



## Analytical Methods

# Determination of cadmium and lead at sub-ppt level in soft drinks: An efficient combination between dispersive liquid-liquid microextraction and graphite furnace atomic absorption spectrometry



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## ABSTRACT

A DLLME method for extraction and preconcentration of Cd and Pb from soft drinks and further determination by GF AAS was developed. Important parameters of DLLME such as the type and volume of dispersive and extraction solvents, concentration of DDTC (complexing agent) and pH were evaluated. Better results were obtained using 500  $\mu\text{L}$  of acetone for Cd and 700  $\mu\text{L}$  of acetonitrile for Pb as dispersive solvents, 60  $\mu\text{L}$  of  $\text{CCl}_4$  as extraction solvent for both analytes and 500  $\mu\text{L}$  of 1.5% DDTC solution. Accuracy was evaluated by recovery assays and ranged from 91 to 113% for Cd and from 95 to 108% for Pb, with RSD below 10 and 7%, respectively. The LODs were 0.006 and 0.072  $\text{ng L}^{-1}$  for Cd and Pb, respectively. The optimized method was applied for the determination of Cd and Pb in soft drinks with different brands and flavours.

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## 1. Introduction

The consumption of soft drinks has increased in the last years around the world. It can be a result of the diversity of brands and flavours available, increasing also the risk of ingestion of compounds considered as non-beneficial to the health (British Soft Drinks Association, 2015; Froes et al., 2009). In this context, some elements such as Cd and Pb are well known due to their toxic properties, which require special attention taking into account mainly the quality control protocols (Nordberg, Sandstrom, Becking, & Goyer, 2002).

The presence of Cd in the organism is related to neurological effects, renal degradation, bone lesions and hypertension in humans. Even at low concentration, Cd is highly toxic and can cause damages in organs such as the kidneys, liver and lungs. On the other hand, Pb affects the central nervous system and may cause delayed growth, renal cancers, intelligence damage and altered behaviour. It is important to mention that the tolerable weekly intake for Cd and Pb is 0.8 and 25  $\mu\text{g kg}^{-1}$  body weight per week, respectively (Nordberg et al., 2002).

Elements such as Cd and Pb have been widely studied and are usually present at low concentration ( $\mu\text{g g}^{-1}$  or  $\text{ng g}^{-1}$ ) in environmental, biological and food samples (Duarte et al., 2007; Nordberg et al., 2002). These low concentration levels require high sensitivity detection techniques and/or efficient sample preparation methods. However, most analytical techniques requires the sample introduction in liquid phase and an efficient sample preparation method is mandatory. On the other hand, the sample dilution is inherent to any digestion method, which impairs the limit of detection (LOD) (Maciel, Knorr, et al., 2014; Maciel, Soares, et al., 2014). Thus, preconcentration methods are the best choice to overcome this limitation.

Dispersive liquid-liquid microextraction (DLLME) was recently developed comprising special characteristics such as simplicity, efficiency, low cost, good enrichment factors, low waste generation and low consumption of organic solvents (Pereira et al., 2013; Rezaee et al., 2006). Due to these features, DLLME has been considered as an alternative for the extraction and preconcentration of trace elements in aqueous samples with some advantages over other well established extraction/preconcentration methods such as cloud point extraction (CPE) (Almendral, Alonso, Porras, Garcia, & Curto, 2004; Manzoori & Bavili-Tabrizi, 2002; Stalikas, 2002), liquid-liquid extraction (LLE) (Almendral et al., 2004;

