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Lead monitoring and control in tobacco products and E-cigarettes by molecular fluorescence

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LEAD MONITORING AND CONTROL IN TOBACCO PRODUCTS AND E-CIGARETTES BY MOLECULAR FLUORESCENCE

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Abstract

A new methodology for lead traces determination has been developed based on the quenching effect on fluorescent emission of the complex formed by the metal with 8-hydroxyquinoline and o-phenanthroline at λ_{em} = 365 nm (λ_{exc} = 250 nm). Experimental variables that influence on fluorimetric sensitivity were optimized using uni-variation assays. The calibration graph using zero[th] order regression was linear from 1.21 µg L^{-1} to 518 µg L^{-1}, with correlation coefficient better than 0.999. Under the optimal conditions, the limits of detection and quantification were of 0.42 µg L^{-1} and 1.21µg L^{-1} respectively. The proposed method showed good sensitivity, adequate selectivity with good tolerance to foreign ions, and was applied to the determination of trace amounts of lead in leachate of tobacco products and refill solutions for e-cigarettes with satisfactory results. The trueness of the recommended procedure was assessed trough parallel analysis of the samples by ICP-MS. The proposed method represents an innovative

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application of luminescence to metal analysis comparable in sensitivity and accuracy to atomic spectroscopies.

Keywords: Lead traces; o-Phenanthroline; 8-Hydroxyquinoline; Molecular Fluorescence; Tobacco products with and without smoke.

1. Introduction

The tobacco exposure and consumption represent an important risk factor of the main chronic diseases non-transmissible (NCTs) which are currently responsible for almost two thirds of deaths worldwide. These diseases mainly include cardiovascular diseases, cancers, diabetes and respiratory diseases, but the use of snuff is not responsible for most of these [1, 2]. In the Americas, NCTs are responsible for 77% of all deaths and within them; the snuff is occasions for 15% of deaths from diseases cardiovascular, 26% of cancer deaths and 51% of deaths due to respiratory diseases [3].

Tobacco smoking causes 40,000 deaths per year in Argentina [4]; an estimated 25.1% of Argentinian adults and 21.5% of girls and 17.4% of boys (13- 15 years old) were smokers in 2012 [5].

Traditionally in the Region of the Americas, attention and actions have been focused mainly on the consumption of cigarettes, leaving aside other tobacco products, such as water pipes and smokeless tobacco; smokeless tobacco is one that is consumed without combustion, either through the nasal route or orally: chewed, sucked or applied to the gums (eg chewing tobacco, chimó, snus, etc.) [6]. Erroneously it is believed that smokeless tobacco is a less harmful alternative to tobacco consumption, besides being less expensive and being seen as an option in places where smoking is prohibited; for this reason, the evolution of their consumption should not be overlooked [7].

Since 2011, a reduced risk product category has appeared on the Argentinian market: the electronic cigarette (e-cigarette). An e-cigarette delivers nicotine and other toxic substances different to that are present in combustible tobacco smoke. Although healthcare professionals mainly see the harm reducing potential of e-cigarettes, others have concerns that e-cigarettes may appeal to youth and form a gateway to tobacco smoking [8]. Researchers have examined smoking initiation among adolescents who experimented with e-cigarettes. They found that those who experimented with e-cigarettes were more likely to start smoking combustible tobacco products over the next year [9, 10].

More than 4,000 chemicals have been isolated from tobacco (hydrocarbons, aldehydes, ketones, aromatic hydrocarbons, heavy metals). Tobacco plants have a special ability to absorb metals from soil and to accumulate it in unusually high concentrations in the leaves. Lead has been identified and quantified both in tobacco and tobacco smoke [11- 14].

Lead has been classified as carcinogenic to humans (Group 1) [15, 17]. This metal ion circulates in the bloodstream and accumulates in tissues and bones, or is eliminated from the body, primarily in urine. The blood brain barrier of children and infants is relatively impermeable to lead but they are at high risk of accumulating lead in the brain and central nervous system that may cause neurodegeneration [18]. It is noteworthy that tobacco exposure is an important source of lead in secondhand tobacco smokers [19].

