Rapid and sensitive gas chromatography ion-trap mass spectrometry method for the determination of tobacco specific *N*-nitrosamines in secondhand smoke

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Abstract

Tobacco-specific nitrosamines (TSNAs) are some of the most potent carcinogens in tobacco and cigarette smoke. Accurate quantification of these chemicals is needed to help assess public health risks. We developed and validated a specific and sensitive

method to measure four TSNAs in both the gas- and particle-phase of secondhand smoke (SHS) using gas chromatography and ion-trap tandem mass spectrometry,. A smoking machine in an 18-m^3 room-sized chamber generated relevant concentrations of SHS that were actively sampled on Teflon coated fiber glass (TCFG) filters, and passively sampled on cellulose substrates. A simple solid-liquid extraction protocol using methanol as solvent was successfully applied to both filters with high recoveries ranging from 85 to 115%. Tandem MS parameters were optimized to obtain the best sensitivity in terms of signal to-noise ratio (S/N) for the target compounds. For each TSNA, the major fragmentation pathways as well as ion structures were elucidated and compared with previously published data. The method showed excellent performances with a linear dynamic range between 2 and 1000 ng mL^{-1}, low detection limits (S/N > 3) of 30-300 pg.ml^{-1} and precision with experimental errors below 10% for all compounds. Moreover, no interfering peaks were observed indicating a high selectivity of MS/MS without the need for a sample clean up step. The sampling and analysis method provides a sensitive and accurate tool to detect and quantify traces of TSNA in SHS polluted indoor environments.

1. Introduction

Secondhand tobacco smoke (SHS), also called environmental tobacco smoke (ETS) or passive smoke, is diluted and dispersed sidestream tobacco smoke that is emitted from cigarettes between puffs. Exposure to SHS is estimated to cause approximately 12% of

the 450,000 deaths each year from tobacco use in the United States (1, 2). In addition, epidemiological data show an increased risk of lung cancer in nonsmokers exposed to SHS [3].

SHS contains many mutagenic and carcinogenic chemicals [4]. Of these, tobacco-specific nitrosamines (TSNAs) are among the most abundant carcinogens identified in tobacco and its smoke [5, 6]. They are formed by nitrosation of nicotine and related tobacco alkaloids during aging, curing, fermentation and combustion [7, 8]. *N*-nitrosonornicotine (NNN), 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and its metabolite 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanol (NNAL), are the most important of the TSNA because of their abundance and strong carcinogenicity [6, 9, 10]. NNK and NNN are classified as human carcinogens [11].

To estimate health concerns of non-smokers' exposed to TSNAs in the indoor environment, it is necessary to develop a fast, sensitive, and selective analytical method that can accurately determine low levels of TSNA in SHS. The most widely used method for TSNA analysis has been gas chromatography (GC) coupled with a thermal energy analyzer (TEA) [12-14]. However, the GC-TEA method has two main disadvantages: (1) it cannot differentiate the co-eluted nitroso-compounds although it is nitroso-specific, and (2) extensive sample preparation, including pre-concentration and clean-up using liquid-liquid (L-L) extraction and/or solid phase extraction (SPE), is necessary due to the limited sensitivity of the method. Recently, LC-MS/MS methods have been developed for TSNA analysis, which provide greater sensitivity and selectivity than the GC-TEA method [15-18]. However, sample matrix effects, can lead to poor analyte recoveries and decreased accuracy and precision. Sample clean up using L-L or SPE [15, 18] can reduce

these effects, but this increases analysis time and limits sample throughput. Another method uses stable isotopically-labeled standards [15-17]. Although this approach is effective, it significantly increases the cost of the analysis, making it impractical for routine use. Recently, Zhou et al. [19] described a new quantitative and sensitive method for the determination of four TSNAs (NNN, NNK, N-nitrosoanatabine (NAT) and N-nitrosoanabasine (NAB)) in mainstream cigarette smoke using gas chromatography coupled to ion-trap tandem mass spectrometry. Despite the high specificity of ion trap (IT) MS^n, a drawback of the method is the need for extensive sample preparation, using L-L extraction, pre-concentration, and SPE clean-up.

