrate this fact we analysed the liquid fraction of four urines with solid fraction. The liquid fraction was analysed before and after sample treatment as described in Section 2.4. The solid fraction was observed with the naked eye. The amount of lead found in the liquid phase was increased after treatment in all studied cases, as may be seen in Table 2. In some of them, the lead concentration increased dramatically. This result confirmed that the acidification of the sample was not enough to extract the lead associated to the solid phase. The aid of the ultrasonic energy was necessary to make the lead extraction feasible. Finally, to assess the complete solid–liquid lead extraction, the solid phase was analysed to detect any non-extracted Pb. The sediments were separated from the liquid phase, dried, weighed and solubilised with a microwave assisted acid pressurized digestion protocol, as explained in the sample treatment section. From the eight solid fractions analysed, lead was detected only in four samples. The amount of lead found in the precipitate was less than 2% of the total lead contained in the sample (lead in the liquid phase and lead in the solid phase). These results confirmed that the solid–liquid ultrasonic extraction was achieved successfully.

Table 2
Lead concentration found in the liquid fraction of the urine of rats before and after treatment with ultrasonic energy ($X \pm$ SD, $n = 3$, μg L^{-1}). Urine A, B and C correspond to rats exposed to lead. Urine D corresponds to control rat.

Sample	Found content	
	Before USLE	After USLE
Urine A	432 ± 26	752 ± 56
Urine B	109 ± 4	833 ± 62
Urine C*	53.3 ± 0.3	60.9 ± 1.2
Urine D	5 ± 2	110 ± 26

* (mg L^{-1}).

Table 3
Lead content in the formaldehyde and in the total mass of brains in each vessel containing formaldehyde, for exposed and non-exposed rats ($X \pm$ SD, $n = 3$, μg).

Sample	Formaldehyde	Total brain
HDA exposed	0.007 ± 0.001	0.211 ± 0.014
NTS exposed	0.007 ± 0.001	0.173 ± 0.010
HDA control	BDL	BDL
NTS control	BDL	BDL

3.4. Analysis of lead in the tissue of the rats' brain

The brain samples of exposed and control rats were preserved in formaldehyde, being different brains collected and preserved in the same recipients, concerning the type of the tissue and the exposure regimen. Therefore, we investigated if lead from the contaminated brains was extracted into the formaldehyde. Lead from brain tissue was extracted using ultrasonic energy as described in Section 2. Data presented in Table 3 shows that the lead found in the formaldehyde was negligible in comparison with the one found in the brain tissue of rats exposed to lead. When non-exposed rats were studied, the lead content of both, formaldehyde and brain, was below the limit of detection of the method. To assess the complete ultrasonic lead extraction from the brain tissue, after sonication, some samples were solubilised with the aid of a microwave assisted acid pressurized digestion protocol. The amount of lead obtained for all the samples studied was below the limit of detection of the method. To further confirm the completeness of the lead extraction, the multi-injection technique was used. This technique makes it possible to reach the lower limits of detection [28], because after the drying step, samples are re-injected several times on the graphite tube. Hence, the amount of metal in the tube is increased by a factor of two or three, depending on the number of re-injections used. In despite of being 3 times re-injected, the levels of lead found were below the limit of detection of the method. To assess the correct performance of the re-injection method, the same samples were then spiked with lead up to 8 μg L^{-1}. Samples were analysed with two and three re-injections. The lead recoveries obtained were ($X \pm$ R.S.D, $n = 3$) 105 ± 4% and 97 ± 4%, respectively. These results further confirm that ultrasonic energy works and it is a powerful tool in the extraction of lead from soft tissue, confirming previous literature on this topic [12,25,29].

3.5. Analytical figures of merit

The equation of standard calibration line was:

$$Y = (0.0047 \pm 0.0002)X + (0.001 \pm 0.003), (R^2 > 0.995, n = 18)$$

where Y is the integrated absorbance (peak area) and X is the Pb concentration in μg L^{-1}. The calibration curve was linear up to 30 μg L^{-1}.

Table 4
Limits of detection and limits of quantification for the different sample treatments assessed.