Atomic spectroscopies have been recognized as the conventional instrumental methods most suitable for the metal traces determination [20, 21]; in many cases, the low levels of toxic metal are incompatible with instrumental sensitivity. Added to this, high prices and expensive instrumental inputs constitute a major constraint for many

control laboratories. The application of luminescent methods to lead traces determination has shown several analytical advantages such as high sensitivity, proper selectivity and dynamic wide range when they have been associated to separation/preconcentration/sensitization steps [22, 23].

In researches previously published, our group have developed alternative analytical methods for the quantification of metal traces (lead, cadmium and nickel) in refill solutions for e-cigarettes [24, 25]. The present paper aims to provide a new methodology for the monitoring of lead with the additional advantages of being simpler and cheaper than the previous ones and not requiring a separation / preconcentration stage prior to the quantitative determination. The new methodology is based in the formation of fluorescent complex of Pb(II) with 8-hydroxyquinoline and o-phenanthroline evidencing a phenomenon of quenching. In addition, the proposed new methodology has been applied to thirty-four real samples of tobacco products (cigarettes, roll your own tobaccos, water pipe tobaccos, oral tobacco products) and e-cigarettes marketed in Argentina.

2. Experimental

2.1 Reagents

Stock solutions of Pb(II) 1×10^{-4} mol L^{-1} were prepared by dilution of 100 µg mL^{-1} standard solution plasma-pure (Leeman Labs, Inc.).

Tris-(hydroxymethyl)-aminomethane (Mallinckrodt Chemical Works, St Louis, USA – 0.01 mol L^{-1}), potassium dihydrophosphate (0.01 mol L^{-1} - Biopack, Buenos Aires, Argentina) sodium tetraborate (Merck & Co., Inc. – 0.01 mol L^{-1}), potassium phthalate acid (Merck & Co., Inc. - 0.01 mol L^{-1}) and acetic acid/acetate (0.01 mol L^{-1}- Mallinckrodt Chemical Works) buffer solutions were prepared. This solution was

adjusted to the desired pH, with aqueous HCl (Merck, Darmstadt, Germany) or NaOH (Mallinckrodt Chemical Works) using a pHmeter (Orion Expandable Ion Analyzer, Orion Research, Cambridge, MA, USA) Model EA 940.

Stock of 8-hydroxyquinoleine solution 1×10^{-6} mol L^{-1} (8-HQ, H.E – Daniel Ltd., England) and o-phenanthroline 1×10^{-6} mol L^{-1} (o-phen, Merck, Darmstadt, Germany) were weekly prepared by dissolution of the appropriate amount in Millie-Q ultrapure water. The stability of solutions were checked by spectrophotometric measurements.

All solutions were prepared with ultrapure water (18MΩ cm^{-1}) obtained from a Milli-Q EASY pure RF (Barnsted, IA, USA). All used reagent were analytical grade.

2.2 Apparatus

Shimadzu RF-5301 PC spectrofluorometer (Shimadzu Corporation Analytical Instrument Division, Kyoto, Japan) equipped with a 150W Xenon lamp and solid sample holder with a GF-UV35 filter were used.

Adjustments of pH were carried out using Orion Expandable Ion Analyzer pH-meter (Orion Research, MA, USA) Model EA 940 with a combined glass electrode.

An Inductively Coupled Plasma Mass Spectrometer, Perkin Elmer 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 PC3 system from ESI (Omaha, NE, USA) (Table 3). Tygon black/black 0.76 mm i.d. and 40 cm length peristaltic pump tubing was used. Auto lens mode on,

peak hopping measure, dwell time of 50 ms, 15 sweeps/reading, 1 reading/replicate, and 6 replicates were used as instrument conditions [26].

2.3 Sampling procedure and samples treatments

2.3.1 Refill solutions for e-cigarettes

Different types of refill liquids purchased via internet with distinct levels of nicotine were studied. Samples were produced no more than three months before tests took place and were stored in a cool and dark place throughout the test duration.

The refill liquids examined were:

• Tobacco USA Mix (18 mg Nicotine).

• Cappuccino (12 mg Nicotine).

