



# New solid surface fluorescence methodology for lead traces determination using rhodamine B as fluorophore and coacervation scheme: Application to lead quantification in e-cigarette refill liquids



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## ABSTRACT

A new environmental friendly methodology based on fluorescent signal enhancement of rhodamine B dye is proposed for Pb(II) traces quantification using a preconcentration step based on the coacervation phenomenon. A cationic surfactant (cetyltrimethylammonium bromide, CTAB) and potassium iodine were chosen for this aim. The coacervate phase was collected on a filter paper disk and the solid surface fluorescence signal was determined in a spectrofluorometer. Experimental variables that influence on preconcentration step and fluorimetric sensitivity have been optimized using uni-variation assays. The calibration graph using zero th order regression was linear from  $7.4 \times 10^{-4}$  to  $3.4 \mu\text{g L}^{-1}$  with a correlation coefficient of 0.999. Under the optimal conditions, a limit of detection of  $2.2 \times 10^{-4} \mu\text{g L}^{-1}$  and a limit of quantification of  $7.4 \times 10^{-4} \mu\text{g L}^{-1}$  were obtained. The method showed good sensitivity, adequate selectivity with good tolerance to foreign ions, and was applied to the determination of trace amounts of Pb(II) in refill solutions for e-cigarettes with satisfactory results validated by ICP-MS. The proposed method represents an innovative application of coacervation processes and of paper filters to solid surface fluorescence methodology.

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## 1. Introduction

Smoking is a major cause of adverse health effects in most countries. In the Argentine alone, an estimated 40,000 people die prematurely from smoking or from exposure to secondhand smoke each year, and others 18,000 have a serious illness caused by smoking [1]. Especially, adolescent smoking is clinically important. In order to prevent the diseases and deaths caused by this addiction, researchers have suggested various replacement alternatives, such as smokeless tobacco and nicotine delivery systems.

Since their launch in 2004, electronic cigarettes (e-cigarettes), an ample range of devices that vaporize nicotine for inhalation operated by battery, have been purchased by millions of people [2]. In recent years, there was a large increase in the use of electronic cigarettes among young people. In the US, a recent report of centers for disease control and prevention (CDC) notes that the

percentage of students in primary and secondary that proved e-cigarettes, doubled between 2011 and 2012, the year in which nearly 2 million students used them. Experts have named this episode as a new phase in smoking and a new route for nicotine addiction in children [3–5].

At present, there is considerable controversy about the health effects of e-cigarettes, especially in comparison to conventional cigarettes. Researchers recently realized concluded that e-cigarette use not discouraged, but on the contrary, has encouraged the consumption of traditional cigarettes among US adolescents [6].

From a public health perspective, the extent to which e-cigarettes may serve as a starter product for non-users of tobacco is a concern [7–10]. The Argentinean National Medicines, Food and Medical Technology Administration (ANMAT) banned sales of e-cigarette; the prohibition are founded on the fact that there is not enough scientific evidence to determine that these (the e-cigarettes) are safe for human consumption, and the fact that they contain variable quantities of nicotine, a highly toxic and addictive drug [11].

From 2009, our research group is performing conscientization workshops of tabaquism in classrooms of secondary schools of our province [12]. In these activities, we use research results realized

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in our laboratory related with contents of cadmium and nickel present in saliva and urine of non-smokers, passive and active smoker subjects [13,14].

Lead is a carcinogen that is present in cigarette smoke [15–17]. Chronic poisoning with this metal leads to a condition known as *saturnism*, characterized by severe anemia, digestive disorders, cardiovascular, nervous and renal failure [18–20].

The determination of lead traces constitutes a fundamental topic in environmental monitoring and toxicological clinical areas. Metal traces are frequently determined by atomic absorption spectrometry, inductively coupled plasma atomic emission spectrometry associated to a previous preconcentration step [21–26]. Coacervation is a surfactant mediated extraction, which has been used for pre-concentrate, enrich and/or recover metal ions as well as organic and biological compounds from aqueous or solid matrices [27,28].

