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Determination of nicotine and N-nitrosamines in house dust by pressurized liquid extraction and comprehensive gas chromatography—Nitrogen chemiluminiscence detection

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ABSTRACT

A novel, highly selective method for the determination of nicotine, N-nitrosamines and tobacco-specific nitrosamines (TSNAs) in indoor dust samples is presented in this study. Samples were extracted by in-cell clean-up pressurized liquid extraction (PLE) that allows high extraction efficiency with moderate consumption of organic solvents. The extracts were analyzed by comprehensive gas chromatography and detected with a nitrogen chemiluminiscence detector (GC × GC-NCD) that provided enhanced selectivity and sensitivity for organic nitrogen containing compounds. Method validation showed good linearity, repeatability and reproducibility (%RSD < 8%). Recovery was higher than 80% for most target compounds and limits of detection lower than 16 ng g^{-1}. The method was used for the determination of the nitrosamine target compounds in house dust samples from both smoking and non-smoking households. All the analytes were found in the samples, nicotine being the most abundant compound in smokers' dust and one of the most abundant in non-smokers' dust. To our knowledge this is the first time that volatile N-nitrosamines and TSNAs have been determined in indoor dust samples. The results demonstrate the presence of these highly carcinogenic compounds in house dust, with inherent human exposure through inhalation and/or involuntary ingestion of house dust.

1. Introduction

House dust has been identified as a major source of environmental contaminants including pesticides, polycyclic aromatic hydrocarbons (PAHs), phthalates, several metals, and other chemicals of human health concern [1,2]. Since contaminants bound to indoor dust are more persistent than those outdoors, indoor dust has been recognized as a significant source of human exposure for an increasing number of pollutants. For instance, the ingestion of house dust has been estimated to be the major route of exposure to some persistent pollutants for children [3]. Furthermore, recently, it has been demonstrated that indoor dust may be the main route of exposure to polybrominated diphenyl ethers for both adults and children [4]. House dust is therefore a key pollutant vector and one which demands further examination for the presence of other contaminants of human health concern.

One of the most important sources of indoor pollution is tobacco smoke. Nicotine is the most abundant organic compound emitted during smoking [5]. It reacts during the burning of tobacco to form tobacco-specific nitrosamines (TSNAs). TSNAs are amongst the most abundant carcinogenic compounds identified in tobacco smoke [6] and they have been related to acute leukemia [7] and lung cancer [8]. Of all TSNAs identified, N′-nitrosonornicotine (NNN) and 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK) are the most prevalent carcinogens in tobacco products and are classified as carcinogenic for humans (Group 1 IARC) [9]. Another interesting TSNA is 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanol (NNAL), which is the main metabolite of NNK and has the same dangers [10]. Moreover, more volatile N-nitrosamines, with genotoxic and carcinogenic properties can be formed in the atmosphere because of the presence of nitrogen-containing species originating during combustion processes [11]. Some studies have also detected the presence of volatile N-nitrosamines, such as N-nitrosodimethylamine and N-nitrosopyrrolidyne, in environmental tobacco smoke [12,13].

Nicotine deposits almost entirely on indoor surfaces and persists for long time [14,15]. This deposited nicotine can also form

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TSNAs by reaction with atmospheric species present in indoor environments, such as ozone, nitrous acid and nitrogen oxides. Given the high levels of nicotine typically found in environments contaminated with tobacco and the low volatility of TSNAs, these contaminants can persist for weeks to months in these indoor environments [5]. Furthermore, several experiments have suggested that airborne NNK concentrations in sidestream cigarette smoke can increase by 50–200% per hour during the first 6 h after cigarettes are extinguished [16]. Whereas direct inhalation of second hand smoke (SHS) is an exposure pathway of concern, non-smokers, especially children, are at risk through contact with surfaces and dust contaminated with residual smoke gases and particles [17] (the so called third hand smoke (THS)) [18]. Several studies have detected nicotine in indoor dust and surfaces [17,19]. However, to our knowledge, there is no information about the presence of TSNAs and volatile N-nitrosamines in indoor dust.

The complexity of dust composition and high adsorption capacity of dust particles requires the use of exhaustive extraction techniques. Furthermore, the extracts obtained from dust samples are complex with a large number of co-extracted interferences. Pressurized liquid extraction (PLE) is an efficient extraction method which can address this problem and, therefore, has been successfully applied for the extraction of organic pollutants in house dust, such as benzotriazole light stabilizers [20], brominated diphenyl ethers [21] and polycyclic aromatic hydrocarbons [22]. Furthermore, the option of integrating an in-cell clean-up active sorbent into the PLE process, has considerably reduced the time and steps needed for sample treatment prior to the analysis [23].