Here we describe an improved analytical approach for determination of TSNAs in SHS based on the use of GC-IT-MS/MS. The method takes full advantage of the specificity and sensitivity of IT-MS^n, where sample preparation involves only extraction of SHS-loaded substrates with methanol followed by centrifugation. We illustrate the method by measuring concentrations of NNN, 4-(methylnitrosamino)-4-(3-pyridyl) butanal (NNA), NNK and NNAL in actual SHS samples. Coupling the high sensitivity/specificity of an ion trap with minimal sample preparation results in a simple assay that greatly increases sample throughput compared with existing methods for determination of TSNAs.

2. Experimental

2.1.   Caution

NNN, NNA, NNK and NNAL are carcinogenic and must be handled with extreme care inside a fume hood with ventilation using appropriate personal protective equipment (nitrile gloves, lab coat, safety glasses).

2.2. Chemicals

NNN, NNA, NNK and NNAL are from Toronto Research Chemicals (Ontario, Canada). Reagent-grade nicotine and quinoline (internal standard, IS) are from Aldrich Chemicals (Milwaukee, WI). HPLC grade methanol and acetonitrile are from Burdick & Jackson (Muskegon, MI).

2.3. Standard and QC sample preparation

Stock solutions of nicotine and TSNAs were prepared by weighing the appropriate standard and dissolving in methanol at concentrations 1-2 mg mL^{-1}. Stock solutions were diluted to 1 and 10 μg mL^{-1}, and stored in the dark at 2-8 $^{\circ}$C in amber glass vials with Teflon-lined caps. Calibration standards were prepared in the range of 2-1000 ng mL^{-1} by dilution of intermediate solutions in methanol. A 32 μg mL^{-1} solution of quinoline was added to all samples to achieve a final IS concentration of 32 ng mL^{-1}. Solvent and matrix blanks were included in all sets of calibration standards. All glassware was washed with a solution of potassium hydroxide in ethanol and rinsed with deionized water.

Positive identification of analytes was achieved by the following criteria: (1) absolute retention times match those of authentic reference standards; (2) the isotope ratio of two characteristic ions agree to within 10-15% of the theoretical values; (3) the S/N ratio is greater than 3 for the selected ions.

2.4. Stainless steel test chamber

SHS was generated in an 18-m^3 environmental chamber using a programmable ignition system connected to a smoking machine (ADL/II smoking system, Arthur D. Little, Inc.) that vented the mainstream smoke out of the chamber (Table 1). The sidestream smoke mixed with chamber air, which was exhausted without recirculation. Nine cigarettes (Marlboro, Altria Group, Inc.) were smoked at equal intervals over a 3-h period with the chamber ventilated at 1 h^{-1}. Total smoking time from ignition to burnout was 4-5 min per cigarette. Three types of environmental samples were collected:

2.4.1. TSNA passive sampling

Passive sampling onto cellulose substrates was used to evaluate the deposition of TSNAs and other SHS-related pollutants onto indoor surfaces. Rectangular cellulose strips (cut from chromatography paper, Whatman Cat. No. 3030-153, 23 cm x 1 cm) were placed inside in the chamber for the 3-h period on a table covered with aluminum foil and at a distance of ~1 m from the cigarettes.

2.4.2. particle-bound TSNA active sampling

Active sampling of SHS particles at 100 L min^{-1} was carried out continuously during the 3-h period using two Teflon-coated glass filters (TCGF, 90 mm diameter) in series. The filters were preceded by a cyclone (URG Corporation, Chapel Hill, NC) to remove particles larger than 2.5 μm diameter. After collection, the TCFG filters were individually wrapped in aluminum foil envelopes, placed in clean plastic bags and stored in the freezer at -20 ^{o}C.

2.4.3. Gas-phase nicotine

Gas-phase nicotine was passively sampled using Tenax-TA sorbent glass tubes. Tenax tubes were analyzed by thermal desorption/gas chromatography as described previously [20].