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Wang & Hansen, 2002) and solid phase extraction (SPE) (Khayatian, Hassannpoor, Nasiri, & Zolali, 2012; Yamini, Hejazi, & Mohammadi, 2003). Nowadays, several variations of original DLLME method have been proposed (e.g., combination with ultrasound, use of low density solvents, etc.), but the classical mode is still the most used (Leonga, Fuh, & Huang, 2014; Rosa et al., 2015). DLLME was initially developed for extraction of organic compounds from environmental aqueous matrices, such as polycyclic aromatic hydrocarbons (PAHs) (Rezaee et al., 2006), pesticides (Farajzadeh, Khoshmaram, & Nabil, 2014) and drugs (Martín, Buchberger, Alonso, Himmelsbach & Aparicio, 2014). Despite most of applications are focused on the extraction of organic compounds, some authors have developed DLLME methods for elements extraction, with the addition of complexing agents (e.g., carbamates) (Jahromi, Bidari, Assadia, Hosseini, & Jamali, 2007; Kocot, Zawisza, & Sitko, 2012; Seeger et al., 2015; Yousefi & Shemirani, 2010). One of the most interesting characteristic of DLLME is the possibility of direct introduction of extract in the analytical instrument, such as UV–Vis spectrophotometry (Maciel, Knorr, et al., 2014; Maciel, Soares, et al., 2014; Wen, Yang, Yan, & Deng, 2011), flame atomic absorption spectrometry (FAAS) (Seeger et al., 2015; Wu et al., 2011; Zhang et al., 2011), graphite furnace atomic absorption spectrometry (GF AAS) (Jahromi et al., 2007; Zhang et al., 2011), inductively coupled plasma optical emission spectrometry (Wu et al., 2011), inductively coupled plasma mass spectrometry (ICP-MS) (Moghim, 2008) and gas chromatography mass spectrometry (Soares, Pereira, Maciel, Vieira, & Duarte, 2013). The excellent enrichment factors provided by DLLME allows the use of routine instruments such as UV–Vis, FAAS and GF AAS, which are usually available in many laboratories.

Taking into account that the direct injection of soft drinks in most analytical instruments (e.g., GF AAS and ICP-MS) is prone to severe interferences, the use of preconcentration methods (e.g., DLLME) is recommended. However, the combination between DLLME and GF AAS has not yet described in literature for determination of Cd and Pb in soft drink. In this work, important parameters for analytes extraction such as the type and volume of dispersive and extraction solvents, sample pH and concentration of diethyldithiocarbamate (DDTC, complexing agent) were evaluated. The proposed method was applied for the determination of Cd and Pb in soft drinks of different brands and flavours.

## 2. Experimental

### 2.1. Instrumentation

The optimization of DLLME method was carried out by analytes determination using a flame atomic absorption spectrometer (model AAS vario 6, Analytik Jena, Germany). However, analytes quantification was performed using a graphite furnace atomic absorption spectrometer (model ZEE nit 60, Analytik Jena) equipped with a transversely heated graphite atomizer with inverse Zeeman-effect background correction system. For measurements by FAAS and GF AAS, hollow cathode lamps of Cd and Pb operated at 4 and 10 mA, respectively, were used. The wavelength was set at 228.8 nm for Cd and at 283.3 nm for Pb, with spectral bandpass of 0.5 nm for both analytes. GF AAS measurements were carried out in transversely heated and pyrolytically coated graphite tubes and platforms specially designed for solid sampling (Analytik Jena). A manual solid sampling system (model SSA 61Z, Analytik Jena) was used for sample/extract introduction into the graphite tube. Integrated absorbance (peak area) was used for all measurements. The operational conditions of GF AAS instrument are shown in Table 1.

For results comparison, samples were digested in a microwave oven (model Multiwave 3000 Microwave Sample Preparation System, Anton Paar, Austria) equipped with eight high-pressure quartz vessels. Digestion was assisted by ultraviolet radiation and a Cd low pressure discharge microwave lamp (Anton Paar) was used inside each quartz vessel. The main operational conditions were adapted from literature (Limbeck, 2006), but using 12 mL of sample and 3 mL of concentrated HNO<sub>3</sub> (65%). Heating program for microwave-assisted digestion combined with ultraviolet radiation (MAD-UV) was performed as follows: i) 400 W (ramp of 10 min and hold for 10 min); ii) 900 W (ramp of 10 min and hold for 10 min); iii) 0 W (cooling 20 min). This approach (MAD-UV) using diluted HNO<sub>3</sub> was selected in order to reduce the sample dilution after digestion, which is observed when conventional MAD is used. After digestion, analytes (monitored isotopes <sup>111</sup>Cd and <sup>208</sup>Pb) were determined by ICP-MS (model Elan DRC II, PerkinElmer, Canada). The radiofrequency power was set at 1300 W and the plasma gas flow rates were 15, 1.2 and 1.10 L min<sup>-1</sup> for principal, auxiliary and nebulizer, respectively. For the determinations using both GF AAS and ICP-MS, argon (99.996%, White Martins, Brazil) was used.