• Ice Mint (0 mg Nicotine).

• Tobacco Whinston (11 mg Nicotine).

• Vanilla (3 mg Nicotine).

• Butter Toffee (0 mg Nicotine).

• Tobacco Camell (18 mg Nicotine).

• Citrus Mix (11 mg Nicotine).

• Cherry (6 mg Nicotine).

• Tobacco USA Mix Marlboro (24 mg Nicotine).

Pb(II) traces present in each sample were determined after simple dilution.

2.3.2 Water pipe tobaccos

Snuff used in narghile or hookah (molasses) were acquired in drugstores of San Luis city (Argentine).

From recently opened packages 0.6 g of molasses (flavors: cola, fresh mint, chocolate, cherry, apple, orange, Arabic coffee and bubble gum) were weighed and each portion was put in contact with in individual leaching solution of acetic acid 1×10^{-3} mol L^{-1} and mechanically shaken during 5 minutes. Supernatants were separated by decantation and the General Procedure was applied to an adequate aliquot of each leachate solution.

2.3.3 Snus

Swedish snus were purchased via internet. The individual packages (flavors: original, fresh mint and chocolate) were opened and homogenized. Weighed portions (0.25 g) of each flavor were placed in individual glass vessels, put in contact with leaching solution of acetic acid 1×10^{-3} mol L^{-1} and shaken during 5 minutes. Supernatants were separated and the General Procedure was applied to an aliquot of each leachate solution.

2.3.4 Dry Rape

Dry rape were acquired in drugstores of San Luis city (Argentine). The individual packages (flavors: chocolate, menthol and anise) were opened and 0.25 g of each flavor was weighed. Each portion was put in individual capsules and a volume of leaching solution (1×10^{-3} mol L^{-1}) was added and manually shaken during 5 minutes. Supernatants were separated and the General Procedure was applied to an aliquot of each leachate solution.

2.3.5 Cigarettes

Commercial cigarettes from different brands were acquired in drugstores of San Luis city (Argentine). Cigarettes from recently opened packages were individually weighed and tobacco was extracted. Afterwards, tobacco was homogenized and 0.6 g of each cigarette was put in individual capsules and a volume of leaching solutions (acetic acid 1×10^{-3} mol L^{-1}) was added and manually shaken during 5 minutes. The General Procedure was applied to an aliquot of each leachate solution.

2.3.6 Fine-cut tobacco

The individual packages of tobacco to assemble (flavors: vanilla, cherry, chocolate and traditional tobacco from two brands) were opened and 0.5 g of each flavor was weighed. Each aliquot was put in individual capsules and a volume of leaching solution (acetic acid 1×10^{-3} mol L^{-1}) and manually shaken during 5 minutes. Supernatants were separated and the General Procedure was applied to an aliquot of each leachate solution.

2.4 General procedure

An adequate aliquot of leachate of tobacco products/diluted refill solutions for e-cigarettes samples (25 µL – 75 µL), Pb(II) aliquot (0, 1.85, 3.91 and 5.97 µg L^{-1}), 100 µL buffer phosphate 1×10^{-3} mol L^{-1} (pH = 6.0), 500 µL o-phen (1×10^{-7} mol L^{-1}), 750 µL 8-HQ (1×10^{-7} mol L^{-1}) were placed in a 10 mL volumetric glass flask. The whole mixture was diluted to 10 mL with Millie-Q ultrapure water. Analyte concentration was determined by fluorescent emission at 305 nm using $\lambda_{exc} = 250$ nm, employed a conventional quartz cell.

2.5 Accuracy study

Adequate volume of leachate of tobacco products / diluted refill solutions for e-cigarettes samples were spiked with increasing amounts of Pb(II) (1.85, 3.91 and 5.97 µg L^{-1}). Pb(II) concentrations were determined by the proposed methodology.

2.6 Precision study

The repeatability (within-day precision) of the method was tested for replicate of samples (n = 6) spiked with 3.91 µg L^{-1} of Pb(II) and the contents were determined by proposed methodology.