2.5.   Extraction procedure

TSNAs sorbed onto cellulose passive samplers were extracted with methanol. Each cellulose substrate was cut in half and weighed. Each half was transferred to a 40-mL amber flask containing 5 mL of methanol spiked with quinoline with final concentration of 32 μg.L^{-1}. Next, the flasks were stirred for 20 min at room temperature. Solutions were centrifuged for 20 min at 10,000 rpm. After centrifugation, particles were discarded and 1-mL aliquots of the supernatant were transferred to amber vials and capped prior to analysis by GC-IT-MS/MS, while the remaining supernatant was archived. TCFG filters were similarly extracted. In preliminary experiments, methanol and acetonitrile were tested as solvents for the extraction of TSNAs. However, methanol yielded higher recoveries of TSNAs. In addition, 20 min extraction time provided the best recoveries for all TSNAs.

2.6.   GC-IT-MS/MS conditions

The GC-IT-MS/MS analyses were performed on a Varian 3800 gas chromatograph (Varian Chromatography Systems, Walnut Creek, CA) equipped with a CP8400 autosampler, Model 4000 ion trap mass detector, GC/MS Workstation v. 6.6 software, and VF-5ms column (30-m length, 0.25-mm ID, 0.25-μm film, Varian part No. CP8944). Helium was used as the carrier gas, at a constant flow rate of 0.8 mL min^{-1}. The injector was operated in splitless mode and programmed to return to split mode 1 min after run

start. The injector temperature was set at 200 ˚C. The GC oven temperature program was as follows: 60 ˚C hold 2 min, ramped to 210 ˚C at 15 ˚C min^{-1}, hold for 6 min, then ramped at 30 ˚C min^{-1} to 300 ˚C and hold for 4 min. The mass spectrometer was operated in the electron ionization (EI) mode at 70 eV. The mass range was scanned from 50 to 350 m/z at 0.82 sec/scan for the full-scan mode.

For MS/MS, all compounds were analyzed using a resonant waveform type. A multi-segment acquisition method, using a series of different scan functions programmed to the retention time windows of TSNAs, was created to program the sequential EI/MS/MS experiments by applying the appropriate ion preparation method (IPM). These IPM processes are based on the Toolkit software and composed of only one microscan with the characteristic MS/MS parameters used for each TSNA. The advantage of this step is the reduction of noise and the increase in selectivity and specifity of the recorded spectra. A low target value (e.g. 5,000 counts) was required to minimize space-charge effects [21]. General parameters were as follows: multiplier voltage offset: +200 V, filament emission current 50 µA, target value 5,000 counts, manifold temperature, 80 ˚C whereas trap and transfer line temperature were set at 200 ˚C.

3. Results and discussion

Our goal was to develop and validate a simple and rapid method for the detection/identification/quantification of TSNA in SHS samples. To achieve maximum sensitivity/selectivity, the method development was carried out following a three step process: i) selection of appropriate precursor/product ions and elucidation of fragmentation patterns; ii) optimization of the IT-MS/MS parameters, and iii) evaluation of the method for the analysis of real SHS samples.

3.1.   Mass spectrometry of TSNAs

To date, only two studies [22, 23] have been devoted to the elucidation of fragmentation patterns of TSNAs in tandem mass spectrometry. These investigations used LC-MS/MS with an electrospray ionization (ESI) source and a triple quadrupole analyzer. However, ESI is characterized as a soft ionization technique, which primarily yields little fragmentation. In addition, the activation processes and collision gas are not the same using a triple quadrupole, which can lead to noticeable differences in MS/MS spectra compared to an ion trap. Therefore dissociation pathways are suggested below to assist interpretation of the MS/MS spectra of the compounds of interest.

Figure 1 shows the EI mass spectra of NNN, NNA, NNK and NNAL, as well as tentative fragmentation pathways for the formation of the main characteristic fragments. Full-scan MS spectra were obtained by GC-IT-MS analysis of a standard solution at concentration 1 μg mL^{-1}. As reported [24], the initial predominant fragmentation step for NNN, NNK and NNAL is due to the loss of a NO$^{\bullet}$ (M-30)$^{+}$ resulting in the formation of products ions at m/z 147, 177 and 179, respectively. In contrast, the ion m/z 177 (M-30)$^{+}$ is apparently absent in the MS spectrum of NNA. This difference may be a consequence of facile cleavage of the CH_3N_2O group, apparently favored by the neighboring pyridine ring. This is consistent with the presence of a characteristic ion m/z 148 (M-59)$^{+}$ in the NNA mass spectrum. For NNAL, another initial fragmentation step is postulated as shown. It involves an intra-molecular protonation of hydroxyl group resulting in an initial loss of H_2O. Subsequent loss of the NO group produces an ion at m/z 161 that may rearrange to form ionized nicotine, as described by Byrd et al [23].