The pH was measured using a pH meter model 781 pH (Metrohm, Switzerland) and when necessary pH was adjusted with diluted solutions of HNO<sub>3</sub> or NH<sub>4</sub>OH. Prior to DLLME procedure, samples were degassed using an ultrasonic bath (model Transsonic TI-H-10, Elma brand, Germany), with a maximum power of 450 W (1.1 W cm<sup>-2</sup>) and frequency of 35 kHz. A mixture of solvents was injected into the sample using a microsyringe (Hamilton, USA) with capacity of 250–1000 µL. Water was distilled, deionized and further purified (resistivity of 18.2 MΩ cm) in a Milli-Q system (Direct-UV 3, Millipore, USA). A centrifuge (model Z 326 K, HERMLE Labortechnik, Germany) was used for separation of phases during DLLME procedure.

### 2.2. Reagents and samples

All reagents used in this work were of analytical grade. Chloroform, carbon tetrachloride, 1,2-dichloroethane, monochlorobenzene and 1,2-dichlorobenzene (Vetec, Brazil) were evaluated as extraction solvents and acetone, acetonitrile, methanol and ethanol (J.T. Baker, USA) were evaluated as dispersive solvents. The manipulation of chlorinated solvents needed a special attention due to their toxicity. Sodium diethyldithiocarbamate (Sigma Aldrich, Brazil) was used as complexing agent. A stock solution of Pd (20 g L<sup>-1</sup>, Sigma-Aldrich, USA) was diluted up to 1000 mg L<sup>-1</sup> in 5% HNO<sub>3</sub>, which was used as chemical modifier. Analytical grade HNO<sub>3</sub> (65%, Merck, Germany) was doubly distilled in a sub-boiling system (model duoPUR 2.01E, Milestone, Bergamo, Italy).

Taking into account that the aim of this work was the development of a method for determination of Cd and Pb in soft drinks, samples were randomly selected considering the brands and flavours commercially available in the local market. Soft drinks were purchased containing the following description: two samples of Regular Cola (named RC I and RC II), two samples of Low Calorie Cola (named LCC I and LCC II), one sample of Regular Guarana (named RG I) and one sample of Regular Lemon (named RL I).

### 2.3. DLLME optimization

For DLLME procedure, 10 mL of sample (RC I was used for method optimization) previously degassed in an ultrasound bath for 30 min. Sample was introduced in a glass conical bottom tube and 500 µL of DDTC solution were added. This solution was manually homogenized (about 10 s) and the mixture containing dispersive (acetone, acetonitrile, methanol or ethanol) and extraction (chloroform, carbon tetrachloride, 1,2-dichloroethane,

**Table 1**

Heating program for the determination of Cd and Pb in soft drinks by GF AAS.

Step	Temperature (°C)	Heating rate (°C s <sup>-1</sup> )	Hold time (s)	Ar flow rate (L min <sup>-1</sup> )
Drying 1 <sup>*</sup>	80 <sup>a</sup>	20	25	0.5
Drying 2	120 <sup>a</sup>	20	25	0.5
Pyrolysis	500 <sup>a</sup> , 700 <sup>b</sup>	100	30	0.5
Atomization	1400 <sup>a</sup> , 1800 <sup>b</sup>	3000	8	0
Cleanout	2400	3000	4	2.0

<sup>\*</sup> Enrichment step.<sup>a</sup> Cadmium.<sup>b</sup> Lead.

monochlorobenzene or 1,2-dichlorobenzene) solvents was quickly injected into the sample solution by using a syringe. After injection, a cloudy solution was instantaneously produced and after about 1 min the solution was centrifuged (3000 rpm for 2 min). In order to reduce interferences during the measurements, a washing step was included in the proposed procedure (Seeger et al., 2015), where the bottom phase (extract) was washed with 5 mL ultrapure water and further submitted to centrifugation step. This washing procedure was repeated as much as necessary. After the last washing, the aqueous phase was not removed. Thus, the capillary of nebulization system of F AAS instrument was introduced into the aqueous phase and the auto zero of baseline was obtained aspirating the aqueous solution. When the integration was started, the capillary was moved to the bottom phase, which was aspirated and the analytical signal was obtained. It is important to mention that the reference solutions for instrument calibration were also submitted to the same procedure.