2.6 Trueness

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

3.   Results and discussion

3.1. Fluorescent Spectra

Previous investigations have shown the feasibility of the formation of ternary complexes between metal ions and different chelating reagents, with its subsequent determination by spectrophotometry or molecular fluorescence [27-29]. Initially, the fluorescence of 8-HQ/Pb (II) and o-phen/Pb (II) systems in aqueous medium were separately explored with promising results for both. However, an improvement in the sensitivity of the method was sought, for which assays were carried out with the objective of evaluating the behavior of the formation of a ternary complex. Effect of 8-HQ addition on o-phen/Pb(II) system was researched. The ternary association formed in aqueous solution showed good sensitivity and stability over time evidencing a quenching effect when the concentration of Pb(II) increased (Fig. 1).

3.2 Optimization of Experimental Conditions

In order to find the optimum experimental conditions for Pb(II)/8-HQ/o-phen association formation, experimental parameters such as pH, nature and concentration of buffer solution, concentrations of chelating reagents, solvent, that affecting the complexation reaction were consecutively investigated.

The first parameter examined was the pH; this parameter plays a unique role on metal-chelate formation and the subsequent metal determination. The results illustrated in **Figure 2**, showed that at pH above 6.0 a maximum quenching effect was obtained. Due to this behavior, the pH value of 6.0 was selected as working value for the following experiences.

The effect of different buffers on the quenching effect fluorescence of Pb(II)/8-HQ/o-phen ternary system was tested. Between the assayed buffer solutions, the best effect quenching fluorescent was obtained using phosphate buffer. The fluorescence response was studied within potassium dihydrophosphate concentration range from 1×10^{-5} to 1×10^{-2} mol L^{-1}. The best performance and optimal stability in the time was achieved for a buffer concentration 1.0×10^{-4} mol L^{-1}.

The concentration effect of 8-HQ and the o-phen chelating reagents on the quantification of Pb(II) were subsequently studied and the results are presented in Figure 3. With these results, a 8-HQ concentration of 7.5×10^{-8} mol L^{-1} and o-phen of 5×10^{-8} mol L^{-1} were chosen as optimal for following assays (**Figure 3**).

3.3 Analytical parameters

3.3.1 Calibration curve and analytical figures of merit

Calibration plots for concentration levels of Pb(II) from 1.21 µg L^{-1} to 518 µg L^{-1} was assayed applying the developed methodology. **Table 1** summarizes the main

characteristics of the calibration plot and optimized experimental conditions, which sustain the proposed procedure for quantification of Pb(II) traces.

The limits of detection (LOD) and quantification (LOQ) were calculated in accordance to the formulas given by the official compendia methods [30], using the relation k(SD)/m where k = 3.3 for LOD and 10 for LOQ. SD represents the standard deviation from 15 replicate blank responses and m is the slope of the calibration curve.

The range of linearity was evaluated by checking the linear regression coefficient (R^2) of the calibration curve. The linearity of the calibration curve was considered acceptable when $R^2 > 0.9997$.

Since no standard or reference material were available by us, the trueness of the proposed methodology was assessed through spiked-recovery tests and by comparison with another independent technique such as ICP-MS.

The reproducibility of the method was evaluated repeating 6 times the proposed approach for each addition in a total of three level of spiked Pb(II) for each sample. The recoveries of Pb(II) and validation with ICP-MS based on the average of three replicate measurements are illustrated in **Table 2.** Obtained results showed satisfactory agreement with adequate precision (%CV).

3.3.2 Interferences study

The effect of foreign common ions on the recovery of Pb(II) was tested. An ion was considered as interferent when it caused a variation in the signal fluorescent of the analyte greater than ±5%. The assayed ions for interferences study were selected considering nature of the samples analyzed. For seven ions studied (Cl., NO_3^-, Na^+ Ca^{2+}, Mg^{2+}, Mn^{2+} and K^{+1}) an excellent tolerance was obtained for interferent/Pb(II) 1000/1 ratio, at optimal working conditions. The most part of cations and anions assayed (CO_3^{2-}

, SO_4^{2-}, Cd^{2+}, Ni^{2+}, Hg^+, Sb^{3+}, Fe^{3+}, Tl^+, Cr^{3+}, Al^{3+}, Cu^{2+} and Co^{2+}) were good tolered at interferent/Pb(III) 100/1 ratio.