In this opportunity, continuing with our contribution in anti-tobacco strategies, a new method for traces Pb(II) determination is proposed. The preconcentration step is based in the formation of coacervate phase using the cationic surfactant cetyltrimethylammonium bromide (CTAB) and potassium iodine, as agent of ionic strength increment. The dye rhodamine B (RhB) is added as fluorophore reagent whose fluorescent signal is increased in the presence of Pb(II). The coacervate phase is collected on a filter paper disk and the solid surface fluorescence signal is determined in a spectrofluorometer. The experimental parameters that influence on separation/preconcentration and determination steps have been optimized and the new methodology has been applied to Pb(II) determination in refill solutions for e-cigarettes.

## 2. Materials and methods

### 2.1. Reagents

Filter papers Blue Ribbon (Whatman, England) 2–5  $\mu\text{m}$  pore size and 4.5 cm diameter were used in coacervate retention studies.

Stock solutions of Pb(II)  $20.72 \times 10^3 \mu\text{g L}^{-1}$  were prepared by dilution of  $100 \mu\text{g mL}^{-1}$  standard solution plasma-pure (Leeman Labs, Inc.).

Buffer sodium tetraborate (Mallinckrodt Chemical Works, New York, Los Angeles, St. Louis, USA)  $1 \text{ mol L}^{-1}$  solution was prepared. The desired pH was obtained by adding  $\text{HClO}_4$  (Merck, Darmstadt, Germany) or NaOH (Mallinckrodt Chemical Works) solution, with the assistance of a pH meter (Orion Expandable Ion Analyzer, Orion Research, Cambridge, MA, USA) Model EA 940.

Stock of RhB solutions  $1 \times 10^{-6} \text{ mol L}^{-1}$  (Fluka AG, Chemische-Fabrik, Buchs SG, Switzerland) solution were weekly prepared by dissolution of the appropriate amount of each, in ultrapure water. The stability of solutions was checked by spectrophotometric measurements.

Sodium dodecylsulfate (SDS) and cetyltrimethylammonium bromide (CTAB) were purchased from Tokyo Kasei Industries (Chuo-Ku, Tokyo, Japan). Sodium cholate ( $\text{C}_{24}\text{H}_{39}\text{O}_5\text{Na}$ , NaC) was purchased from Sigma (St. Louis, MO 63103, EEUU). Solution of CTAB  $1 \times 10^{-2} \text{ mol L}^{-1}$  was prepared by dissolution of the appropriate amount in ultrapure water.

Glass materials were previously washed with a 10% v/v  $\text{HNO}_3$  solution and then with ultrapure water. All used reagent were analytical grade.

### 2.2. Apparatus

Spectrofluorimetric measurements were made using a Shimadzu RF-5301 PC spectrofluorophotometer equipped with a

150 W Xenon lamp and 1.00 cm quartz cells and solid sample holder. A combined glass electrode and a pH meter (Orion Expandable Ion Analyzer, Orion Research, Cambridge, MA, USA) Model EA 940 were used for pH adjustments.

An inductively coupled plasma mass spectrometer, PerkinElmer SCIEX, ELAN DRC-e (Thornhill, Canada) was used for validation measurements. The argon gas with minimum purity of 99.996% was supplied by Air Liquide (Córdoba, Argentina). An HF-resistant and high performance Teflon Nebulizer model PFA-ST, was coupled to a quartz cyclonic spray chamber with internal baffle and drain line cooled with the PC<sup>3</sup> system from ESI (Omaha, NE, USA) (Table 1). Tygon black/black 0.76 mm i.d. and 40 cm length peristaltic pump tubing was used. The instrument conditions were as follows: auto lens mode on, peak hopping measure mode, dwell time of 50 ms, 15 sweeps/reading, 1 reading/replicate, and 3 replicates [29].