The type of dispersive solvent (acetone, acetonitrile, methanol and ethanol) and extraction solvent (chloroform, carbon tetrachloride, 1,2-dichloroethane, monochlorobenzene and 1,2-dichlorobenzene) were evaluated. After the choice of dispersive and extraction solvents, the respective volumes were also evaluated from 100 to 900 µL and from 10 to 120 µL. Other important parameters were also investigated such as pH (2–6) and concentration of complexing agent (0–2.5%).

For the exclusion of outliers in replicates, the Grubbs test was used (Microsoft Excel<sup>®</sup> software, Microsoft, Version 2010). The GraphPad InStat (Version 3.00, 1997) software was used for application of *t*-Student test (comparison of two averages) and Tukey-Kramer test (comparison of three or more averages). A 95% significance level was adopted for all comparisons.

### 3. Results and discussion

The experiments for optimization of DLLME method were performed by using F AAS for analytes determination and operational conditions were adapted from previous work (Seeger et al., 2015). For this purpose, 10 mL of spiked samples (RC I) with final concentration of 10 µg L<sup>-1</sup> were employed for both analytes. However, for the evaluation of sample pH and DDTc concentration for Cd, samples were spiked at final concentration of 5 µg L<sup>-1</sup>.

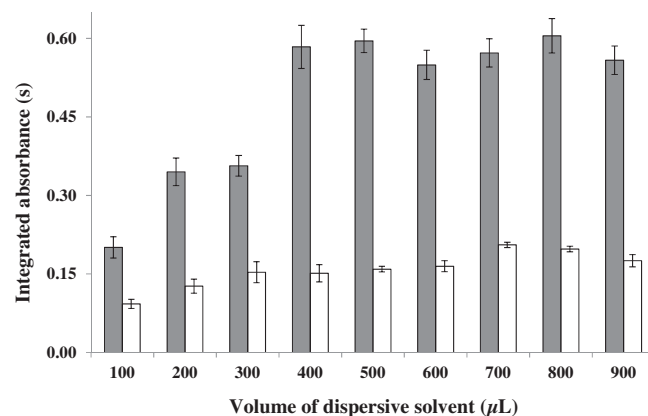
#### 3.1. Evaluation of the type of dispersive and extraction solvents

Initially, the selection of the type of dispersive and extraction solvents was performed combining acetone, acetonitrile, methanol or ethanol (dispersive solvents) with chloroform, carbon tetrachloride, 1,2-dichloroethane, monochlorobenzene or 1,2-dichlorobenzene (extraction solvents). The volume of dispersive and extraction solvents was arbitrarily chosen as 500 and 100 µL, respectively. For choosing the better combination between dispersive and extraction solvents, a visual comparison (qualitative) was performed taking into account the lowest amount of solids around

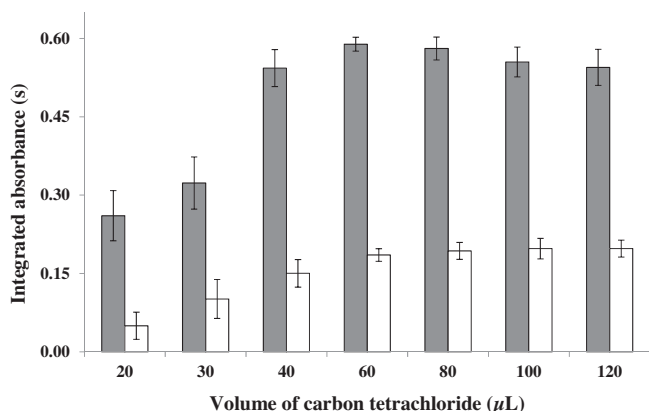
the drop. Then, the extracts containing the better combination of solvents were introduced in the nebulization system of F AAS instrument, taking into account the highest absorbance and lowest background signals. For Cd and Pb, better results (not showed) were obtained using carbon tetrachloride (extraction solvent) for both analytes combined with acetone and acetonitrile (dispersive solvent), respectively.