After scanning the full MS spectra, the first step of the IT-MS/MS optimization procedure was the selection of an appropriate precursor ion for each TSNA. To achieve the highest sensitivity, the most abundant ion (the base peak) was selected. . Thus, ions m/z 147 for NNN, m/z 148 for NNA, m/z 177 for NNK and m/z 148 for NNAL were selected. To elucidate the identity of the main characteristic product ions (Figure 1), we worked out possible structures and compared these with literature data [22-24] For NNN, the main product ions of m/z 147 were m/z 145, 118 and 105, which arise from loss of molecules H_2, C_2H_4 followed by H$^{\bullet}$ radical, and C_3H_6 respectively. NNA precursor ion m/z 148 looses a CO moiety and produces the ion m/z 120, which can fragment further to give ion m/z 92. For NNK, three major product ions (m/z 146, 159, 106) were observed in the collision induced dissociation CID spectrum of precursor ion m/z 177. The predominant product ion m/z 146 arises from loss of methylamine (CH_3NH_2) whereas an elimination of H_2O molecule yields the ion m/z 159. Another fragmentation pathway is the cleavage of C-C in position α nearby the carbonyl moiety leading to the formation of m/z 106. In a similar manner, the precursor ion of NNAL m/z 148 looses a C_3H_6 molecule and forms the stable product at m/z 106.

The proposed fragmentation pathways, displayed in Figure 1, are in agreement with the product ion structures and the mass spectra obtained. However, the mechanistic basis postulated for the formation of some characteristic ions requires further investigation.

3.2.   Optimization of IT-MS/MS parameters

The next steps were to select the most appropriate mass transition for each TSNA congener and to optimize the ion trap MS/MS parameters for maximum sensitivity and specifity. These parameters included the resonant excitation voltage (REV) and "q" value referring to the RF voltage. Results are summarized in Table 2.

The isolation window (m/z) and excitation time (ms) were also investigated. . However, no statistical differences were found in the S/N ratio of target ions after changing these parameters, so default values were used as follows: isolation window 3 m/z and excitation time 20 msec.

3.2.1. Resonant excitation voltage (REV)

In IT-MS, the resonant excitation voltage (REV), also known as collision energy, refers to the radio-frequency (RF) voltage applied to the end-cap electrodes of the IT-MS, which produces ions from the precursor ions through CID.

Four series of injections of a standard solution of four TSNAs (100 pg μl $^{-1}$ for each congener) were made for the purpose of optimizing signal intensities of product ions by varying REV between 0 and 0.8 V, stepped by 0.2 V. Figure 2 illustrates the change in the relative abundance of product ions and their sum with REV for each TSNA congener ("q" value was set at 0.40 and other parameters as default values).

For all TSNAs, the signal of precursor ion decreased strongly when REV was increased from 0.2 to 0.4 V, indicating a relatively simple fragmentation. In addition, the relative abundances of product ions reached their highest values at a specific REV between 0.4 and 0.6 V. Thereafter, when REV was increased, the relative abundance decreased for all TSNA congeners. This is because the high energy not only causes the precursor ions to fragment, but also causes the product ions to dissociate. For each TSNA, an optimum

REV value was selected, corresponding to the maximum of relative abundances of major product ions (see table 2).

To provide supplementary structural information and avoid false positive identification, the precursor ion was also acquired if its relative abundance was higher than 10% at optimum REV (0.4 V for NNN and 0.38 for NNA). Unfortunately, for NNK and NNAL, the maximum of relative abundances was obtained for REV higher than 0.4 V. Therefore, for these compounds, the optimum REV value was selected to maximize the sensitivity without taking into account the precursor ion. Nevertheless, apart from the product ion chosen for the quantitation (see Table 2), at least one additional product ion was also acquired in order to confirm the identity of each analyte.