		Urine-solid phase (μg g^{-1})	Urine-liquid phase (μg L^{-1})	Brain (μg g^{-1})
USLE	LOD	–	0.7	0.35
	LOQ	–	2.1	1.05
MWD	LOD	1.75	–	1.75
	LOQ	5.25	–	5.25

The injection volume of sample was 20 μL and 5 μL for the chemical modifier solution. Time to complete the sample treatment using USLE or MWD was of 10 min and 4 h respectively.

Table 5
Concentration of lead found in urine and brain samples treated with the USLE protocol. Formaldehyde was measured directly by ETAAS..

Exposed rats	Urine	NTS	HDA	Formaldehyde NTS**	Formaldehyde HDA**
	752 ± 56**	2.3 ± 0.2	3.1 ± 0.2	4.4 ± 0.6	4.8 ± 0.3
	833 ± 62**	2.4 ± 0.1	3.2 ± 0.3		
	60.9 ± 1.2	2.1 ± 0.2	3.4 ± 0.3		
	27.4 ± 2.0	2.4 ± 0.1	3.5 ± 0.2		
	5.5 ± 0.1	2.7 ± 0.1	3.0 ± 0.2		
	4.3 ± 0.4	1.9 ± 0.2	3.2 ± 0.3		
Control rats	BQL	BDL	BDL	BDL	BDL
	BDL	BDL	BDL		
	BDL	BDL	BDL		
	225 ± 23**	BDL	BQL		
	110 ± 26**	BDL	BQL		
	BDL	BDL	BQL		

*(mg L^{-1}; $n = 3$).
** (μg L^{-1}; $n = 3$).

Regarding the method, the limit of detection (LOD) was 0.7 μg L^{-1} and the limit of quantification (LOQ) was 2.1 μg L^{-1}. These limits were calculated using the criterion of [*X*] + 3SD for LOD and [*X*] + 10SD for LOQ, [*X*] being the mean absorbance signal of 10 blank measurements and SD, the corresponding standard deviation of ten measurements. Taking into consideration those instrumental limits, the limits of detection and the limits of quantification of the sample treatments described in Section 2 are presented in Table 4.

Within bath and between baths precision were calculated using the calibrator material. The values were 5% ($n = 9$) and 7% ($n = 3$), respectively.

3.6. Analytical results

The sample treatment developed was applied to a number of rats under study, as shown in Table 5. When brains of exposed and non exposed rats are compared, lead accumulation is clearly observed in the brain tissue. Furthermore, the levels of lead in urine of exposed rats are also high in comparison with rats which have not been exposed, thus indicating that the levels of lead in urine can be used as an indicator of exposure to lead. This result is in agreement with the literature on this topic.

4. Conclusions

A rapid and simple ultrasonic-based methodology to determine lead in brain and urine from rats has been proposed. The new methodology allows handling mg of brain sample in a few minutes. The volume required to extract the lead from the brain was 1 mL, and can potentially be reduced to 250 μL, by just changing the ultrasonic tip used. Furthermore, the lead content in urine that had developed a precipitate can be easily determined by acidification (nitric acid, 10% v/v) and ultrasonication (6 min) of the sample.

Acknowledgements

J.L. Capelo-Martínez acknowledges the support of the Xunta de Galicia (Spain) for their Parga-Pondal Research contract. D. Guimarães acknowledges support of the Fundação para a Ciência e Tecnologia (FCT) for the Grant (SFRH/BD/38788/2007). J.P. Santos, D. Guimarães and M.L. Carvalho acknowledge the support by FCT- Fundação para a Ciência e a Tecnologia (Portugal) under the Project No. PEstOE/FIS/UI0303/2011, financed by the European Community Fund FEDER through the COMPETE – Competitiveness Factors Operational Programme. The authors acknowledge support of REQUIMTE, and Carla Rodrigues and Nuno Costa for the technical support. H. M. Santos and G. Vale acknowledge the post-doctoral fellows given by the Fundação para a Ciência e Tecnologia (FCT) with numbers SFRH/BPD/73997/2010 and SFRH/BPD/73117/2010 respectively.

