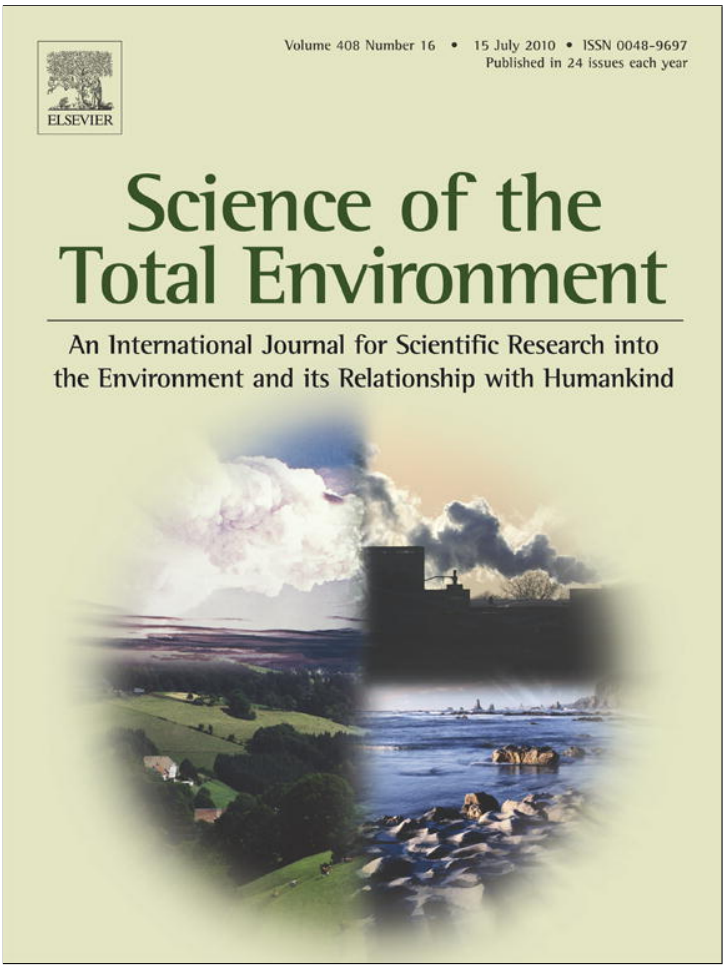

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Cadmium monitoring in saliva and urine as indicator of smoking addiction

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ABSTRACT

Cadmium is one of the many substances that may be acquired through active and passive smoking of tobacco. Saliva and urine are proposed for cadmium monitoring of non-smokers, second hand smokers, smokers and tobacco chewing appertaining to San Luis citizens without occupational exposition. Biological samples were collected by the same subjects, under strict proceeding instructions of sampling. Physical characteristics of samples were observed and checked with commercial test. Samples were analyzed using an adapted molecular fluorescence methodology with a previous extraction step. Stability of biological samples was daily studied for a period of one month. The method was successfully validated for accuracy, precision, linearity, specificity, and sensitivity. The simplicity and low coefficient of variance confirm the suitability of the method for urinary and salivary cadmium analyses. On the other side, the obtained results are in concordance with previous national epidemiological dates.

1. Introduction

Smoking habit represents the main cause of human disease and death. Every year, 5.4 million smokers die and, if precautionary measures are not taken, it is expected that this number will increase more than 8 million in 2020. In Argentina, tabaquism has the highest level in South America causing 40,000 deaths/year. Today, in San Luis, 33.4% of adults are smokers (Encuesta Nacional de Factores de Riesgo, 2006) and the beginning age is between 12 and 13 years old, with a similar consumption to adults (Table 1, Fig. 1). An additional risk is that tobacco addiction can act as an open door to other drugs such as cocaine, heroin, etc.

Actually, with the objective of providing young people in scholar age the opportunity of knowing the effects of tobacco on health, we are realizing several activities for raising them awareness based on seminars, practices and answers/questions. Figs. 2 and 3 show partial results of these conscientization activities and evidence the presence of tabaquism addiction in this social group. We consider that only with trustworthy information, tomorrow, they could take the correct decision with respect to smoking habit.

Passive smokers or second hand smoke (SHS) exposure (Table 2) is an associated problem to tobacco addiction; fortunately some cities have regulations to protect non-smokers in working and public places; however, 55% of young people are exposed in their own homes.

There are numerous harmful substances in tobacco; several toxic metals are found among these substances and may be acquired through active and passive smoking. Cadmium is not regarded as essential to human life; otherwise, cadmium is now known to be extremely toxic and accumulates in humans mainly in the kidneys for a relatively long time, from 20 to 30 years (Ramirez, 2002). Cigarettes are especially dangerous because cadmium is efficiently absorbed when inhaled; cadmium oxide generated during the burning of cigarettes is highly bioavailable (Arain et al., 2008). Approximately, 10% of the inhaled cadmium oxide is deposited in lung tissues, and another 30–40% is absorbed into the systemic blood circulation of smokers. Cadmium levels in the blood of smokers are 4–5 times higher than in non-smokers and 2–3 times greater than in their kidneys (Satarug and Moore, 2004). There are elevated serum cadmium levels in smokers resulting in glomerular dysfunction (Cooper, 2006).