Several techniques have been used for the analytical determination of nicotine and N-nitrosamines, the most common of which is gas chromatography (GC). GC has been coupled with different detectors such as, mass spectrometry (MS) [19], especially ion trap MS [24,25]; thermal energy analysis (TEA), which is the more widely used method for TSNA determination [26]; and nitrogen specific detectors, e.g. the nitrogen–phosphorous [27] or nitrogen chemiluminiscence detector (NCD) [28]. Comprehensive gas chromatography (GC × GC), which has an increased separation power and much better sensitivity and peak resolution than one dimensional GC, has been used for the determination of organic contaminants in complex matrices [29]. Moreover, the combination of an element specific detector can provide more resolution of the target analytes with enhanced sensitivity. In this sense, a GC × GC-NCD has recently been used for determining organic nitrogen compounds (ON) in aerosol samples, showing higher selectivity and sensitivity than mass spectrometry detectors [30].

Hence, the aim of this study is the development of a selective analytical method for the determination of nicotine and N-nitrosamines (9 volatile N-nitrosamines and 5 TSNAs) in indoor dust based on in-cell clean-up PLE, followed by GC × GC-NCD determination. Parameters affecting the efficiency and selectivity of the analytical method are discussed. Dust samples collected from non-smoking and smoking households were analyzed with the proposed method. To our knowledge, this study represents the first time that the occurrence of N-nitrosamines, including TSNAs, in house dust has been reported.

2. Experimental

2.1. Chemical standards

The standards of the target compounds involved a mixture of 9 nitrosamines at 2000 mg L^{-1} in methanol [EPA 8270/Appendix IX Nitrosamines Mix, from Sigma–Aldrich, (Steinheim, Germany) including N-nitrosodimethylamine (NDMA), N-nitrosomethylethylamine (NMEA), N-nitrosodiethylamine (NDEA), N-nitrosodi-*n*-propylamine (NDPA), N-nitrosomorpholine (NMor), N-nitrosopyrrolidine (NPyr), N-nitrosopiperidine (NPip), N-nitrosodi-*n*-butylamine (NDBA) and N-nitrosodiphenylamine (NDPhA) and an the individual standard of nicotine, also from Sigma–Aldrich. TSNAs of the N′-nitrosonornicotine (NNN), N′-nitrosoanatabine (NAT), N′-nitrosoanabasine (NAB), 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK) and 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanol (NNAL) from Fluka (Buchs, Switzerland). The standards had a minimal purity of 97% except for NNAL, of which the purity was ≥92%. Fig. 1 shows the chemical structure of the target compounds.

The standard solutions of the target compounds were prepared in methanol and the diluted mixtures in ethyl acetate (GC grade with >99.9% purity, SDS, Peypin, France). Other solvents used in the optimization of the method (acetone and dichloromethane) were also GC grade from SDS. Hyflo Super Cel diatomaceous earth for filling the extraction cells of the pressurized liquid extraction equipment was supplied by Sigma–Aldrich, C_{18} (Bond Elute LRC, 10 CC) was from Varian (Harbor City, CA, USA), silica (230–400 mesh) and alumina (70–230 mesh) were provided by Merck (Darmstadt, Germany) and florisil (60–100 mesh) by Fluka. The sorbents were first conditioned at 400 °C for 6 h and then stored in closed vials, at room temperature, before their use. Helium gas of purity 99.999% was used for the chromatographic analysis.

2.2. Sample collection and preparation

House dust was collected from non-smoking and smoking private homes, using conventional vacuum cleaners in regular use in the households between July and December 2010. The collected dust was next sieved with a stainless steel sieve and the fraction under 100 μm was stored in amber glass vials and kept at 4 °C until analysis.

Method optimization and validation was carried out using spiked samples of house dust. Spiked samples were prepared by adding different volumes of standard solution of the target compounds in ethyl acetate, adding enough volume of solvent to cover the entire sample. The mixture was accurately homogenized and kept in a cupboard funnel at room temperature until the solvent had completely evaporated and then aged for at least one week. It was then stored in amber glass vials at 4 °C before being extracted.

2.3. Pressurized liquid extraction

Extractions were performed using an ASE 200 Accelerated Solvent Extraction system (Dionex, Sunnyvale, CA, USA) in 11 mL stainless steel extraction cells. Ethyl acetate was used as the extraction solvent. Under the optimized conditions, 0.5 g of sieved house dust were mixed and dispersed in a mortar with 1 g of silica. Next, the extraction cells were filled with two cellulose filters placed at the bottom of the cell, followed by 1 g of diatomaceous earth, the dispersed sample, and then more diatomaceous earth until the cell was full. The extraction was carried out at 100 °C, with the cells pressurized at 1500 psi, using 3 consecutive static cycles of 10 min each. The flush volume was 100% and the purge time 100 s. The extracts were then filtered with a 0.45 μm nylon syringe filter, evaporated to a volume of ca. 0.5 mL, and made up to 1 mL with ethyl acetate.

The PLE process was optimized using house dust samples spiked at a final concentration of 20 μg g^{-1} of each target compound. In order to assess possible contamination, procedural blanks were also performed by filling the cells with only 1 g of silica and diatomaceous earth. No signal of the target compounds was found in these blanks.