3.4 Applications

To establish the applicability of proposed method, it was applied to Pb(II) quantification in samples of different type of tobacco consumption. The results obtained showed an adequate precision (see **Table 2**).

Robustness of the assay was evaluated by repeating the analysis six times for each sample. **Table 2** shows the recovery results achieved. The results obtained indicate that the proposed method is suitable for Pb(II) determination in all studied samples. As there were not available Certified Materials, in order to check the accuracy of the proposed method, a comparative analysis by ICP-MS was carried out using the conditions cited in **Table 3**. Results obtained for replicate samples (n = 6) with proposed method and ICP-MS technique were statistically compared (t-test) and no significant differences (p = 0.05, DF = 3) were observed.

Additionally, it is important to realize a comparation of the concentrations of lead found by other researchers [31-37] and those reported in this paper. In all studied samples, Pb(II) contents were in the same order as those informed by other researchers, showing few significative variations. This fact put in evidence, one again, that the consumption of that all forms of tobacco use (with or without smoke) represent a severe risk to health.

4. Conclusions

Atomic spectroscopies represent the most important techniques for metal trace analysis, but they require specialized and very expensive instruments. The development

of new simple and rapid analytical methods for Pb(II) traces determination continues being an actual topic due to toxicological relevance of metal. The proposed methodology symbolizes a promising approach for Pb(II) monitoring with low operation cost, simplicity of instrumentation and non-polluting solvents. Samples of tobacco products with and without smoke marketed in Argentina were successfully analyzed with an average recovery close to 100%. The method was validated by ICP-MS technique and it's applied at real samples with good tolerance to regular foreign tobacco constituents. Under the light of this and other studies in this field, the high concentrations of lead in tobacco products found and the subsequent risks on health deeper investigation is necessary into the characteristics of the healthy tobacco products and their regulation in benefit of consumers. We considered that only with trustworthy information, tomorrow, they could take the correct decision with respect to smoking habit.

Acknowledgements

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Figure captions

Figure 1: Fluorescence spectra of Pb(II)/8-HQ/o-phen systems.

Figure 2: Influence of pH on Pb(II)/8-HQ/o-phen.

Figure 3: Effect of 8-HQ and o-phen concentrations on the Pb(II)/8-HQ/o-phen

fluorescent signal.

Table 1: Optimal experimental conditions and analytical parameters for Pb(II)/8-HQ/o-phen determination.

Parameters	Studied range	Optimal conditions
pH	5-12	6
Concentration buffer phosphate	1×10^{-5}- 1×10^{-2} mol L^{-1}	1×10^{-4} mol L^{-1}
Concentration 8-HQ	1×10^{-9}- 1×10^{-6} mol L^{-1}	7.5×10^{-8} mol L^{-1}
Concentration o-phen	1×10^{-9}- 1×10^{-6} mol L^{-1}	5×10^{-8} mol L^{-1}
LOD	-	0.42 µg L^{-1}
LOL	-	$1.27 - 518$ µg L^{-1}
R^2	-	0.9997

Table 2: Recovery studies by lead determination in refill solutions for e-cigarettes, tobacco of conventional cigarettes, tobacco to make cigarettes, snuff used in WP or narghile (molasses), snus and dry rape.

Sample	Pb(II) added ($\mu g\ L^{-1}$)	Proposed methodology		ICP-MS	%RE*
		Pb(II) found ±CV ($\mu g\ L^{-1}$)	Recovery (%, n=6)	Pb(II) found ±SD ($\mu g\ L^{-1}$)	
1	- 1.85 3.91 5.97	0.53±0.04 2.37±0.03 4.46±0.06 6.51 ±0.02	- 98.11 103.77 101.89	–	-
2	- 1.85 3.91 5.97	0.57 ±0.02 2.45±0.05 4.46±0.09 6.53±0.01	- 101.75 96.49 98.25	0.55±0.01	3.51
3	- 1.85 3.91 5.97	1.26 ± 0.01 3.09± 0.04 5.14± 0.03 7.22 ±0.02	- 98.41 97.62 99.21	1.30±0.02	3.07
4	- 1.85 3.91 5.97	2.57±0.08 4.45±0.03 6.49±0.07 8.52±0.04	- 101.17 100.39 99.22	2.55±0.04	0.78
5	- 1.85 3.91 5.97	1.77 ±0.02 3.64±0.04 5.67±0.08 7.76±0.07	- 101.13 99.43 101.13	1.73±0.001	2.26
6	- 1.85 3.91 5.97	2.08 ±0.03 3.95±0.04 5.97±0.02 8.07±0.07	- 100.96 99.04 100.96	2.15±0.04	3.26