Heavy metals are frequently determined by atomic techniques such as atomic absorption spectrometry, inductively coupled plasma (ICP)-atomic emission spectrometry and ICP-mass spectrometry (Anthemidis et al., 2004; Cerutti et al., 2003; Coelho and Arruda, 2005; Manzoori and Karim-Nezhad, 2004; Linge, 2007) at ppb levels. Particularly, cadmium determination in biological and environmental samples at ultra-trace levels, has been supported by ET-AAS with in-atomizer trapping (Lampugnani et al., 2003). Recently, a new methodology for determination of cadmium at ultra-trace levels has been developed (Talio et al., 2009). The analytical advantages with respect to the application of molecular fluorescence to cadmium determination are associated to high sensitivity, proper selectivity and wide dynamic range. On the other side, the separative step (CPE,

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Table 1
Number of daily smoked cigarette according to age and sex groups (San Luis, Argentina). Source: Encuesta Nacional de Factores de Riesgo, 2006.

Adult age group	Sex		Average smoked cigarettes
	Women	Men	
18–24	8.3	10.2	9.6
25–34	12.2	13.3	12.7
35–49	10.7	19.1	15.3
50–64	11.9	17.3	14.4
More of 65	24.9	13.2	17.1

cloud point extraction) gives high efficacy of extraction, operative simplicity and safety due to no exposure to toxic solvents when it is compared to other traditional extraction procedures (Moreda Pineiro et al., 2001).

Non-invasive sampling procedures are very attractive options for pediatric and aged patients. It has been demonstrated that salivary and urinary contents reflect levels of biomarkers (Esteban and Castaño, 2009; Aps and Martens, 2005; Van Nieuw Amerongen et al., 2004; Soo-Quee and Choon-Huat, 2007; Phillip and Bentley, 2001; Esteban and Castaño, 2009); particularly, saliva samples have been used to substitute blood samples or as additional tool in diagnosis of certain diseases. Salivary monitoring has been used to explore environmental pollutants (Thaweboon et al., 2005; Luconi et al., 2006; Luconi et al., 2001).

The aim of the present research is to bring an actual look in the state of tobacco addiction using urinary and salivary cadmium contents as indicators of exposition. With this purpose, saliva and urine samples of smoker, second hand smoker, non-smoker and tobacco chewing subjects belonging to San Luis were analyzed using an adapted molecular fluorescence methodology. Taking into account the biologic nature of samples, specific experimental variables related to stability and dilution test as well as the proper sampling procedure, between others must be studied and optimized.

2. Experimental

2.1. Reagents

Urine samples were tested using Urine Strip-Wiener lab. (Rosario, Argentina).

$1 \cdot 10^{-6}$ mol L^{-1} Cd(II) stock solutions were prepared by dilution of 100 μg mL^{-1} standard solution plasma-pure (Leeman Labs, Inc.).

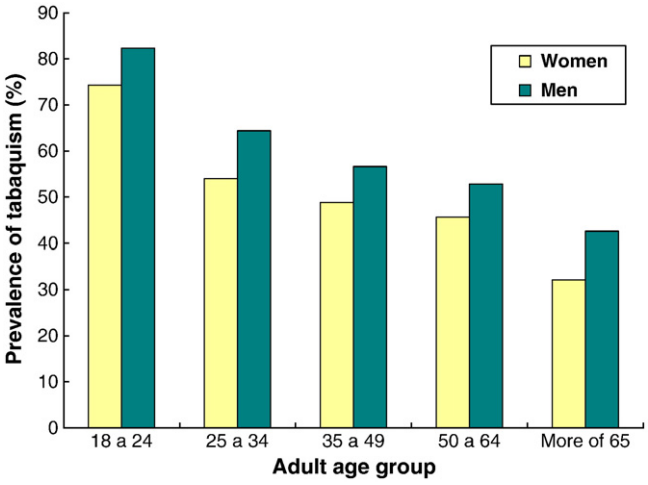


Fig. 1. Prevalence of tabaquism (%), according to age and sex groups in San Luis. (Encuesta Nacional de Factores de Riesgo. Sección TABACO. Ministerio de Salud de la Nación, Argentina 2006. www.msal.gov.ar/tabaco).