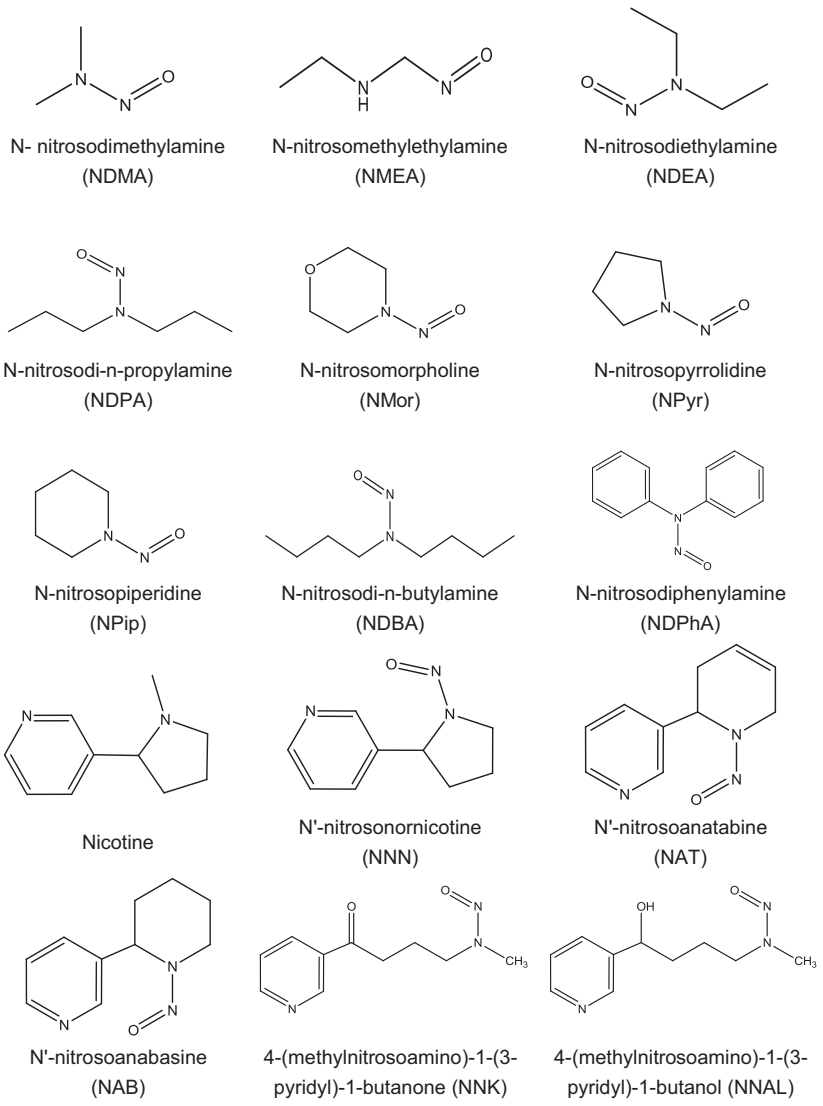

Fig. 1. Chemical structures of the target N-nitrosamines, nicotine and tobacco-specific nitrosamines.

2.4. Chromatographic analysis

In this study, two chromatographic systems were used to analyze the extracts. For the optimization of PLE conditions, a one dimensional GC–MS system was used. The GC–MS equipment was a 6890N GC and 5973 *inert* MS from Agilent Technologies (Palo Alto, CA, USA), equipped with a Zebron ZB-50 capillary column (30 m × 0.25 mm × 0.25 μm) provided by Phenomenex (Le Pecq Cedex, France). For the GC–MS analysis, the inlet was set at 200 °C and injections (1 μL of extracts) were performed in pulsed splitless mode at a pressure of 30 psi for 2 min. The helium carrier gas flow was set at 1 mL min^{-1}. The oven temperature program began at 40 °C for 2 min, was then increased to 100 °C at 15 °C min^{-1}, and next to 250 °C at 20 °C min^{-1} and kept at that temperature for 3 min. The GC–MS interface was set at 280 °C. The MS-detector was in the selective ion monitoring mode (SIM) operating at an electron impact energy of 70 eV.

Once the PLE conditions were optimized, the method validation and the quantification of the samples were done in a GC × GC–NCD system that consisted of a 7890 gas chromatograph and an 255 Nitrogen Chemiluminiscence Detector, both from Agilent. The GC was equipped with a secondary oven to fit the second column, and a modulator between first and second GC columns based on a Leco (Cheshire, UK) liquid nitrogen two stage cold jet system. The modulator and the secondary oven operated at +15 °C above the GC oven temperature and the modulation period was 5 s. The first column was a non-polar BPX5 (30 m × 0.32 mm i.d. × 0.25 μm film thickness) and the second column a BPX50 (1.5 m × 0.10 mm i.d. × 0.10 μm film thickness) both from SGE Analytical Science (VIC, Australia).