### 2.3. General procedure

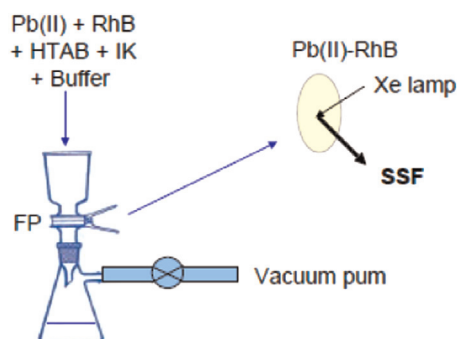
Adequate volume of sample/standard Pb(II) solution ( $7.4 \times 10^{-4}$ – $3.4 \mu\text{g L}^{-1}$ ), 1 mL RhB  $1 \times 10^{-6} \text{ mol L}^{-1}$ , 500  $\mu\text{L}$  CTAB  $1 \times 10^{-2} \text{ mol L}^{-1}$ , 1.5 mL KI  $1 \times 10^{-2} \text{ mol L}^{-1}$  and 1 mL buffer solution  $1 \times 10^{-2} \text{ mol L}^{-1}$  (pH=9.2) were putted in volumetric flask and the whole mixture was diluted to 10 mL with ultrapure water. The system was manually shaken (30 s) and filtered through disk of filter paper (FP) with the assistance of a vacuum pump. PF was dried at room temperature and reserved in dried ambient (20–25 °C) to follow step. Pb(II) concentration was determined on FP by solid surface fluorescence at  $\lambda_{\text{em}}=568 \text{ nm}$  ( $\lambda_{\text{exc}}=520 \text{ nm}$ ), using a solid sample holder (see Fig. 1).

### 2.4. Selection and treatment of samples

A wide variety of liquids is available on the market, mainly differing in their flavoring and content of propylene glycol (PG) and glycerol. All types of studied refill liquids were purchased via

**Table 1**  
Instrument settings and data acquisition parameters for ICP-MS.

Sample introduction	Nebulizer model PFA-ST, coupled to a quartz cyclonic spray chamber with internal baffle and drain line, cooled with the PC <sup>3</sup> system from ESI (Omaha, NE, USA)
Sample uptake rate ( $\mu\text{L min}^{-1}$ )	200
RF power (W)	1200
Nebulizer gas flow rates ( $\text{mL min}^{-1}$ )	0.77
Interface	Ni cones (sampler and skimmer)
Isotope	$^{208}\text{Pb}$
Scanning mode	Peak hopping
Dwell time (ms)	50 in standard mode
Number of replicate	3



**Fig. 1.** Schematic representation of general procedure of developed methodology.

Internet with different levels of nicotine: no nicotine, 11, 12 and 18 mg mL<sup>-1</sup> nicotine. Samples were produced no more than three months before the tests took place and where stored in a cool and dark place throughout the duration of the tests.

The refill liquids examined were as follows:

- Tobacco USA Mix (18 mg nicotine).
- Cappuccino (12 mg nicotine).
- Ice Mint (0 mg nicotine).
- Tobacco Whinston (11 mg nicotine).

### 2.5. Interferences study

Different amounts of ions which may be present in samples (1/100 and 1/1000 Pb(II)/interferent ratio) were added to the test solution containing 2.5 µg L<sup>-1</sup> Pb(II) and general procedure was applied.

### 2.6. Accuracy study

Adequate volume of refill liquid e-cigarette samples were spiked with increasing amounts of Pb(II) (0.25–2.50 µg L<sup>-1</sup>). Pb(II) concentrations were determined by proposed methodology.

### 2.7. Precision study

The repeatability (within-day precision) and reproducibility (between-day precision) of the methodology were tested for replicate of samples (*n*=4) spiked of Pb(II) and the contents were determined during 3 days by proposed methodology.

### 2.8. Validation

Pb(II) contents in samples was determined by ICP-MS, using operational conditions previously consigned in apparatus item.

## 3. Results and discussion

The fluorescence excitation and emission spectra of the RhB dye prepared in the buffer solution (sodium tetraborate) were obtained and compared with the spectra in the presence of the Pb(II) toxic heavy metal in the same medium. Dye emission remained unchanged when metal was added.

In order to achieve the preconcentration of metal traces, a scheme of coacervation was applied. In order to achieve the preconcentration of metal traces, a scheme of coacervation was applied. The coacervation technique is based upon the phase separation behavior exhibited by surfactant solutions. Upon alteration of temperature or addition of appropriate additives (such as salt, acid, other surfactants, polymers or organic compounds, etc.), solutions of surfactant separate to form an extract, small volume surfactant-rich phase (coacervate) and the remaining bulk aqueous phase of higher volume. An analyte specie initially present in solution which binds to the micelle entity thus becomes concentrated in the surfactant-rich coacervate phase.

Between available surfactants, CTAB, SDS, NaC were studied, together and alone. The ability of different substances to induce phase separation and coacervate formation in aqueous solutions of surfactants at room temperature was surveyed; the following reagents were assayed: NaCl, KI, CaCl<sub>2</sub>, HCl (0.01–0.1 mol L<sup>-1</sup>). The coacervate phase generated was then collected on solid supports with using a vacuum pump. The best results respect to Pb(II) quantitative retention and reproducibility were obtained when CTAB/KI couple were used in coacervation scheme.