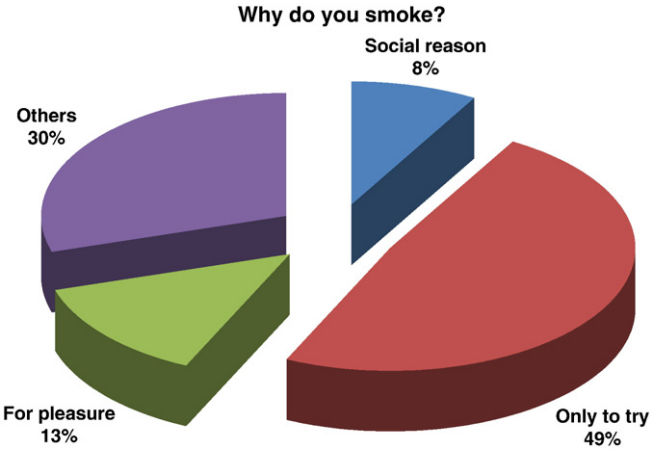


Fig. 2. Reasons invoked by young people in scholar age. Data obtained from our activities in San Luis city schools, March–April 2009.

Extractant solution of surfactant PONPE 7.5 (polyethyleneglycol-mono-*p*-nonylphenylether, Tokyo Kasei Industries, Chuo-Ku, Tokyo, Japan) was prepared as it is indicated in Talio et al. (2009).

$1 \cdot 10^{-2}$ mol L^{-1} Tris (Mallinckrodt Chemical Works, New York, Los Angeles, St. Louis, USA) solution was prepared. This solution was adjusted to the desired pH, with aqueous $HClO_4$ (Merck, Darmstadt, Germany) or NaOH (Mallinckrodt Chemical Works, New York, Los Angeles, St. Louis, USA).

$1 \cdot 10^{-6}$ mol L^{-1} eosin (eo, sodium bromofluorescein, $C_{20}H_6O_5Br_4Na_2$) stock solution (H.E — Daniel Ltd., England) and a $1 \cdot 10^{-5}$ mol L^{-1} o-phenanthroline (o-phen, 1,10-phenanthroline, $C_{12}H_8N_2$, Merck, Darmstadt, Germany) were weekly prepared by dissolution of the appropriate amount in ultrapure water.

All used reagents were of analytical grade.

2.2. Apparatus

Fluorescence measurements were made using a Shimadzu RF-5301 PC spectrofluorometer equipped with a 150 W Xenon lamp and 1.00 cm quartz cells. Instrument excitation and emission slits were adjusted to 3 nm.

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.

A centrifuge was used in biological sample processing to accelerate the phases separation process.

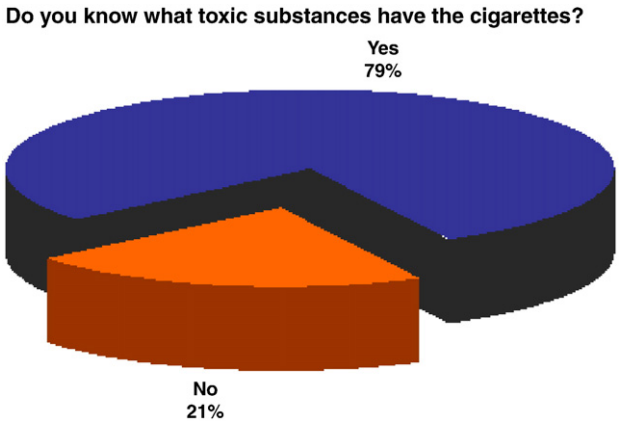


Fig. 3. Knowledge of cigarette toxins. Data obtained from our activities in San Luis city schools, March–April 2009.

Table 2
Percentage of subjects exposed to SHS according to age and sex groups (San Luis, Argentina). Source: Encuesta Nacional de Factores de Riesgo, 2006.

Adult age group	Sex		(%) Mean
	(%) Women	(%) Men	
18–24	74.3	82.3	78.5
25–34	54.0	64.4	58.9
35–49	48.8	56.6	52.7
50–64	45.6	52.8	48.6
More of 65	34.0	42.6	58.8

Table 3
Characteristics of studied subjects attending to smoking habit.

Group	Subjects	Smoked cigarettes/days
1	8	0
2	7	SHS
3	6	5
4	7	10
5	6	20
6	9	40
7	2	TChH

Urinary sediments were typified using Eclipse E200 Microscopy (Nikon Instruments, Inc.).

All used glass materials were previously washed with a 10% v/v HNO_3 water solution and then with ultrapure water.

2.3. Biological sample collection

Studied subjects were interrogated using a written test, in order to obtain information related to smoking habit (frequency, time of addiction, etc.), age, sex, occupational situation, etc.

Saliva of seven subjects and first morning urine samples of forty subjects were collected from occupationally unexposed subjects. Biological samples were collected, between 8 and 10 h to reduce possible circadian contributions, into cadmium-free polystyrene test tube. Samples were centrifuged for 10 min at 1000 *g* and processed immediately after arriving to the laboratory. The obtained samples (10 mL approximately for each sample) were centrifuged for 10 min; the sediments (2 mL approximately for each sample) were discarded. The supernatants (5 mL approximately) were reserved.