Extracts (1 μL) were injected into the GC × GC-NCD using a Gerstel automated liquid injector (Gerstel, Mulheim an der Ruhr, Germany). Injections were performed in pulsed splitless mode at a temperature of 210 °C and a pressure of 30 psi for 2 min. The initial temperature of the first dimension column was 55 °C for 1 min and the subsequent temperature program was a heating rate of 5 °C min^{-1} until 255 °C was reached and held isothermally for a further 1 min. The initial temperature of the second dimension column was 70 °C for 1 min and a 5 °C min^{-1} heating rate was used until 270 °C was reached and held isothermally for further 1 min. Helium was used as a carrier gas at a constant flow of 1 mL min^{-1}.

Pyrolysis of the analytes in the NCD was carried out at 900 °C under a hydrogen flow rate of 4 mL min^{-1} and an oxygen flow rate of 10 mL min^{-1}. Data from the NCD was collected at 50 Hz over the entire course of the analysis. Fig. 2 shows a GC × GC-NCD chromatogram of 1 ng standard mixture of the target compounds. The

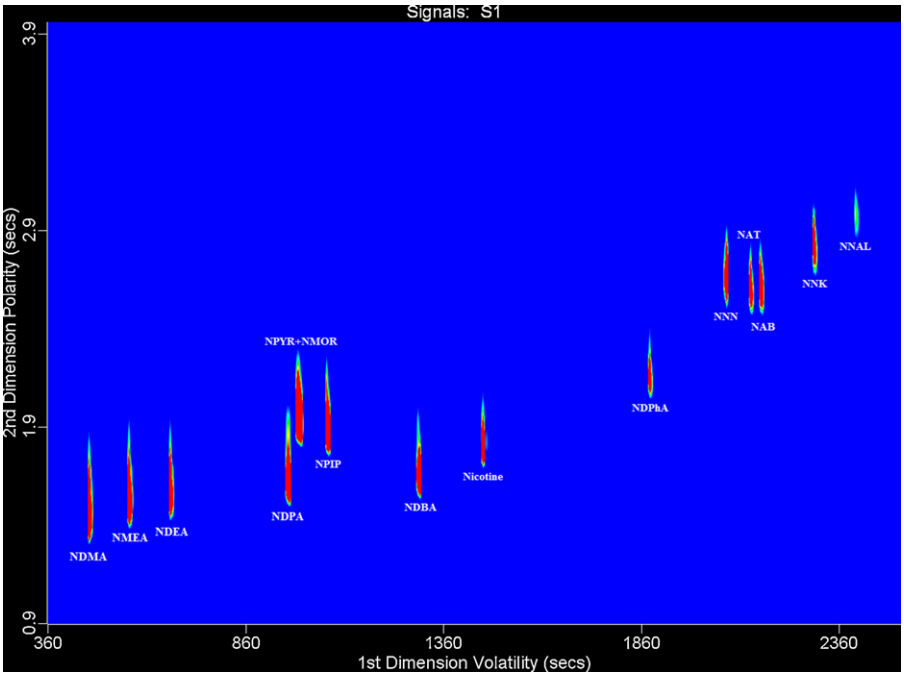

Fig. 2. GC × GC-NCD chromatogram of 1 ng standard mixture of the target compounds.

chromatogram is presented as a contour plot with the retention times on columns 1 (indicating decreasing volatility) and 2 (indicating increasing polarity) on the *X* and *Y*-axes, respectively. Each spot on the chromatogram represents an individual ON compound, except for NPyr and NMor, which were quantified together.

3. Results and discussion

3.1. Chromatographic analysis

In this study, two chromatographic systems were used. First, the extracts obtained in the PLE optimization were analyzed using a one dimensional GC–MS. The use of a midpolarity phase capillary column (a 50% diphenyl/50% dimethyl polysiloxane) allowed the separation of NMor and NPyr that usually coelute when using more apolar GC columns. However, low sensitivity was achieved with the GC–MS system and, therefore, GC × GC-NCD was selected for the method validation and the analysis of the house dust samples. The inlet temperature was set at 210 °C in both chromatographic methods because TSNAs degraded in the injector at higher temperatures.

The main parameters to optimize for NCD are pyrolysis temperature and hydrogen and oxygen flow rates. As seen in Fig. 1 nitrosamines are characterized by a N–NO bond, which is the weakest in the molecule and therefore can be selectively broken under moderate conditions. Previous studies demonstrated that the higher responses of the most volatile N-nitrosamines were obtained at an oxygen rate of 5 mL min^{-1} and a pyrolysis temperature of 450 °C, without the use of hydrogen [31]. However, higher oxygen flow rates and pyrolysis temperatures (10 mL min^{-1} and 900 °C) with a hydrogen flow rate of 4 mL min^{-1} provided better responses for other organic nitrogen compounds (ON) [30]. In this study, the NCD parameters were optimized by injecting 5 replicates of a standard solution containing 10 μg L^{-1} of the 15 ON compounds in ethyl acetate. The highest responses for all target ON compounds were obtained at a pyrolysis temperature of 900 °C using hydrogen and oxygen at flow rates of 4 mL min^{-1} and 10 mL min^{-1}, respectively.