The effect of salt addition on the extraction efficiency was investigated by the addition of KI in the concentration range of 1 × 10<sup>-4</sup>–1 × 10<sup>-2</sup> mol L<sup>-1</sup>. The results showed that by increasing of KI concentration up to 1 × 10<sup>-3</sup> mol L<sup>-1</sup>, the extraction efficiency increased, may be due to the effect of salt on the phase separation and retention of coacervate phase on solid support. Consequently, a 1.5 × 10<sup>-3</sup> mol L<sup>-1</sup> of KI was selected for further studies.

Among the solid supports assayed, filter paper (blue ribbon) showed a satisfactory retention and was chosen as optimal due to, between others, its low cost. As can be seen in Fig. 2, RhB solid surface fluorescence shows an increased response in the presence of Pb(II).

Taking into account that the interaction of metal with RhB is pH-dependent, equilibrium pH experimental parameter was optimized. The effect of this parameter was evaluated preparing systems maintaining constant Pb(II) concentration and other parameters, with varying pH from 4.0 to 12.5. Fig. 3 shows the found behavior. At acidic pH values, the emission signal was maintained low, beginning to increase at pH 6. The maximum emission was showed between pH 8.5 and 10. For pH very alkaline, the emission again decayed. So, a pH value of 9.2 was chosen as optimal.

The effect of the nature of the buffer on the analytical signal was also evaluated. The best results with respect to sensitivity and reproducibility were obtained using sodium tetraborate 1 × 10<sup>-3</sup> mol L<sup>-1</sup> (pH=9.2).

The chelating reagent concentration was also studied for optimum generation of the Pb(II)/RhB association. The effect of dye concentration on the analytical signal was evaluated by preparing systems with the toxic heavy metal and different concentrations of RhB. Using RhB 1 × 10<sup>-7</sup> mol L<sup>-1</sup>, the best results were obtained taking into account sensitivity and reproducibility.

### 3.1. Analytical figures of merit

The limit of detection (LOD) of the proposed method was studied under optimal experimental conditions by applying the procedure for blank solutions. The detection limits based on three times the standard deviations of the blank (*N*=15) was 2.2 × 10<sup>-4</sup> µg L<sup>-1</sup>.

The calibration graph resulted linear in the range of 7.4 × 10<sup>-4</sup>–3.4 µg L<sup>-1</sup> for Pb(II). Table 2 summarizes the main characteristics of calibration plot and optimized experimental

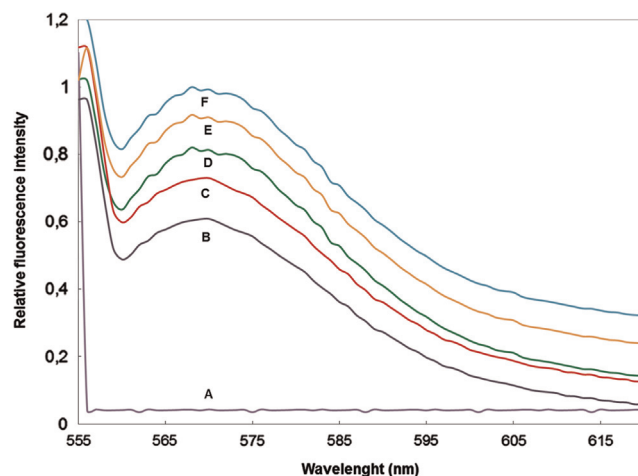


Fig. 2. Emission spectra for Pb(II) quantification. A: filter paper; B: filter paper with  $C_{\text{RhB}}=1 \times 10^{-7}$  mol L<sup>-1</sup>;  $C_{\text{HTAB}}=5 \times 10^{-4}$  mol L<sup>-1</sup>;  $C_{\text{KI}}=1.5 \times 10^{-3}$  mol L<sup>-1</sup>;  $C_{\text{buffer borax}}=1 \times 10^{-3}$  mol L<sup>-1</sup>, pH 9.22; C: Idem B with Pb(II) 0.30 µg L<sup>-1</sup>. D: Idem B with Pb(II) 0.65 µg L<sup>-1</sup>. E: Idem B with Pb(II) 1.25 µg L<sup>-1</sup>. F: Idem B with Pb(II) 2.50 µg L<sup>-1</sup>. Conditions:  $\lambda_{\text{em}}=568$  nm;  $\lambda_{\text{exc}}=520$  nm; (slits 3/3). Other experimental conditions are described under procedure.

conditions, which sustain the proposed procedure for quantification of Pb(II) traces.