#### 3.2. Effect of solvents volume

The volume of dispersive solvent (acetone for Cd and acetonitrile for Pb) was evaluated from 100 to 900 µL, using 100 µL of carbon tetrachloride (extraction solvent). As can be seen in Fig. 1, the extraction efficiency for Cd from 100 to 300 µL was significantly lower than other volumes (400–900 µL). In the same way, good extraction efficiency was observed for Pb from 300 to 900 µL. Thus, considering the highest analytical signals and lowest relative standard deviation (RSD), 500 µL of acetone and 700 µL of acetonitrile were chosen for extraction of Cd and Pb, respectively. Then, the volume of carbon tetrachloride (extraction solvent) was evaluated from 20 to 120 µL for both analytes. Results are shown in Fig. 2. In addition to the low extraction efficiency for Cd and Pb using 20 and 30 µL of carbon tetrachloride, the extract handling was difficult. The RSD was significantly higher for these volumes, ranging from 15 to 19% for Cd and from 36 to 52% for Pb. Taking into account that from 40 to 120 µL no significant difference (*p* > 0.05) was observed for Cd and Pb, 60 µL of carbon tetrachloride was chosen, because using 40 µL the recovery was slightly lower, with relatively high RSDs and extract handling was somewhat difficult. In addition, this volume range is in agreement with those reported in literature (from 17 to 1000 µL) (Jahromi et al., 2007; Jia, Han, Liu, Duan, & Chen, 2010; Ma, Du, Zang, Li, & Wang, 2009).



**Fig. 1.** Effect of the volume of dispersive solvent for extraction of Cd (■) and Pb (□) and determination by FAAS. DLLME conditions: 10 mL of spiked sample (10 µg L<sup>-1</sup>), acetone for Cd and acetonitrile for Pb as dispersive solvent, 100 µL of carbon tetrachloride (extraction solvent), 500 µL of 2% (m/v) DDTc solution and centrifugation at 3000 rpm for 2 min. Error bars represent the standard deviation (n = 5).



**Fig. 2.** Effect of the volume of extraction solvent for Cd (■) and Pb (□) and determination by F AAS. DLLME conditions: 10 mL of spiked sample ( $10 \mu\text{g L}^{-1}$ ), 500  $\mu\text{L}$  of acetone for Cd and 700  $\mu\text{L}$  of acetonitrile for Pb as dispersive solvent, carbon tetrachloride as extraction solvent, 500  $\mu\text{L}$  of 2% (m/v) DDTC solution and centrifugation at 3000 rpm for 2 min. Error bars represent the standard deviation ( $n = 5$ ).

### 3.3. Effect of pH

The pH plays an important role in the formation of metal-complexing agent as well as in the extraction efficiency. The pH range commonly used for complexation of Cd and Pb by DDTC and extraction using DLLME as sample preparation method is about pH 3 to 10 and most specifically from pH 4 to 6 (Jahromi et al., 2007; Jia et al., 2010; Ma et al., 2009; Yousefi & Shemirani, 2010). The study of pH effect was performed ranging from 2 to 6. Except for Pb at pH 2, the pH range evaluated showed no significant difference ( $p > 0.05$ ) on extraction efficiency for both analytes. It is important to point out that this negligible effect of pH on extraction efficiency has been described by some authors (Jahromi et al., 2007; Jia et al., 2010; Ma et al., 2009). Thus, taking into account that the pH of samples ranged from 2.5 to 4.0, any adjustment was required prior to extraction by DLLME.

### 3.4. Effect of DDTC concentration

DDTC was used as complexing agent due to its good affinity by many elements, including Cd and Pb. The concentration of complexing agent is an important parameter for DLLME method because the extraction efficiency is dependent of the complete complexation of analyte(s) and it must be present at enough concentration. The effect of DDTC concentration was investigated using 500  $\mu\text{L}$  of this solution with concentration ranging from 0.5 to 2.5% (m/v). Considering the absorbance signal and lower RSDs, 500  $\mu\text{L}$  of 1.5% DDTC solution was chosen for analytes complexation. It is important to mention that, despite of some works have shown the influence of interfering ions on extraction efficiency, this study was not carried out once all optimization was performed using sample matrix.