7	- 1.85 3.91 5.97	3.12±0.01 4.97±0.06 7.02±0.02 9.12±0.04	- 100.00 99.68 100.96	3.02±0.05	3.20
8	- 1.85 3.91 5.97	2.76±0.01 4.59±0.04 6.64±0.06 8.75±0.02	- 99.27 98.91 100.72	-	-
9	- 1.85 3.91 5.97	0.97±0.02 2.84±0.04 4.86±0.05 6.91±0.05	- 102.06 97.94 96.91	0.95±0.02	2.06
10	- 1.85 3.91 5.97	1.25±0.03 3.07±0.07 5.20±0.08 7.20±0.02	- 97.60 103.2 98.40	1.22±0.02	2.40
11	- 1.85 3.91 5.97	2.88±0.02 4.76±0.03 6.76±0.07 8.84±0.01	- 101.04 98.96 99.65	2.85±0.002	1.04
12	- 1.85 3.91 5.97	2.49±0.07 4.31±0.02 6.36±0.01 8.47±0.03	- 98.79 98.39 100.40	2.75±0.001	9.45
13	- 1.85 3.91 5.97	1.89± 0.08 3.72±0.04 5.82±0.01 7.85 ±0.07	- 98.94 102.65 99.47	2.05±0.004	7.80
14	- 1.85 3.91 5.97	3.14±0.06 4.97±0.01 7.07±0.03 9.10±0.04	- 102.55 100.64 99.68	3.24±0.002	3.08
15	- 1.85 3.91 5.97	3.44±0.07 5.25 ±0.02 7.33 ±0.01 9.45±0.06	- 98.84 99.42 101.16	3.74±0.001	8.02
16	- 1.85 3.91 5.97	1.33 ±0.01 3.20 ±0.02 5.25±0.03 7.32±0.06	- 101.50 100.75 101.50	–	-
17	- 1.85 3.91 5.97	3.41±0.05 5.28±0.04 7.30±0.07 9.35±0.02	- 100.58 99.41 99.12	3.45±0.02	1.16

18	- 1.85 3.91 5.97	2.74±0.03 4.61±0.04 6.66±0.06 8.82±0.05	- 100.73 100.37 104.01	-	-
19	- 1.85 3.91 5.97	1.23±0.03 3.10±0.06 5.17±0.05 7.16±0.02	- 101.63 102.44 96.75	1.30±0.07	5.38
20	- 1.85 3.91 5.97	2.91±0.01 4.77±0.04 6.80±0.01 8.87±0.02	- 100.34 99.31 99.65	2.75±0.01	
21	- 1.85 3.91 5.97	3.39±0.01 5.25±0.05 7.32±0.02 9.39±0.08	- 100.29 100.59 10.88	-	-
22	- 1.85 3.91 5.97	2.69±0.05 4.55±0.05 6.63±0.02 8.64±0.04	- 100.37 101.15 99.25	2.91±0.01	
23	- 1.85 3.91 5.97	2.88±0.04 4.75±0.07 6.77±0.05 8.86±0.04	- 100.70 99.30 100.35	2.94±0.01	2.04
24	- 1.85 3.91 5.97	1.05±0.02 2.92±0.04 4.99±0.02 7.01 ±0.07	- 101.90 102.86 99.05	1.10±0.02	4.54
25	- 1.85 3.91 5.97	1.75 ±0.09 3.58±0.06 5.68±0.04 7.70±0.01	- 98.86 101.15 98.86	1.81±0.09	3.51
26	- 1.85 3.91 5.97	2.64±0.03 4.50±0.07 6.53±0.03 8.60±0.01	- 100.38 99.24 99.62	–	-
27	- 1.85 3.91 5.97	2.08±0.08 3.95±0.01 6.02±0.01 8.02±0.04	- 100.96 101.44 98.55	2.15±0.02	3.25
28	- 1.85 3.91 5.97	3.77±0.06 5.60±0.04 7.66±0.02 9.76±0.09	- 99.47 99.46 100.53	-	-