Table 3 shows the main analytical parameters of conventional methodologies by Pb(II) determination in different matrixes. As can be seen, the new method displayed adequate selectivity and sensitivity, comparable with other informed methodologies with the advantage of using a low-cost instrument.

### 3.2. Interferences study

The effect of foreign ions on the recovery of Pb(II) was tested. Different amounts of ions commonly present in samples were added to the test solution containing  $2.5 \mu\text{g L}^{-1}$  of Pb(II), and the developed procedure was applied. An ion was considered as interference, when it caused a variation in the fluorescent signal of the test system greater than  $\pm 5\%$ . The tolerance limits of various foreign ions are given in Table 4. These results demonstrate that large excess amounts of some common cations and anions do not interfere on the determinations of the analyte, putting in evidence the adequate selectivity of the developed methodology.

### 3.3. Applications

The usefulness of the proposed method was evaluated for the determination of the analyte in refill liquid solution for e-cigarettes. In order to check the accuracy of the proposed method, and

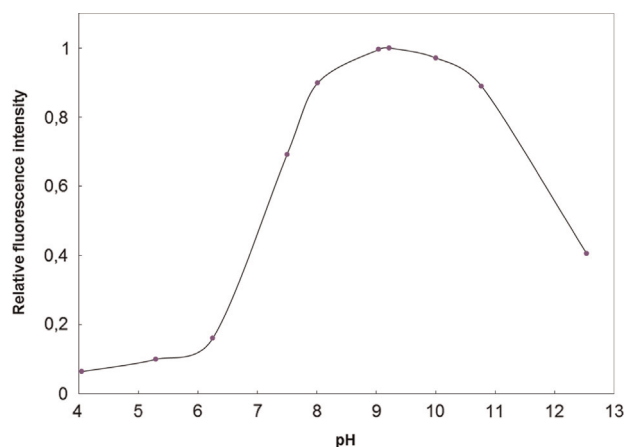


Fig. 3. Influence of pH on lead determination. Conditions:  $\lambda_{em} = 568 \text{ nm}$ ;  $\lambda_{exc} = 520 \text{ nm}$ ;  $C_{RhB} = 1 \times 10^{-7} \text{ mol L}^{-1}$ ;  $C_{HTAB} = 5 \times 10^{-4} \text{ mol L}^{-1}$ ;  $C_{KI} = 1.5 \times 10^{-3} \text{ mol L}^{-1}$ ;  $C_{buffer \text{ borax}} = 1 \times 10^{-3} \text{ mol L}^{-1}$ , pH 9.22;  $C_{Pb(II)} = 2.5 \mu\text{g L}^{-1}$ . Other experimental conditions are described under procedure.

Table 2  
Experimental conditions and analytical parameters for lead determination by SSF.

Parameters Support	Studied range	Optimal conditions
	Cellulose acetate, nylon, Teflon, filter paper	Filter paper (blue ribbon)
pH	4.0–12.5	9.22
Buffer sodium tetraborate	$1 \times 10^{-4}$ – $0.5 \text{ mol L}^{-1}$	$1 \times 10^{-3} \text{ mol L}^{-1}$
RhB concentration	$1 \times 10^{-8}$ – $1 \times 10^{-5} \text{ mol L}^{-1}$	$1 \times 10^{-7} \text{ mol L}^{-1}$
CTAB concentration	$1 \times 10^{-5}$ – $1 \times 10^{-2} \text{ mol L}^{-1}$	$5 \times 10^{-4} \text{ mol L}^{-1}$
KI concentration	$1 \times 10^{-4}$ – $1 \times 10^{-2} \text{ mol L}^{-1}$	$1.5 \times 10^{-3} \text{ mol L}^{-1}$
LOD	–	$2.2 \times 10^{-4} \mu\text{g L}^{-1}$
LOQ	–	$7.4 \times 10^{-4} \mu\text{g L}^{-1}$
LOL	–	$7.4 \times 10^{-4}$ – $3.4 \mu\text{g L}^{-1}$
$r^2$	–	0.999

Table 3  
Analytical parameters of methodologies for Pb(II) determination.