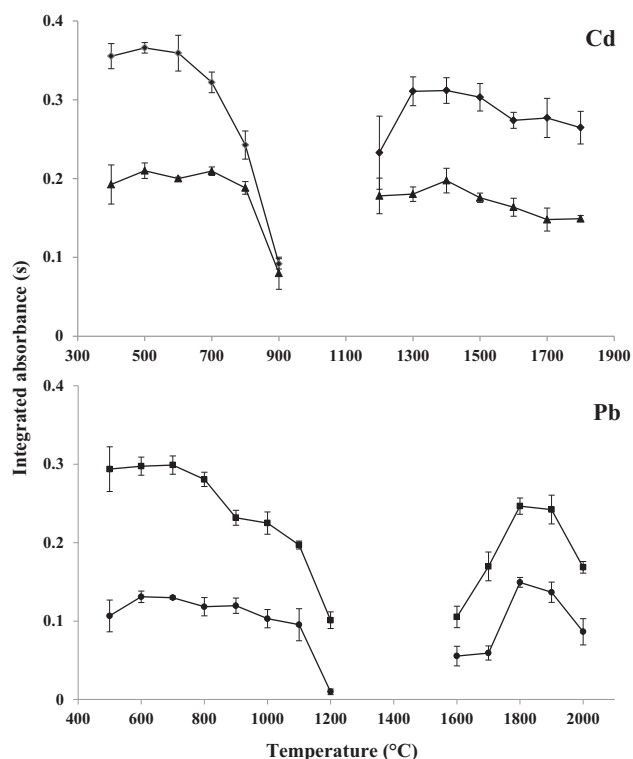
After optimization of the main parameters that affect the proposed DLLME method [500  $\mu\text{L}$  of acetone for Cd (dispersive), 700  $\mu\text{L}$  of acetonitrile for Pb (dispersive), 60  $\mu\text{L}$  of carbon tetrachloride (extraction) for both analytes, no pH adjustment and 1.5% DDTC solution], the calibration of F AAS instrument was performed. Using these conditions, the extraction efficiency for Cd and Pb was 96 and 97%, with RSD lower than 3.9 and 5.4%, respectively. It is important to point out that the reference solutions used for instrument calibration were submitted to the optimized DLLME method. Then, samples were also submitted to DLLME method, but concentration of Cd and Pb in all analysed samples was below the

LOD (0.25 and  $0.50 \mu\text{g L}^{-1}$ , respectively). Limits of detection were calculated as 3 times the standard deviation for 10 consecutive measurements of blank solutions, divided by the slope of the calibration curve. Despite of these LODs were considered suitable, taking into account the use of F AAS as detector, it was investigated the use of GF AAS as a more powerful detection technique. In this purpose, the heating program of graphite furnace was carefully optimized.

### 3.5. Pyrolysis and atomization curves

For the determination by GF AAS, the main steps (especially pyrolysis and atomization temperature) of heating program of GF AAS instrument must be evaluated. The establishment of optimum pyrolysis temperature is important for matrix removal, keeping the analyte available and free from matrix components during the atomization, in order to reduce the background signal. As Cd and Pb are well known as volatile elements, the optimization of pyrolysis and atomization temperatures was performed using a Pd solution ( $2 \mu\text{L}$  of  $1000 \text{ mg L}^{-1}$ ) as chemical modifier. Due to the low volume of graphite platform, quantification was performed by pipetting of extract in two aliquots (about 25  $\mu\text{L}$  each). The second aliquot was added after finishing “Drying 1” step (Table 1), by stopping the heating program. After adding the second aliquot, the heating program was run up to the end.

The heating program for pyrolysis and atomization curves was evaluated from 400 to 1200 °C (pyrolysis) and from 1200 to 2000 °C (atomization). These curves were carried out for reference solutions (15 and 300  $\text{ng L}^{-1}$  for Cd and Pb, respectively) and samples, both submitted to DLLME method prior to detection by GF



**Fig. 3.** Pyrolysis and atomization curves for Cd and Pb by GF AAS: a) Cd using reference solution (◆) and sample (▲) and b) Pb using reference solution (■) and sample (●). DLLME conditions: reference solutions of Cd ( $15 \text{ ng L}^{-1}$ ) and Pb ( $300 \text{ ng L}^{-1}$ ), 10 mL of sample; 500  $\mu\text{L}$  of acetone for Cd and 700  $\mu\text{L}$  of acetonitrile for Pb as dispersive solvent, 60  $\mu\text{L}$  of carbon tetrachloride as extraction solvent; 500  $\mu\text{L}$  of 1.5% (m/v) DDTC solution and centrifugation at 3000 rpm for 2 min. Error bars represent the standard deviation ( $n = 3$ ).