With the purpose of assuring to obtain representative samples, subjects received detailed information about the collection protocol.

Saliva specimen: in order to minimize the possibility of contamination with food debris and airborne particles, the subjects were instructed to thoroughly rinse their mouths three times, first with 1.5% citric acid solution (a salivation stimulant) and then twice with ultrapure water. After 10 min, the collection was started. Subsequently, saliva was allowed to accumulate on the floor of the mouth and the subjects were instructed to spit into a test tube. Each saliva collection period was 20 min. Subjects were also informed about the procedures for storing samples, till arriving to laboratory.

Urine specimen: in order to prevent subsequent interferences, subjects were instructed as follows:

1) Do not take vitamins or aggregated minerals 36 h before urine collection.
2) Do not drink tap water during 24 h previous to sample collection.
3) The first-void urine is preferred as sample and only the medium fraction is collected.
4) Samples must be directly remitted to laboratory for analysis; if it is not possible, they must be preserved at 4 °C until analysis.

2.4. Protection of human subjects

Written informed consents were obtained from all participants.

2.5. Physical characterization and semi-quantitative determination of clinical parameters in biological samples

Biological samples were physically characterized, namely colour, odor and appearance, presence of sediment, blood and mucus, in order to establish variables that could affect the obtained results.

Urine samples were tested using commercial reagent strips and sediments were observed using an optical microscopy.

2.6. Dilution test

5 mL of each biological sample was taken and dilutions were carried out to obtain dilution factors of 1/2, 1/4, 1/8, 1/16 and 1/20.

2.7. Proposed methodology

Adequated aliquots of saliva/urine/standard solution ($2.80\cdot10^{-3}$–2.80 μg L^{-1}), 100 μL buffer Tris solution $1\cdot10^{-2}$ mol L^{-1} (pH = 7.6), 250 μL o-phen ($1\cdot10^{-5}$ mol L^{-1}), 500 μL eo ($1\cdot10^{-6}$ mol L^{-1}) and 500 μL of extractant solution, were placed in a 10 mL graduated centrifuge tube. The whole mixture was diluted to 10 mL with ultrapure water. The system was kept at 40 °C for 15 min in thermostatic bath for equilibration and then centrifuged for 5 min at 3500 rpm at approximately 1000 *g*. After being cooled at −18 °C during 5 min the surfactant rich phase became a viscous gel and the aqueous phase could be poured off (Fig. 4). The surfactant phase in the

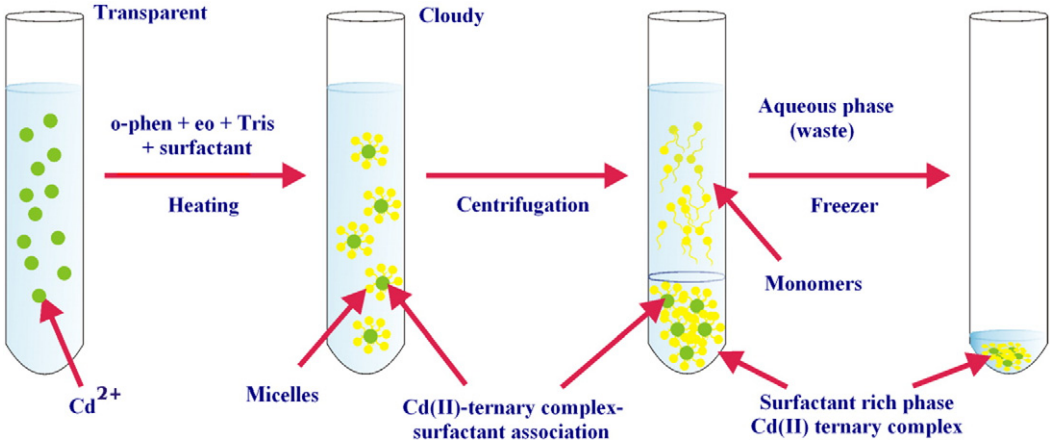


Fig. 4. Schematic representation of cadmium complex CPE.

Table 4
Stability test applied to saliva and urine samples of subject 1 (non-smokers group).

Time (h)	S–Cd ($\mu g L^{-1}$)	U–Cd ($\mu g L^{-1}$)	CV
0	0.45	0.55	0.01
24	0.45	0.56	0.03
48	0.46	0.55	0.03
72	0.44	0.57	0.03
168 (seven days)	0.45	0.54	0.04
360 (fifteen days)	0.47	0.57	0.04
720 (thirty days)	0.43	0.53	0.09

tube was then made up to 3 mL by adding 300 µL of buffer Tris pH 7.6, 1 mL of absolute ethanol and ultrapure water. Fluorescent emission was measured at $\lambda_{em} = 545$ nm using $\lambda_{exc} = 525$ nm.

2.8. Interferences study

Different amounts of common cations were added to the test solution containing 1.70 µg L^{-1} of Cd(II) and the proposed methodology was applied.