References

[1] J. Schwartz, D. Otto, Arch. Environ. Health 42 (1987) 153–160.
[2] R. Ronchetti, P. Van den Hazel, G. Schoeters, W. Hanke, Z. Rennezova, M. Barreto, M.P. Villa, Acta Paediatr. 95 (2006) 45–49.
[3] E.C. Banks, L.E. Ferretti, D.W. Shucard, Neurotoxicology 18 (1997) 237–281.
[4] J. Schwartz, Environ. Res. 65 (1994) 42–55.
[5] J. Millos, M. Costas-Rodríguez, I. Lavilla, C. Bendicho, Anal. Chim. Acta 622 (2008) 77–84.
[6] E. Engstrom, A. Stenberg, S. Senioukh, R. Edelbro, D. Baxter, I. Rodushkin, Anal. Chim. Acta 521 (2004) 123–135.
[7] D. Halls, J. Anal. Atom. Spectrom. 10 (1995) 169–175.
[8] P. Parsons, W. Slavin, Spectrochim. Acta B 54 (1999) 853–864.
[9] J. Capelo, S. Catarino, A. Curvelo-Garcia, M. Vaiao, J. AOAC Int. 88 (2005) 585–591.
[10] S. Catarino, J. Capelo, A. Curvelo-Garcia, R. De Sousa, J. Int. Sci. Vigne Vin. 40 (2006) 91–100.
[11] L. Amoedo, J. Capelo, I. Lavilla, C. Bendicho, J. AOAC Int. 85 (2002) 212–218.
[12] L. Amoedo, J. Capelo, I. Lavilla, C. Bendicho, J. Anal. Atom. Spectrom. 14 (1999) 1221–1226.
[13] G. Vale, S. Pereira, A. Mota, L. Fonseca, J. Capelo, Talanta 74 (2007) 198–205.
[14] T. Jerman, P. Trebse, B. Vodopivec, Food Chem. 123 (2010) 175–182.
[15] M. Romdhane, C. Gourdon, Chem. Eng. J. 87 (2002) 11–19.
[16] T. Agusa, T. Matsumoto, T. Ikemoto, Y. Anan, R. Kubota, G. Yasunaga, T. Kunito, S. Tanabe, H. Ogi, Y. Shibata, Environ. Toxicol. Chem. 24 (2005) 2107–2120.
[17] K. Kannan, T. Agusa, E. Perrotta, N.J. Thomas, S. Tanabe, Chemosphere 65 (2006) 2160–2167.
[18] P. Bustamante, P. Bocher, Y. Chérel, P. Miramand, F. Caurant, Sci. Total Environ. 313 (2003) 25–39.
[19] Q. Pasha, S.A. Malik, N. Shaheen, M.H. Shah, Biol. Trace Elem. Res. 134 (2010) 160–173.
[20] D.C. Montgomery, Design and Analysis of Experiments, 6th ed., Wiley, Hoboken (Great Britain), 2005.
[21] A.I. Khuri, T. Mathew, B.K. Sinha, Statistical Tests for Mixed Linear Models, Wiley, New York, Chichester, 1998.
[22] C. Burrini, A. Cagnini, Talanta 44 (1997) 1219–1223.
[23] A. Volynsky, B. Spivakov, Y. Zolotov, Talanta 31 (1984) 449–458.
[24] E. Tserovsky, S. Arpadjan, J. Anal. Atom. Spectrom. 6 (1991) 487–491.
[25] C. Maduro, G. Vale, S. Alves, M. Galesio, M. da Silva, C. Fernandez, S. Catarino, M. Rivas, A. Mota, J. Capelo, Talanta 68 (2006) 1156–1161.
[26] X. Hu, Int. J. Environ. Anal. Chem. 91 (2011) 263–271.
[27] K. Provazi, B. Campos, D. Espinosa, J. Tenorio, Waste Manage. 31 (2011) 59–64.
[28] I. Lavilla, A. Mosquera, J. Millos, J. Cameselle, C. Bendicho, Anal. Chim. Acta 566 (2006) 29–36.
[29] J. Capelo, C. Maduro, C. Vilhena, Ultrason. Sonochem. 12 (2005) 225–232.