A potentially important advantage of using NCD as a detector is that it produced an equimolar response to organic nitrogen compounds. ON compounds responded with equal nitrogen responses confirming the response of the detector to be independent of the molecular structure or other functionality [30,32]. However, in this study, two of the target compounds did not show equimolarity, namely nicotine and NNAL, possibly because of degradation reactions occurring during pyrolysis of these compounds. Therefore, standard calibration curves of each compound were used to quantify the samples.

3.2. PLE optimization

Initial PLE experiments were carried out in order to determine the optimal extraction solvent. Taking into account the solubility of the target compounds, four solvents were tested as extraction solvents: dichloromethane, ethyl acetate, methanol and acetone. For these experiments, 0.5 g of pooled house dust spiked at 20 μg g^{-1} mixed with 1 g of diatomaceous earth were extracted at 80 °C and 1500 psi in one single cycle of 5 min. In all the experiments, the flush volume and purge time were fixed at 100% and 100 s, respectively. Extracts were filtered with a 0.45 μm nylon syringe filter, evaporated to a volume of ca. 0.5 mL, and adjusted to 1 mL with the extraction solvent and analyzed by GC–MS. Fig. 3 shows the extraction efficiencies for the four solvents for nine representative compounds. The responses in Fig. 3 were normalized with respect to the responses obtained with ethyl acetate. It was found that the higher the polarity of the extraction solvent, the more complex the visual appearance of the corresponding extracts. In this sense, methanol extracts were cloudy and difficult to filter. As seen in Fig. 3, in general the best results were obtained when using ethyl acetate, therefore, it was used as extraction solvent thereafter.

As commented in the introduction, house dust is a complex matrix, which contains a large range of organic contaminants [1]. In order to enhance selectivity and eliminate interferences from the extracts, four sorbents were tested as in cell clean-up sorbents: C_{18}, silica, alumina and florisil. Fractions of 0.5 g of pooled house dust spiked at 20 μg g^{-1} where mixed and dispersed in a mortar

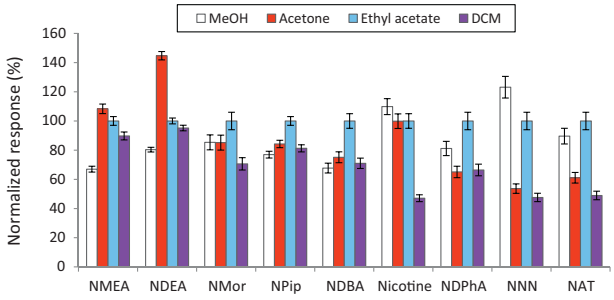

Fig. 3. Influence of the solvent on the efficiency of the extraction process for nine representative compounds ($n = 3$, 1 cycle of 5 min at 80 °C, 1500 psi, 100% flush volume and 100 s purge time). Normalized responses to those achieved using ethyl acetate.

with 1 g of the above sorbents. For these experiments the PLE conditions were the same than those described above for the solvent optimization (1 single cycle of 5 min at 80 °C and 1500 psi) using ethyl acetate as extraction solvent. The chromatograms obtained by GC–MS with each sorbent were compared with those obtained with diatomaceous earth (DE), which is a non-retentive inert sorbent. Fig. 4 shows the normalized responses obtained with the different clean-up sorbents for nine representative target compounds with respect to the DE extracts (without clean-up). As seen in the figure, the least retentive sorbent for the target compounds was silica, the responses obtained for the target compounds with 1 g of this sorbent being similar to those obtained with DE. The signal of some compounds was higher with silica than with DE because the elimination of interferences made the peaks of the target compounds better to quantify. Florisil was in general the most retentive sorbent. Regarding the reduction of co-extracting interferences, C_{18} did not lead to any significant reduction of them in comparison with diatomaceous earth; however cleaner extracts and less complex GC–MS chromatograms were achieved with silica, florisil and alumina. In light of these results, silica was chosen as the clean-up sorbent for the next experiments. Next the optimal amount of silica to be used was tested from 0.5 to 2.0 g. Amounts higher than 1 g led to a slight decrease in the responses. Therefore, 1 g of silica was fixed for the in-cell clean-up.

The effects of extraction temperature, time and number of cycles on the efficiency of the extraction were simultaneously evaluated using a multifactorial design $3^2\ 2^1$ of 18 experiments. Table 1 summarizes the factors and levels selected for the design. The factor levels were selected based on previous house dust studies [20,23,33]. Statistical analysis was carried out with Statgraphics-Plus 5.1 (Magnugistic, Rockville, MD, USA). In all experiments, the pressure was set at 1500 psi (enough to maintain ethyl acetate in a liquid state in this range of temperatures), flush volume at 100% and purge time at 100 s (until the extraction cell content was completely dry).