2.9. Accuracy study

5 mL of biological samples was spiked with increasing amounts of Cd(II) (0.34–4.50 µg L^{-1}). Cadmium contents were determined by proposed methodology.

2.10. Precision study

The repeatability (within-day precision) of the method was evaluated preparing saliva and urine replicate samples ($n = 6$) containing 0.45 µg L^{-1} and 0.547 µg L^{-1} of cadmium, respectively, and cadmium contents were determined by proposed methodology.

2.11. Recovery procedure

5 mL saliva/urine was spiked with increasing amounts of Cd(II) (0.34–4.50 µg L^{-1}) and treated following proposed methodology.

2.12. Stability test of biological samples

5 mL of biological samples coming from subject 1 (non-smokers group) was spiked with increasing amounts of Cd(II) (0.34–4.50 µg L^{-1}). Metal contents were determined by proposed methodology at different times (1 day, 5 days, 1 week, 2 weeks, and 1 month) using preservation in a refrigerator at 4 °C. None stabilizer was added by the risk of incorporating analyte as impurity, attending to bibliographic recommendation (Seiler et al., 1994).

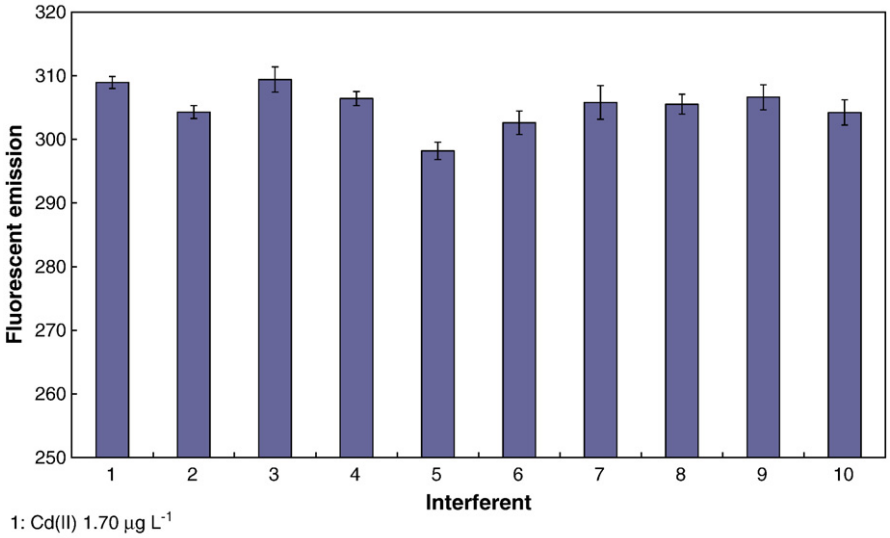
3. Results

Subjects with different addictions to tobacco were selected to evaluate S–Cd and U–Cd contents. Taking into account the most facility of obtaining urine samples, forty subjects were studied. Among them, only seven subjects with different addiction levels, were requested for saliva sampling. Attending to smoking habits, the studied subjects can be described as can be seen in Table 3.

3.1. Physical and chemical characterizations of biological samples

Once in the laboratory, biological samples were observed and characterized with respect to physical appearance (colour, odor and appearance, presence of sediment, and blood and mucus) in order to establish variables that could interfere in the determinations. All processed samples can be namely considered within the normal physical parameters.

At once, samples were centrifuged for 10 min at 1000 *g*. Supernatants were put into two polyethylene tubes and one portion of each sample was reserved for cadmium examination.



1: Cd(II) 1.70 µg L^{-1}
2: Cd(II) in presence of Bi(III)
3: Cd(II) in presence of Zn(II)
4: Cd(II) in presence of Cu(II)
5: Cd(II) in presence of Ca(II)
6: Cd(II) in presence of Cr(III)
7: Cd(II) in presence of Mg(II)
8: Cd(II) in presence of Ba(II)
9: Cd(II) in presence of Sr(II)
10: Cd(II) in presence of Fe(III)

Fig. 5. Tolerances of cations in 100/1 (interferent/Cd(II)) ratio ± %CVs (I) have been included for each interferent.

Urine samples of the other tubes were tested using commercial reagent strips and clinical parameters pH, urobilinogen, glucose, ketones, bilirubin, proteins, nitrite, blood, specific gravity and leucocytes were determined. Processed samples can be mainly considered within the normal clinical parameters.

Urine sediments were observed using optical microscope and classified with the intention of verifying the existence of some correlation between type of sediment and tobacco addiction level. The more frequent sediments were leukocytes, piocites, hematies, normal cells, mucus, bacteria, and crystals of different types (urates, uric acid and oxalates).

3.2. Stability studies of biological samples

Biological samples were spiked with increasing amounts of Cd(II) and were processed by the proposed methodology at different times, meanwhile they were preserved in a refrigerator at 4 °C. From the results (Table 4), it can be inferred that both biological samples have optimal stability for Cd(II) determination during thirty day study period.