**Table 2**Determination of Cd and Pb in commercial soft drinks by GF AAS after DLLME. Results represent the mean and standard deviation ( $n = 3$ ).

Sample	Cd ( $\text{ng L}^{-1}$ )				Pb ( $\text{ng L}^{-1}$ )			
	Concentration	Added	Found	Recovery (%)	Concentration	Added	Found	Recovery (%)
Regular Cola I	$9.92 \pm 0.45$	10	$20.9 \pm 0.20$	110	$103 \pm 3$	120	$240 \pm 12$	108
Regular Cola II	$9.40 \pm 0.25$	10	$18.6 \pm 0.34$	92	$96.7 \pm 6.2$	120	$213 \pm 6$	97
Low Calorie Cola I	$2.13 \pm 0.20$	10	$12.8 \pm 0.58$	107	$44.0 \pm 3.1$	120	$170 \pm 18$	104
Low Calorie Cola II	$6.46 \pm 0.19$	10	$15.6 \pm 0.51$	91	$115 \pm 2$	120	$229 \pm 14$	95
Regular Guarana I	$8.69 \pm 0.61$	10	$19.1 \pm 0.60$	104	$77.5 \pm 2.9$	120	$203 \pm 11$	105
Regular Lemon I	$0.310 \pm 0.007$	10	$11.6 \pm 0.80$	113	$7.41 \pm 0.52$	120	$131 \pm 4$	103

AAS. Results are shown in Fig. 3. For Cd, a decreasing on analytical signal from 700 and 800 °C was observed for reference solution and sample, respectively. Even the results being statistically similar ( $p > 0.05$ ) using 500 or 600 °C, 500 °C was selected as pyrolysis temperature based on the lower RSDs. The atomization temperature for Cd showed a similar behaviour for reference solution and sample. Thus, 1400 °C was chosen as atomization temperature.

For Pb, a similar behaviour for pyrolysis and atomization curves was observed. Lead was thermally stable during pyrolysis up to 700 and 1100 °C for reference solution and sample, respectively. The atomization curve for both solutions was exactly the same, with 1800 °C as optimum temperature. Thus, taking into account the same criteria (high analytical signal and low RSD) used to choose the pyrolysis temperature, 700 °C was selected for pyrolysis and 1800 °C for atomization for Pb determination. Considering that a similar behaviour was observed for both analytes using reference solution and sample, calibration was performed with aqueous standards.

### 3.6. Optimized DLLME procedure

Aliquots of 10 mL of soft drink and 500  $\mu\text{L}$  of 1.5% DDTC solution were placed in a 15 mL glass tube with a conical bottom. A mixture containing the dispersive (500  $\mu\text{L}$  of acetone for Cd and 700  $\mu\text{L}$  of acetonitrile for Pb) and extraction (60  $\mu\text{L}$  of carbon tetrachloride) solvent was quickly injected into the sample solution with aid of a 1 mL microsyringe. After injection, a cloudy solution was formed, which was centrifuged at 3000 rpm for 2 min, and small droplets of extraction solvent containing complexed analytes were deposited at the bottom of the glass tube. In order to reduce some interference during the measurements, the bottom phase (extract) was washed with ultrapure water (5 mL and centrifugation at 3000 rpm for 2 min). The aqueous phase was removed and extract was analyzed by GF AAS.

### 3.7. Determination of Cd and Pb in soft drinks by GF AAS after DLLME

Initially, in order to evaluate the possibility of performing a direct analysis of soft drinks by GF AAS, samples were directly weighed (about 25  $\mu\text{L}$ ) into the platform of solid sampling system. However, for all samples, even using Zeeman-effect the background signal was not corrected and the measurement was not been performed. It is important to mention that some experiments were carried out evaluating the pyrolysis and atomization temperatures (results not showed), but results were unsatisfactory. This fact, combined with the low LODs, is one of several evidences to use DLLME for minimizing sample matrix effects.