Method	Comments	Reference
DLLME–ETAAS	LOL: 0.1–20 ng/mL ( $r^2 > 0.999$ ) RSD < 3.2% LOD = 39 ng/L LOQ = 0.1 ng/mL Applied to biological and water samples	[21]
DLLME–ETAAS	LOL: 0.05–1 $\mu\text{g/L}$ Correlation coefficient = 0.9994 RSD = 2.5% LOD = 0.02 $\mu\text{g/L}$ Applied to water samples	[22]
ICP–OES	LOD = 0.16 $\mu\text{g/L}$ RSD = 3.0% Recovery (%) = 97.6–97.8 Applied to water samples	[23]
FAAS	LOD = 0.02 $\mu\text{g L}^{-1}$ RSD = 10.0% Recovery (%) = 93–100 Applied to tap water samples	[24]
GF–AAS	Studied ranged from 1.33 to 3.61 $\mu\text{g g}^{-1}$ dry weight with an average of 2.46 $\mu\text{g g}^{-1}$ . Applied to different cigarette brands	[25]
ICP–MS	Studied ranged from $\leq 3$ to 10.9 ng/m <sup>3</sup> . Applied to vaping of electronic cigarettes samples	[26]
This method	LOL = $7.4 \times 10^{-4}$ – $3.4 \mu\text{g L}^{-1}$ LOD = $2.2 \times 10^{-4} \mu\text{g L}^{-1}$ LOQ = $7.4 \times 10^{-4} \mu\text{g L}^{-1}$ $R^2 = 0.999$ Recovery (%) = 96.33–106.66. Applied to refill solutions (e-cigarettes) samples	–

Table 4  
Tolerance limits of interfering species in Pb(II) determination.

Interferent/Pb(II) mole ratio	Interferent specie
1000:1	$\text{Na}^+$ , $\text{K}^+$ , $\text{Cl}^-$
100:1	$\text{Cd}^{2+}$ , $\text{Ca}^{2+}$ , $\text{Mg}^{2+}$ , $\text{Ni}^{2+}$ , $\text{Zn}^{2+}$ , $\text{Co}^{2+}$ , $\text{Cu}^{2+}$ $\text{Hg}^{2+}$ , $\text{Fe}^{3+}$ , $\text{As}^{3+}$ , $\text{Cr}^{3+}$ , $\text{CO}_3^{2-}$ , $\text{SO}_4^{2-}$ , $\text{NO}_3^-$

Conditions:  $\lambda_{em} = 568 \text{ nm}$ ;  $\lambda_{exc} = 520 \text{ nm}$ ;  $C_{RhB} = 1 \times 10^{-7} \text{ mol L}^{-1}$ ;  $C_{HTAB} = 5 \times 10^{-4} \text{ mol L}^{-1}$ ;  $C_{KI} = 1.5 \times 10^{-3} \text{ mol L}^{-1}$ ;  $C_{buffer \text{ borax}} = 1 \times 10^{-3} \text{ mol L}^{-1}$ , pH 9.22;  $C_{Pb(II)} = 2.5 \mu\text{g L}^{-1}$ . Other experimental conditions are described under procedure.

since there were not available certified materials, a parallel analysis with ICP–MS was carried out following the conditions published in the previous works [29–31]. Comparison between the results obtained of Pb(II) contents for replicate of samples ( $n=4$ ) with proposed method and ICP–MS technique were statistically compared ( $t$ -test) and no significant differences ( $p=0.05$ ,  $DF=3$ ) were observed. The operational conditions for the ICP–MS analysis are listed in Table 1.

Sample aliquots (0.100 mL) were spiked with increasing amounts of Pb(II). Obtained results showed satisfactory agreement with adequate precision. The reproducibility of the method was evaluated repeating the proposed methodology, 4 times ( $n=4$ ) for each sample. Table 5 shows the recovery results achieved for each sample. Obtained results indicate that the proposed method is suitable for determination of this analyte in such studied samples.