3.3. Dilution test

In order to establish the proper volume of each sample for realize Cd(II) determination, different sample volumes were taken and diluted with ultrapure water. The adequate dilution for each sample was that which signal falls into the linearity range of the developed methodology.

3.4. Interferences study

The effect of foreign ions on the recovery of Cd(II) was tested. An ion was considered as interferent when it caused a variation in the fluorescent signal of the analyte greater than ±5%. In the optimal conditions, Li^+, Na^+, K^+ can be present at 10,000:1 excess to Cd(II) without interfere. Fig. 5 and Table 5 show the obtained tolerance results for a group of regular cations and anions, respectively.

3.5. Analytical performance and applications

The accuracy of the methodology was performed using the standard addition method. The reproducibility of the method was evaluated repeating the proposed approach, 6 times for each sample. The recoveries of Cd(II) in each type of sample, for one individual by group based on the average of replicated measurements, are illustrated in Tables 6 and 7. Table 8 presents the real concentrations of SCd and UCd for the same studied individual previously showed.

UCd and SCd contents showed a good correlation ($r^2 = 0.988$) for subjects identified as belonging to group 1, 2, 3, 4, 5, and 6 (Fig. 6). The obtained results for SCd and UCd can be good correlated with the total number of smoked cigarettes by each studied individual (Fig. 7), taking an average of ten years of tobacco addiction.

Table 5
Anions tolerance study for 1000/1 anion/Cd (II) ratio.

Anion	Fluorescent emission	%CV
CO_3^{2-}	313.20	1.52
SO_4^{2-}	313.84	0.95
NO_3^-	313.04	1.13
CH_3COO^-	311.40	0.87
Cl^-	306.64	1.24
Br^-	305.81	1.47
I^-	304.79	0.81
F^-	314.95	1.54

Table 6
Salivary cadmium. Recovery study for one subject by group.

Sample	Cd(II) added (μg L^{-1})	Cd(II) found ± CV (μg L^{-1})	Recovery (%, $n = 6$)
1[a]	–	0.45 ± 0.02	–
	1.70	2.15 ± 0.05	100.00
	3.35	3.80 ± 0.05	100.00
	4.50	4.88 ± 0.07	98.50
2[b]	–	0.83 ± 0.05	–
	1.10	1.97 ± 0.02	103.62
	2.25	3.06 ± 0.30	99.11
	3.35	4.30 ± 0.05	102.36
3[c]	–	1.80 ± 0.06	–
	1.10	2.90 ± 0.05	100.00
	3.35	5.10 ± 0.10	98.50
	4.50	6.22 ± 0.03	98.25
4[d]	–	1.10 ± 0.06	–
	1.10	2.22 ± 0.07	100.00
	1.70	2.82 ± 0.10	101.81
	2.25	3.45 ± 0.20	104.44
5[e]	–	1.67 ± 0.04	–
	0.55	2.22 ± 0.08	100.00
	1.10	2.80 ± 0.03	102.70
	2.25	3.94 ± 0.09	100.89
6[f]	–	1.18 ± 0.06	–
	1.70	2.96 ± 0.10	104.70
	2.25	3.53 ± 0.10	104.44
	3.35	4.54 ± 0.05	100.29
7[g]	–	1.41 ± 0.06	–
	1.10	2.53 ± 0.05	101.81
	1.70	3.15 ± 0.10	102.35
	2.25	3.70 ± 0.03	101.77

Sample volume (mL) a: 5; b: 3; c: 2.5; d: 1:5; e: 1; f: 0.5; and g: 0.25.
n = Number of replicates = 6.

4. Discussion

The best way to prevent the epidemic of tabaquism is to conserve the health of our childhood and teens teaching the bad consequences of this habit. The education in an early age is fundamental to assimilate all the activities of prevention and promotion of health.

Table 7
Urinary cadmium. Recovery study for one subject by group.

Sample	Cd(II) added (μg L^{-1})	Cd(II) found ± CV (μg L^{-1})	Recovery (%, $n = 6$)
1[a]	–	0.55 ± 0.03	–
	1.10	1.66 ± 0.05	100.90
	3.35	3.95 ± 0.07	101.50
	4.50	5.13 ± 0.04	101.77
2[b]	–	0.79 ± 0.04	–
	1.10	1.90 ± 0.07	100.90
	3.35	4.30 ± 0.10	104.77
	4.50	5.35 ± 0.09	101.33
3[c]	–	1.39 ± 0.04	–
	0.55	1.94 ± 0.09	100.00
	2.25	3.59 ± 0.10	97.78
	3.35	4.76 ± 0.07	100.59
4[d]	–	1.09 ± 0.06	–
	2.25	3.25 ± 0.07	99.11
	3.35	4.41 ± 0.10	99.10
	4.50	5.69 ± 0.20	102.22
5[e]	–	0.92 ± 0.04	–
	0.55	1.48 ± 0.08	101.81
	3.35	4.23 ± 0.03	98.80
	4.50	5.51 ± 0.09	102.00
6[f]	–	1.40 ± 0.06	–
	2.25	3.60 ± 0.10	97.77
	3.35	4.78 ± 0.10	100.89
	4.50	6.02 ± 0.05	102.66
7[g]	–	2.15 ± 0.06	–
	1.10	3.26 ± 0.05	100.90
	3.35	5.55 ± 0.10	101.49
	4.50	6.72 ± 0.03	101.55

Sample volume (mL): a: 5; b: 1.5; c: 1; d: 0.5; e: 0.25; f: 0.25; and g: 0.25.
n = Number of replicates = 6.