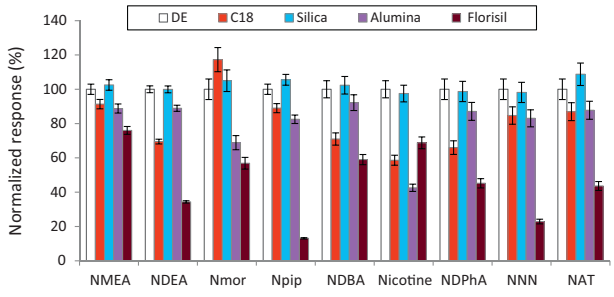

Fig. 4. Influence of the kind of clean-up sorbent on the efficiency of the extraction process for nine representative compounds ($n = 3$, same extraction conditions as in Fig. 1). Normalized responses to those achieved using silica.

Table 1
Factors and levels selected for the $3^2\ 2^1$ design.

Factors	Lower	Intermediate	Upper
Temperature (°C)	80	100	120
Time (min)	5	10	15
Cycles	1	–	3

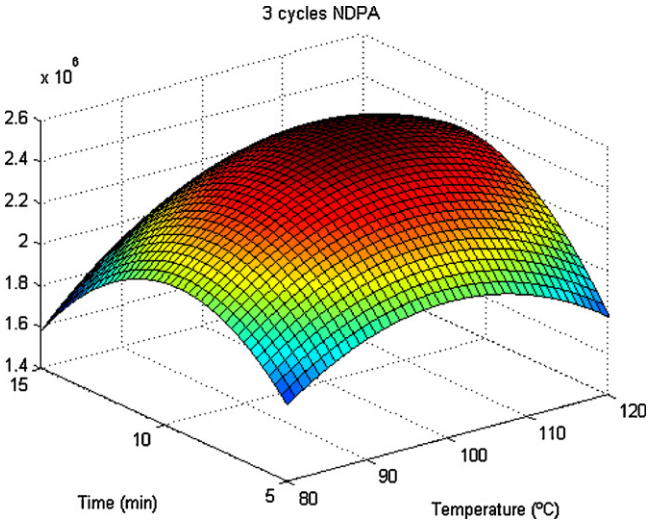

Fig. 5. NDBA response surface for the extraction temperature against the extraction time for 3 cycles.

As an example of the results, Fig. 5 shows the fitted response surface for NDBA at 3 cycles. Similar response surfaces were obtained for most of the target compounds. In general, time increases the chromatographic responses up to 10 min, except for NDMA whose responses were higher using longer extraction times. Regarding the effect of temperature, in general, responses were greater at higher temperatures. However, for most compounds at 10 min of extraction responses at 120 °C were similar of slightly lower than those obtained at 100 °C. Furthermore, the chromatograms of the dust extracts at 120 °C showed more co-extraction interferences, which made the quantification of the target compounds difficult. Therefore, 10 min of extraction time and a temperature of 100 °C were selected as a compromise between the results. Finally, since the number of cycles of 10 min each had a slightly positive effect for most compounds, three cycles were selected thereafter. The optimized conditions for the PLE extraction are summarized in Table 2.

3.3. Method validation

Once PLE parametres were optimized, the method was validated using GC × GC-NCD determination. First the main instrumental parameters of the GC × GC-NCD system were evaluated, which are summarized in Table 3. The instrumental limits of detection (LODs)

Table 2
Optimized conditions for the pressurized liquid extraction of nitrosamines in house dust.

Pressurized liquid extraction	
Amount of sample	0.5 g
Solvent	Ethyl acetate
Cell volume	11 mL
Static time	10 min
Temperature	100 °C
Pressure	1500 psi
No. extraction cycles	3
Flush volume	100%
Purge time	100 s

Table 3
Main instrumental parameters for the GC × GC-NCD system: first and second dimension retention times (t_R), limit of detection and linear range expressed in pg ($n = 10$).

Compound	1st t_R (s)	2nd t_R (s)	LOD (pg)	Linear range[a] (pg)
NDMA	470	1.46	1.7	7–25,000
NMEA	565	1.54	1.7	7–40,000
NDEA	670	1.58	1.7	7–15,000
NDPA	970	1.62	1.2	5–40,000
NPYR	995	1.94	1.7	7–25,000
NMOR	995	1.94	1.6	7–25,000
NPIP	1070	1.88	1.5	6–30,000
NDBA	1300	1.64	1.9	8–40,000
Nicotine	1460	1.8	7.3	32–20,000
NDPhA	1880	2.16	3.0	13–25,000
NNN	2075	2.58	4.3	19–25,000
NAT	2135	2.60	3.0	13–20,000
NAB	2160	2.56	2.8	12–25,000
NNK	2295	2.82	4.2	18–25,000
NNAL	2400	3.08	7.1	31–15,000

[a] LOQ: the lowest value of the linear range of each compound.

and limits of quantification (LOQs) were calculated based on the standard deviation at low concentrations (10 pg of each target compound). LODs were calculated according to EPA protocol 40 CFR 136 [34] multiplying the Student t-value ($n = 10$, 95% confidence level) per the standard deviation of 10 replicates and LOQs as ten times the standard deviation. The combination of comprehensive GC with the NCD detection demonstrated a high sensitivity allowing limits of detection (LODs) and limits of quantification (LOQs) at low pg levels, ranging from 1.7 to 7.3 pg and from 7.2 to 31.5 pg, respectively. Linearity was good for all the target compounds with correlation coefficient values (r^2) between 0.9915 and 0.9994 for the linearity ranges shown in Table 3. Instrumental repeatability was under 5% for a low calibration level (50 pg, $n = 5$).