In order to determine the repeatability (within-day precision) of the method, refill liquid solution for e-cigarettes replicate

**Table 5**  
Recuperation and validation studies by lead determination in refill solutions (e-cigarettes) samples.

Sample	Pb(II) added ( $\mu\text{g L}^{-1}$ )	Proposed methodology		ICP-MSPb(II) found $\pm$ SD ( $\mu\text{g L}^{-1}$ )
		Pb(II) found $\pm$ CV ( $\mu\text{g L}^{-1}$ )	Recovery (%), $n=4$	
1	–	0.030 $\pm$ 0.02	–	0.028 $\pm$ 0.01
	0.25	0.282 $\pm$ 0.03	106.66	
	0.75	0.778 $\pm$ 0.08	93.33	
	–	0.015 $\pm$ 0.06	–	
2	0.25	0.264 $\pm$ 0.04	93.33	
	0.75	0.766 $\pm$ 0.03	106.66	
	–	1.25 $\pm$ 0.02	–	
3	1.25	2.53 $\pm$ 0.09	102.40	1.24 $\pm$ 0.13
	2.50	3.73 $\pm$ 0.07	98.40	
	–	2.55 $\pm$ 0.03	–	
4	1.25	3.84 $\pm$ 0.02	101.57	2.52 $\pm$ 0.04
	2.50	5.01 $\pm$ 0.01	98.43	

1 – Tobacco USA mix (18 mg nicotine), 2 – Cappuccino (12 mg nicotine), 3 – Ice mint (0 mg nicotine), 4 – Tobacco whinston (11 mg nicotine).

**Table 6**  
Lead contents in e-cigarettes and conventional cigarettes.

Samples	Pb(II) found ( $\mu\text{g}/\text{e-cigarette}$ ) this work	Pb(II) content ( $\mu\text{g}/\text{cigarette}$ ) reference [15]
1	0.13	0.17
2	0.10	
4	0.19	

samples ( $n=4$ ) were analyzed by proposed methodology. The precision was better than 0.032 CV for Pb(II) determination. The reproducibility (between-day precision) was also evaluated over 3 days by performing four determinations each day with a CV of 0.067.

With the aim of verify that e-cigarettes really represent an less harmful habit that smoking, results were expressed as Pb(II) content/e-cigarettes and were compared with the informed by other authors [26] for conventional cigarettes (Table 6). In all studied samples, Pb(II) contents in e-cigarette liquids were in the same order as conventional cigarettes. This fact put in evidence that the consumption of e-cigarettes does not represent a healthy option.

#### 4. Conclusions

The proposed methodology showed to be an alternative environmental friendly, simple, economical, rapid, and precise for determination of Pb(II) traces using RhB dye. The coacervation scheme was use for retention/preconcentration of analyte on filter paper resulting a powerful tool for sensitive determination of metal in studied samples. The good tolerance at high levels of regular foreign constituents put in evidence the high selectivity and versatility of the new methodology. Precision and accuracy were tested and validated by ICP-MS with good agreement. The

reached sensitivity was comparable at those arrived with atomic spectroscopies employing a simple and inexpensive instrumental.

The developed methodology was successfully applied to refill liquid solution for e-cigarettes, proving to be sources of Pb(II). The impact of vaping on health and the composition of refill liquids require therefore further research into the product characteristics. For the benefit of consumers, quality and safety requirements of e-cigarettes and refill liquids should be harmonized.