Table 8
Real salivary and urinary cadmium contents for one subject by group.

Subject	Dairy cigarettes	S–Cd ± CV (μg L^{-1})	U–Cd ± CV (μg L^{-1})
1	0	0.45 ± 0.083	0.55 ± 0.094
2[a]	0	1.38 ± 0.156	2.63 ± 0.104
3	3	3.84 ± 0.206	6.95 ± 0.167
4	10	5.57 ± 0.095	10.92 ± 0.083
5	20	8.36 ± 0.114	18.50 ± 0.100
6	40	12.05 ± 0.096	28.08 ± 0.078
7	TChH	28.32 ± 0.180	43.20 ± 0.104

[a] SHS.

Cadmium is a cumulative nephrotoxicant that is absorbed from food and cigarette smoking (Cooper, 2006). The preponderant biological matrixes for its determinations have been serum, blood and urine (Campillo et al., 1999).

Saliva and urine represent easily accessible body fluids using non-invasive sampling procedures and they can reflect levels of biomarkers (Esteban and Castaño, 2009; Aps and Martens, 2005; Van Nieuw Amerongen et al., 2004; Soo-Quee and Choon-Huat, 2007; Esteban and Castaño, 2009); particularly salivary monitoring has been used to explore environmental pollutants (Thaweboon et al., 2005; Luconi et al., 2006; Luconi et al., 2001).

4.1. Physical and chemical characterizations of biological samples

Biological samples of studied subjects showed normal clinical parameters; only few presented pathological characteristics, as follows:

- Two subjects gave positive assay for glucose and ketone.
- Two subjects gave positive assay for bilirubin.
- Six showed traces of protein.
- Two subjects gave positive assay for nitrite.
- Eight subjects showed presence of hematies, which were confirmed by microscopic observation.

Pathological parameters don't interfere with cadmium determination. Developed methodology was applied without trouble at total of biological samples, confirming the robustness of new methodology.

4.2. Stability studies of biological samples

Previous researchers have pointed out stability studies of biological samples referring to organic and inorganic metabolites (Quiñones et al., 2006). The present work gives a study of the effect of storage time on saliva and urine samples destined to cadmium quantification. The obtained results show a satisfactory stability of thirty days (Table 4). This report results are very important in clinical analysis laboratory and epidemiological control where often the high number of samples forces a substantial storage time.

4.3. Dilution test

5000 μL of each biological sample was taken and dilutions were carried out to obtain dilution factors of 1/2, 1/4, 1/8, 1/16 and 1/20. Dilution test was of 5000 μL for subjects with minor exposure and of 0.125 μL for the most exposed subjects. These dilution factors were adopted for the following studies.

Cadmium contents were determined by the proposed methodology, employing the obtained volume samples through test dilution, in order to work within the linearity range of the calibration of the developed methodology.

4.4. Interferences

The cations Co(II), Pb(II) and Al(III) interfere in the determination when they are present in a ratio 1:1 (foreign ion/Cd(II)) and must be separated before applying the developed methodology.

The obtained results show the good tolerance of the proposed methodology.

4.5. CPE spectral advantages in urine determinations

Urine is a strongly fluorescent sample; CPE step removes the highly fluorescent matrix with the additional advantage that allows determining the analyte without other previous treatment (Wang et al., 2007). Urine samples were tested using the developed methodology with good results; this fact is evidence of the versatility of the new methodology for quantifying cadmium trace in different biological matrixes.