29	- 1.85 3.91 5.97	0.63±0.02 2.50±0.06 4.52±0.02 6.59 ±0.01	- 103.17 96.83 98.41	-	-
30	- 1.85 3.91 5.97	1.76±0.06 3.60±0.04 5.65±0.03 7.73±0.03	- 99.43 98.86 100.00	1.85±0.01	4.86
31	- 1.85 3.91 5.97	0.95 ± 0.07 2.77± 0.03 4.88± 0.08 6.90 ±0.01	- 96.84 102.10 97.89	1.01±0.06	5.94
32	- 1.85 3.91 5.97	2.64±0.05 4.47± 0.02 6.54± 0.03 8.63 ±0.04	- 99.24 99.62 100.75	2.60±0.04	1.51
33	- 1.85 3.91 5.97	1.47 ±0.04 3.33± 0.03 5.37± 0.02 7.41 ±0.03	- 100.68 99.32 97.95	-	-
34	- 1.85 3.91 5.97	2.02 ±0.01 3.85± 0.05 5.96±0.07 8.01±0.01	- 99.00 101.48 100.99	2.15±0.01	6.05

*%RE= 100 × (|measured value − actual value|)/actual value.

Refill solutions for e-cigarettes 1: Vanilla (3 mg Nicotine); **2:** Tobacco Camell (18 mg Nicotine); **3**: Ice Mint (0 mg Nicotine);**4**: Tobacco Whinston (11 mg Nicotine); **5**: Tobacco USA Mix (18 mg Nicotine):**6**: Butter Toffee (0 mg Nicotine);**7**: Cappuccino (12 mg Nicotine); **8**: Citrus Mix (11 mg Nicotine);**9**: Cherry (6 mg Nicotine);**10**: Tobacco USA Mix Marlboro (24 mg Nicotine).

Traditional tobacco of commercial cigarettes: 11-Tobacco blond (Tobacalera Massalin Particulares); 1**2-**Tobacco blond (Tobacalera Massalin Particulares); 1**3-** Black tobacco (Tobacalera Nobleza Piccardo); 1**4-**Tobacco blond-convertible (Tobacalera Massalin Particulares); 1**5**- Convertible blond tobacco (Tobacalera Nobleza Piccardo). **Tobacco to assemble cigarettes: 16-** Flavor vanilla; 1**7-**Flavor cherry; 1**8-** Flavor chocolate; 1**9-** Traditional tobacco Brand 1; **20-** Traditional tobacco Brand 2.

Molasses: Flavors: **21:** Cola; 2**2:** Chocolate; 23: Fresh mint; 2**4:** Cherry**; 25**: Apple; 2**6**: Orange; 2**7**: Arabic coffee; 2**8:** Bubblegum.

<u>**Snus**</u>**:** flavors: **29:** Original; **30:** Fresh mint; **31:** Chocolate. **Dry rape:** flavors: **32:** Chocolate; **33:** Menthol; **34:** Anise.

Table 3: Instrument settings and data acquisition parameters for ICP-MS

Sample uptake rate (μL min^{-1})	200
Sample introduction	Nebulizer model PFA-ST, coupled to a quartz cyclonic spray chamber with internal baffle and drain line, cooled with the PC^3 system from ESI (Omaha, NE, USA)
RF power (W)	1200
Nebulizer Gas flow rates (mL min^{-1})	0.77
Interface	Ni cones (sampler and skimmer)
Isotope	^{208}Pb
Scanning mode	Peak hopping
Dwell time (ms)	50 in standard mode
Number of replicate	6

Highlights

- New methodology for lead traces determination does not require a separation / preconcentration stage.