The proposed method was applied in soft drinks with different brands and flavours for the determination of Cd and Pb by GF AAS. The concentration of Cd and Pb in samples is shown in Table 2. Cadmium concentration ranged from 0.31 to  $9.92 \text{ ng L}^{-1}$  and Pb ranged from 44 to  $115 \text{ ng L}^{-1}$ . Accuracy was evaluated by analyte spiking (Table 2) and recoveries ranged from 91 to 113% for Cd and from 95 to 108% for Pb. Another alternative to evaluate the accuracy was performed by using MAD-UV and analytes determination by ICP-MS. However, even ICP-MS being a powerful detection technique, it was not possible to determine Cd and Pb in soft drinks because the concentration was below the LOD (42 and  $166 \text{ ng L}^{-1}$ , respectively).

The main analytical features of proposed method comparing to data from the literature are shown in Table 3. The LODs were estimated as three times the standard deviation of blanks ( $n = 10$ ) and were 0.006 and  $0.072 \text{ ng L}^{-1}$  for Cd and Pb, respectively. These extremely low LODs were possible due to the combination between the high detection power of GF AAS and high preconcentration factor (about 200 times) of DLLME. In comparison to data from literature, these LODs are extremely low and provide a suitable determination of Cd and Pb in soft drinks (El-Shahawi & Al-Saidi, 2013; Moghimi, 2008). In addition, the precision of proposed method was lower than 10 and 7% for Cd and Pb, respectively. The

**Table 3**

Comparison of analytical features of microextraction methods.

Microextraction method	Analyte	Sample	Extraction solvent	Dispersive solvent	LOD ( $\text{ng L}^{-1}$ )	Detection technique	References
IL-DLLME	Cd	Saline water	75 mg [Hmim] [PF <sub>6</sub> ]	500 $\mu\text{L}$ ethanol	30	F AAS	Yousefi and Shemirani (2010)
DLLME-SFO	Cd	Water, beverage and cereal	100 $\mu\text{L}$ 1-dodecanol	1000 $\mu\text{L}$ methanol	0.3	F AAS	Wu et al. (2011)
DLLME	Cd	Water	30 $\mu\text{L}$ C <sub>2</sub> Cl <sub>4</sub>	500 $\mu\text{L}$ THF	1200	F AAS	Karim-Nezhad, Ahmadi, and Zare-Dizajdizi (2011)
DLLME	Cd	Water	34 $\mu\text{L}$ CCl <sub>4</sub>	500 $\mu\text{L}$ methanol	0.6	GF AAS	Jahromi et al. (2007)
DLLME	Cd and Pb	Water	17 $\mu\text{L}$ CCl <sub>4</sub>	500 $\mu\text{L}$ methanol	0.5 (Cd) and 1.6 (Pb)	ICP-MS	Jia et al. (2010)
DLLME	Cd and Pb	Soft drinks	60 $\mu\text{L}$ CCl <sub>4</sub>	500 $\mu\text{L}$ acetone (Cd) and 700 $\mu\text{L}$ acetonitrile (Pb)	0.006 (Cd) and 0.072 (Pb)	GF AAS	This work

Abbreviations: DLLME, dispersive liquid-liquid microextraction; DLLME-SFO, dispersive liquid-liquid microextraction based on solidification of floating organic droplet; IL-DLLME, ionic liquid-based dispersive liquid-liquid microextraction; THF, tetrahydrofuran.

calibration range (considering the sample volume and preconcentration factor) was linear from 0.02 to 0.40 and from 0.25 to 3.50 ng L<sup>-1</sup>, with determination coefficient (r) of 0.998 and 0.996 for Cd and Pb, respectively. The matrix effect was also investigated by comparison of calibration curve with reference solutions and with analyte spike, both submitted to the proposed method. The matrix effect was not observed, once the difference in the slope of calibration curves was not higher than 5%.

#### 4. Conclusions

The proposed DLLME method combined with GF AAS could be considered as a good alternative for Cd and Pb determination at sub-ppt level in soft drinks. The feasibility of using DLLME for sample preparation was demonstrated and parameters were carefully optimized for determination of Cd and Pb by GF AAS. Instrument calibration requires only submitting the reference solution to the DLLME method prior to the analytes determination. When comparing the performance of DLLME and GF AAS with MAD-UV and ICP-MS, several advantages could be mentioned such as low cost, simplicity, lower LODs (about 3 orders of magnitude), good accuracy and precision.

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