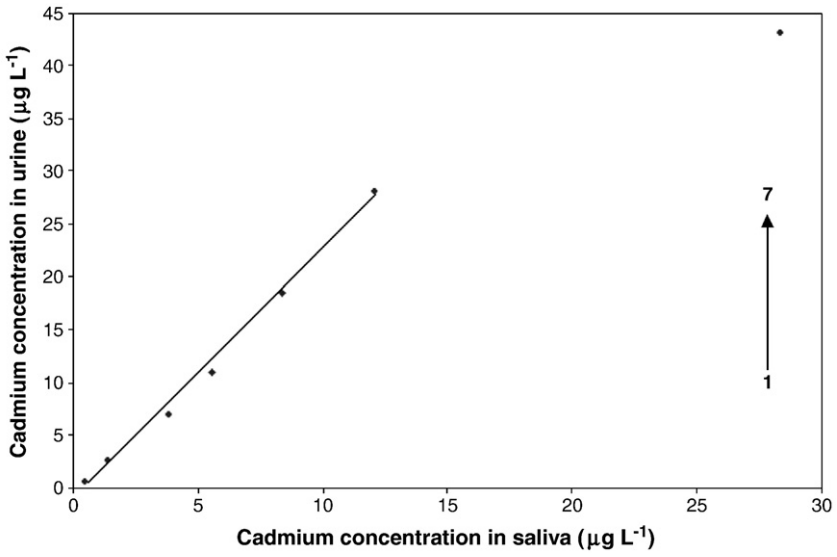


Fig. 6. Correlation between urinary and salivary cadmium concentrations for each group of subjects under study.

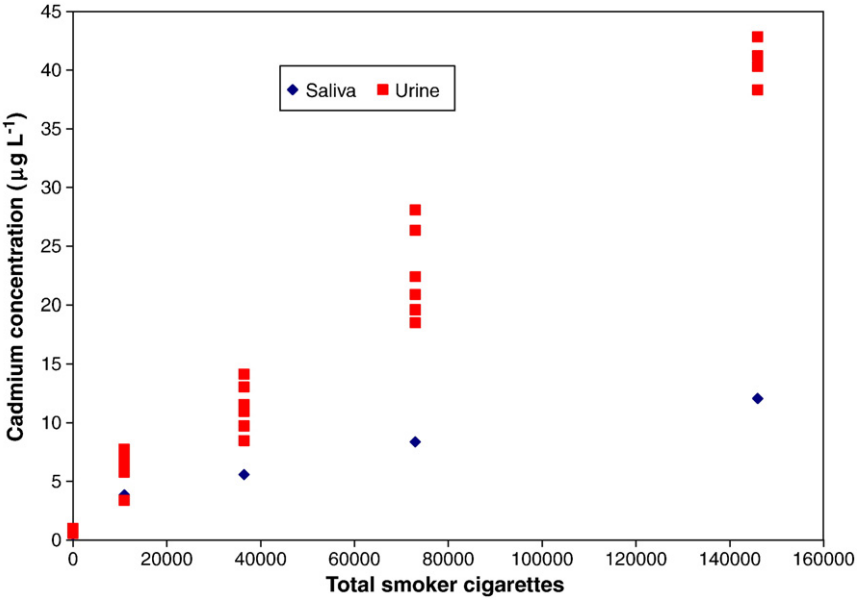


Fig. 7. Contents of SCd and UCd in studied subjects, according to total number of smoked cigarettes (ten years was taken as average of smoking habit).

4.6. Salivary and urinary cadmium contents related to tobacco habit

The obtained results (Tables 6 and 7) showed that the proposed method is suitable for determination of Cd(II) in urinary and salivary samples, for all range of studied concentrations and it can be successfully applied to Cd(II) monitoring.

Smoker subjects have higher S–Cd and U–Cd concentrations than non-smoker subjects (Fig. 7). The result for subjects of group 7 (TChH) puts in evidence high grade of exposition. Also, it has been just established that active and passive smoking are toxic to renal function, in the case of subjects with TChH, the obtained correlation between UCd and SCd contents is still lower than the other results; it could be attributed to kidney failure and permanent damages due to accumulation of heavy metal and exposure to other tobacco toxic substances.

5. Conclusions

The aim of the present work has been to determine traces of cadmium in saliva and urine samples of smokers, second hand smokers, non-smokers and chewing tobacco habit subjects of San Luis city (Argentina), using a simple combined methodology. The method showed good sensitivity and adequate selectivity and was successfully applied to the determination of trace amounts in both biological samples with good tolerance to regular foreign constituents; it represents a promising approach in the area of environmental monitoring with low operation cost, simplicity of instrumentation and non-polluting solvents.

Both biological samples show to be stable during one month for metal determination. Results of UCd could be successfully correlated with the SCd contents. Additional investigations are required for arriving at valid conclusions with respect to the incidence of sex and age over exposition level. Considering that high salivary concentration of the carcinogen cadmium in considered smoker samples may contribute to the pathologic effects, efforts should be made by the control agencies and health agents to discourage the consumption of cigarettes and the tobacco chewing habit.

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Glossary

Cloud point extraction: CPE
o-phenanthroline: o-phen
Eosin: eo
Polyethyleneglycolmono-p-nonylphenylether: PONPE 7.5
Limit of detection: LOD
Limit of quantification: LOQ
Limit of linearity: LOL
% Coefficient of variance: %CV
Inductively coupled plasma mass spectrometry: ICP-MS
Electrothermal Atomic Absorption Spectroscopy: ETAAS
Salivary cadmium: SCd
Urinary cadmium: UCd
Second hand smoker: SHS
Tobacco chewing habit: TChH