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#### References

- [1] A. Pichon-Riviere, A. Alcaraz, A. Bardach, F. Augustovski, J. Caporale, F. Caccavo, Carga De Enfermedad Atribuible Al Tabaquismo En Argentina. Documento Técnico IECS N° 7, Instituto De Efectividad Clínica Y Sanitaria, Buenos Aires, Argentina, 2013.
- [2] D. Purkayastha, BAT ramps-up e-cigarette expansion as sales go up in smoke international business times (July 31, 2013). ([http://www.thefreelibrary.com/BAT Ramps-up E-cigarette Expansion as Sales Go Up in Smoke.-a0338323170](http://www.thefreelibrary.com/BAT+Ramps-up+E-cigarette+Expansion+as+Sales+Go+Up+in+Smoke.-a0338323170)) (accessed 30.12.14).
- [3] (<http://www.perfil.com/ciencia/Crecen-las-evidencias-en-contra-del-uso-de-los-cigarrillos-electronicos-20140412-0035.html>) (accessed 30.12.14).
- [4] K.E. Farsalinos, R. Polosa, Ther. Adv. Drug Saf. 5 (2014) 67–86.
- [5] P. Hajek, J.F. Etter, N. Benowitz, T. Eissenberg, H. McRobbie, Addiction 109 (2015) 1801–1810.
- [6] L.M. Dutra, S.A. Glant, JAMA Pediatr. 168 (2014) 610–617.
- [7] N.K. Cobb, D.B. Abrams, N. Engl. J. Med. 21 (2011) 193–195.
- [8] C.K. Yamin, A. Bitton, D.W. Bates, Ann. Intern. Med. 153 (2010) 607–609.
- [9] J. Foulds, S. Veldheer, A. Berg, Int. J. Clin. Pract. 64 (2011) 1037–1042.
- [10] J. Foulds, A.C. Schmelzer, M.B. Steinberg, Int. J. Clin. Pract. 64 (2010) 142–146.
- [11] ([http://www.ficargentina.org/index.php?option=com\\_content&view=article&id=92:la-anmat-prohibio-el-cigarrillo-electronico-en-argentina&catid=61:archivo-de-noticias&Itemid=17&lang=en](http://www.ficargentina.org/index.php?option=com_content&view=article&id=92:la-anmat-prohibio-el-cigarrillo-electronico-en-argentina&catid=61:archivo-de-noticias&Itemid=17&lang=en)) (accessed 30.12.14).
- [12] M. Talio, M. Luconi, A. Masi, L. Fernández, Sci. Total Environ. 15 (2010) 3125–3132.
- [13] M. Talio, M. Luconi, L. Fernández, J. Pharm. Biomed. Anal. 52 (2010) 694–700.
- [14] M. Talio, M. Luconi, L. Fernández, Microchem. J. 99 (2011) 486–491.
- [15] P.M. Lutz, E.A. Kely, T.D. Brown, T.J. Wilson, G. Brock, R.E. Neal, Toxicology 291 (2012) 43–50.
- [16] R.S. Pappas, G.M. Polzin, C.H. Watson, D.L. Ashley, Food Chem. Toxicol. 45 (2007) 202–209.
- [17] K. Swami, C.D. Judd, J. Orsini, Spectrosc. Lett. 42 (2009) 479–490.
- [18] M. Jakubowski, Int. J. Occup. Med. Environ. Health 24 (2011) 1–7.
- [19] P. Liang, H. Sang, Anal. Biochem. 380 (2008) 21–25.
- [20] D.C. Bellinger, Int. J. Environ. Res. Public Health 8 (2011) 2593–2628.
- [21] P. Liang, H. Sang, Anal. Biochem. 380 (2008) 21–25.
- [22] M.T. Naseri, M. Reza, M. Hosseini, Y. Assadi, A. Kiani, Talanta 75 (2008) 56–62.
- [23] Q. He, Z. Hu, Y. Jiang, X. Chang, Z. Tu, L. Zhang, J. Hazard. Mater. 175 (2010) 710–714.
- [24] U. Divrikli, A.A. Kartal, M. Soylak, L. Elci, J. Hazard. Mater. 145 (2007) 459–464.
- [25] W. Schober, K. Szendrei, W. Matzen, H. Osiander-Fuchs, T. Di Heitmann, R.A. Schettgen, H. Jörres, Int. J. Hyg. Environ. Health 217 (2014) 628–637.
- [26] M.W. Ashraf, J. Arts Sci. Commer. 2 (2011) 140–147.
- [27] G. Jia, L. Li, J. Qiu, X. Wang, W. Zhu, Y. Sun, Z. Zhou, Spectrochim. Acta A 67 (2007) 460–464.
- [28] Z. Sosa Ferrera, C. Padron Sanz, C. Mahugo Santana, J.J. Santana Rodríguez, Trends Anal. Chem. 23 (2004) 469–479.
- [29] F. Moyano, Microchem. J. 112 (2014) 17–24.
- [30] M. Savio, M.S. Ortiz, C.A. Almeida, R.A. Olsina, L.D. Martinez, R.A. Gil, Food Chem. 159 (2014) 433–438.
- [31] E.R. Verni, F. Moyano, L.D. Martinez, A.V. Lapiere, R.A. Gil, J. Anal. At. Spectrom. 28 (2013) 1655–1659.