- It is an important contribution at green chemistry.

- This methodology showing good reproducibility with low operation cost.

- The trueness of the recommended procedure was assessed by ICP-MS.

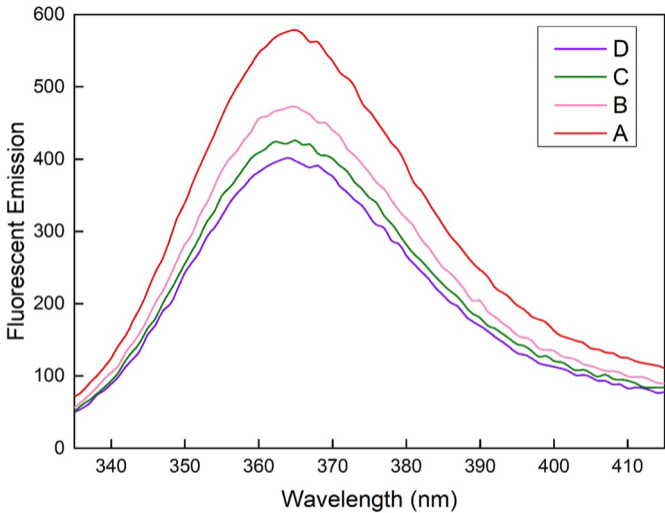- It was applied to in leachate of tobacco products with and without smoke and refill solutions for e-cigarettes.
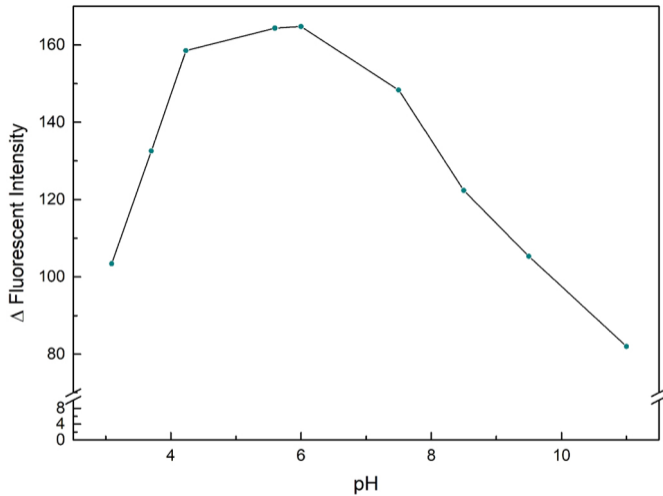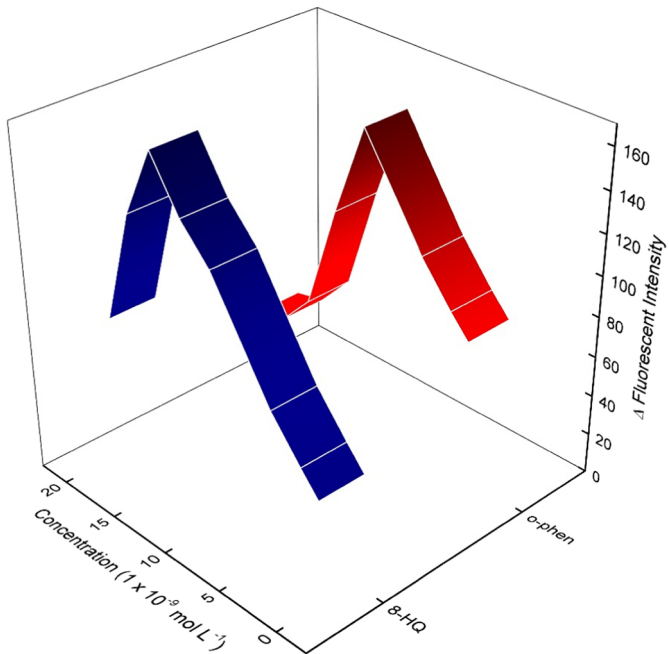
Figure 1

Figure 2

Figure 3