The whole method (in-cell clean-up PLE GC × GC-NCD) parameters were evaluated with spiked samples of house dust at a low and a high calibration level (1 and 50 μg g^{-1}, respectively). Repeatability and reproducibility between days were below 8% RSD for all the target compounds ($n = 5$). To calculate recoveries, the peak areas obtained by spiking the pooled house dust at the two calibration levels were compared with a calibration curve obtained by direct injection of the standards. As shown in Table 4 recoveries were similar for both levels and were higher than 80% for the majority of the target compounds. The most volatile N-nitrosamines, such as NDMA, NMEA and NDEA showed lower recoveries probably because were partially lost during the evaporation process. Since recovery values were similar for both calibration levels, no matrix effects were observed and quantification was performed by external calibration. Method detection limits (MDLs) ranged between 2.5 ng g^{-1} for NDPA and 15.8 ng g^{-1} for nicotine and method quantification limits (MQLs) were from 11 ng g^{-1} to 68 ng g^{-1} for the same compounds. A previously reported MDL of nicotine in house dust [19] using a GC and nitrogen–phosphorus detection was similar (20 ng g^{-1}).

Table 4
Method parameters: recoveries of pooled spiked house dust sampes spiked at 1 μg g^{-1} and 50 μg g^{-1}, method detection limit (MDL) and method quantification limit (MQL), expressed in ng g^{-1}.

Compound	Recovery		LOD (ng g^{-1})	LOQ (ng g^{-1})
	1 μg g^{-1}	50 μg g^{-1}		
NDMA	56	53	5.8	25.3
NMEA	76	75	4.5	19.5
NDEA	82	79	4.1	17.7
NDPA	97	93	2.5	10.9
NPYR	98	102	4.0	17.4
NMOR	86	82	3.3	14.1
NPIP	81	80	3.6	15.5
NDBA	85	81	4.5	19.4
Nicotine	92	88	15.8	68.5
NDPhA	101	97	6.0	25.9
NNN	99	101	8.7	37.5
NAT	98	99	6.0	25.9
NAB	98	101	5.6	24.4
NNK	106	103	8.4	36.4
NNAL	101	98	14.2	61.6

Table 5
Average, maximal and minimal concentrations of the target compounds found in the samples, expressed in μg g^{-1}.

Compound	Non-smoking			Smoking		
	Average	Max	Min	Average	Max	Min
NDMA	0.45	1.11	0.10	0.29	0.79	0.48
NMEA	0.77	2.89	0.05	0.35	0.63	0.17
NDEA	0.92	2.81	0.23	0.32	0.90	0.03
NDPA	0.26	0.48	0.14	0.67	1.85	0.08
NMor/NPyr	0.11	0.15	n.d.	0.25	0.39	n.d.
NPip	0.67	0.85	n.d.	0.54	0.99	n.d.
NDBA	0.11	0.13	n.d.	1.22	1.98	n.d.
Nicotine	1.91	2.83	0.95	14.7	21.6	1.27
NDPhA	2.08	4.15	1.00	4.46	13.7	0.12
NNN	0.12	0.23	<MQL	0.31	0.46	n.d.
NAT	0.35	0.42	n.d.	3.28	6.54	n.d.
NAB	0.25	0.39	n.d.	0.67	0.93	n.d.
NNK	0.55	0.68	0.42	0.89	2.29	0.24
NNAL	2.02	3.68	n.d.	n.d.	n.d.	n.d.
Total	9.60	12.3	3.53	26.3	38.9	3.12

n.d., values under the method detection limit; <MQL, values under the method quantification limit.

3.4. Analysis of house dust samples

The optimized in-cell clean-up PLE GC × GC-NCD method was applied for the determination of nicotine and the target N-nitrosamines in house dust samples from smoking and non-smoking homes (four samples of each). As an example, Fig. 6 shows the separation of ON compounds using GC × GC-NCD from one of the non-smoking samples. Over 60 ON compounds including Nicotine, N-nitrosamines and TSNAs were seen. Peak identification is based primarily on retention times of standard compounds. The signals corresponding to the target compounds were found in this sample. As it can be seen, despite the complexity of the extracts, very simplified chromatograms were obtained using the optimized method.

A summary of the average, maximal and minimal concentrations found in the non-smoking and smoking households' dust samples is presented in Table 5. NPyr and NMor were quantified together because of their similar retention times. As expected, total concentrations of these ONs were in general higher in smokers' samples than in those of non-smokers', the average total concentrations being approximately 3-fold higher in smokers' house dust.

In some of the samples, all the target compounds were detected. The most abundant ON in non-smoking samples was NDPhA with values up to 4.15 μg g^{-1}. Other abundant N-nitrosamines in these samples were NMEA (up to 2.89 μg g^{-1}) and NDEA (up to 2.81 μg g^{-1}). Nicotine was also one of the most abundant ONs in non-smokers' dust with concentrations ranging from 1 μg g^{-1} to 2.83 μg g^{-1}. It was however the most abundant compound in smokers' dust samples with concentrations from 1.27 μg g^{-1} to 21.6 μg g^{-1} (more than 7-fold higher than in non-smokers' dust). Nicotine concentrations found here were lower than those found in house dust samples in Baltimore (USA), that showed median nicotine concentrations of 11.7 μg g^{-1} in non-smoking homes and 43.4 μg g^{-1} in smoking homes [19].

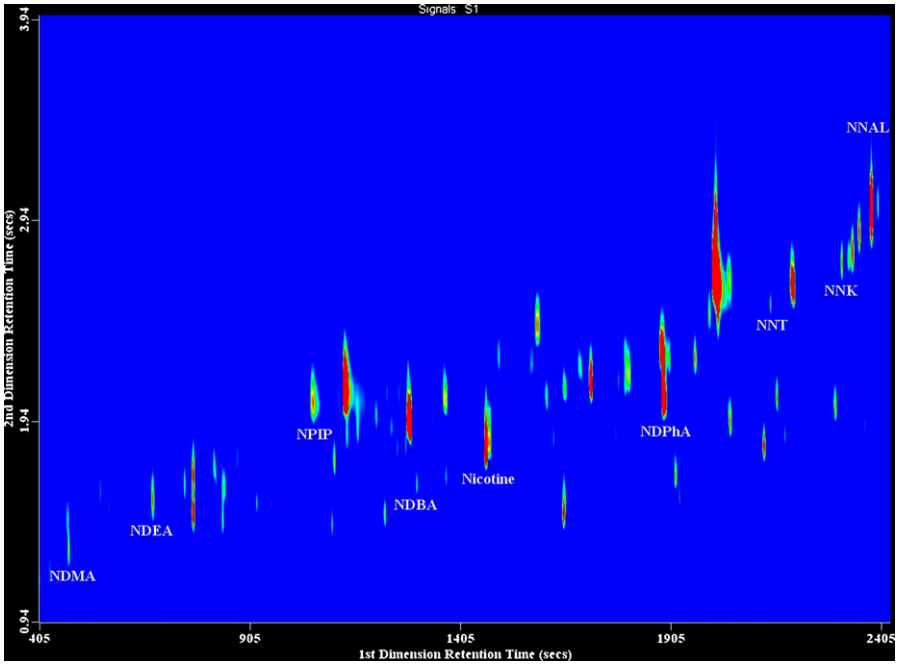

Fig. 6. GC × GC-NCD chromatogram of non-smokers' household dust. The peaks of the indentified target compounds are indicated.

Regarding TSNAs, the most abundant were NAT and NNK with values of up to 6.54 $\mu g\,g^{-1}$ and 2.29 $\mu g\,g^{-1}$, respectively in smokers' dust. However, NNAL was only detected in 3 non-smoking samples (in concentrations up to 3.68 $\mu g\,g^{-1}$) but was not detected in any of the smoking samples. As commented in the introduction, NNAL is the main degradation product of NNK, however NNAL concentrations did not show correlation with NNK concentrations in these house dust samples. The presence of nicotine and TSNAs in non-smokers' dust, may demonstrate the influence of outdoor pollution in indoor environments. In addition, smoking visitors could have visited these non-smokers' houses and contributed to the house dust with their dead skin, fabric fibres and hairs. Nevertheless, further investigation and determination of these TSNAs in more house dust samples is needed.

As commented before, previous studies have only determined nicotine in house dust [17,19], but none of the N-nitrosamines selected in this study have been previously reported in this matrix. This study has demonstrated the presence of these highly carcinogenic target ON compounds (nicotine, N-nitrosamines and TSNAs) in both non-smoking and smoking household dust and therefore there is inherent human exposure to these compounds through inhalation and/or ingestion of house dust.

4. Conclusions

This study developed an efficient method for simultaneously determining nicotine, seven volatile N-nitrosamines and five tobacco-specific nitrosamines in indoor dust. In-cell clean-up pressurized liquid extraction was used for the extraction of the analytes from the dust samples with recovery higher than 80% for most target compounds. The high selectivity and sensitivity of the comprehensive gas chromatography followed by nitrogen chemiluminiscence detection provided limits of detection and quantification at low ng g^{-1} levels.

The reliability of the method was demonstrated through the determination of these tobacco related compounds in several smoking and non-smoking house dust samples. Results showed that the total average concentrations of the target compounds in smoking house dust was nearly three times higher than those obtained in non-smoking homes. This study demonstrates for the first time the presence of nitrosamines in smoking and non-smoking house dust and therefore the inherent human exposure to these highly carcinogenic compounds.

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