On the other hand, to improve the specificity of the detection, only daughter ions characteristic of the individual analytes were used for quantification. The parent or precursor ion was systematically excluded from the quantification analysis since it could originate from several molecules and consequently has a low specifity. The quantitation ions were selected based on the criteria of peak intensity and ion specificity as well as potential interferences from other compounds (see Table 2). Thus, at optimum REV, the most abundant mass transition with a higher product mass was selected for quantitation. This decreased chances of co-eluting interferences, which are more common at low *m/z* values, as well decreased background noise in the chromatogram.

3.2.2. Optimization of 'q' value

In the MS^n mode, the 'q' value refers to the RF voltage of constant frequency and variable amplitude that is applied to the ring electrode of the ITMS, governing the low end of the product ion m/z range and also the extent of fragmentation. A higher 'q' value

allows more energy to be deposited into the precursor ion, but some product ions may be rejected out of the trap. In contrast, a lower 'q' value provides less energy and causes fewer collisions of the ions, but it ensures that the product ions stay in the trap. In this work, optimization of 'q' value was performed by multiple injections of TSNA standards under the optimal REVs. The results showed that the signal intensities of most TSNAs increased with increasing 'q' from 0.25 to 0.45 and then decreased beyond 0.5. Optimal 'q' levels for TSNA were found to be in the range of 0.39 - 0.46, as shown in Table 2.

3.3.   Method Performance

We evaluated the following performance criteria: extraction recoveries, linearity, accuracy, precision, sensitivity, selectivity and stability of analytes.

3.3.1. Extraction recoveries

Since the tobacco smoke matrix always contains TSNAs, estimation of method recovery was performed using blank matrix spikes. Blank cellulose substrates and TFGC filters were spiked, in triplicate, with 500 ng of each TSNA standard and quinoline (IS) to represent levels expected in extracts of indoor samples. All samples were extracted, prepared and analyzed using the procedure described above. Extraction efficiency of each TSNA was determined by calculating the mean of the experimentally measured amounts and dividing by the nominal amount. Table 3 presents recovery results for both TCFG and cellulose extracts. The recoveries ranged between 85 and 115%, with good reproducibility, as shown by the low relative standard deviation values (RSD < 10%). No further clean up of the extracts was required as the ITD chromatogram was highly specific and no overlaps in the chromatogram occurred during analysis.

3.3.2. Linearity

Quantification was performed by means of 5-point calibration curves for which the concentrations of a mixture of TSNAs standards ranged from 2 to 1000 ng mL^{-1} whereas the concentration of IS was set at 32 ng.mL^{-1}. For all TSNAs, linear response was obtained with typical values for the correlation coefficient (R^2) higher than 0.998.

3.3.3. Selectivity

Selectivity was evaluated by analyzing a different set of blank samples of TCFG and cellulose filters and monitoring the absence of interferences with S/N ratios higher than 3. No significant interfering peaks were observed in the ion chromatogram time windows monitored for each compound, and no false positive responses were observed for any of the TSNAs. Considering that no clean-up step is required during sample preparation, the selectivity of the IT-MS/MS method is extremely powerful, mainly due to the appropriate choice of mass transitions and MS/MS parameters. Although not evaluated here, real indoor air samples may have interfering peaks or coeluting compounds; however, the IT-MS/MS is still expected to provide adequate selectivity because the appropriate specific mass transition (parent ion – fragment ions) was selected for each TSNA.

3.3.4. Accuracy and precision

The accuracy of the method was evaluated by taking two aliquots of the same prepared smoke sample and then spiking one of the aliquots with a known concentration of each TSNA. The concentrations of fortified matrix spikes were approximately five times the TSNA concentrations in the smoke samples. Accuracy was calculated as the mean of the experimentally determined concentration (subtracted from the unspiked measured

concentration) from replicate analysis divided by the nominal concentration. As shown in Table 3, accuracy for all analytes was > 90% except for NNAL (77%). The lower accuracy for NNAL could be related to the high SD for NNAL extraction (see Table 3), as well as to possible loss of analyte during preparation and analysis. We expect that the accuracy of NNAL can be improved if a nitroso-compound such as NNPA or a deuterated nitrosamine were available for use as an internal standard.

The precision of the method was determined by calculating the relative standard deviation (RSD) of six replicate measurements collected from the chamber. All RSDs were acceptable and less than 10% (see Table 3) indicating a good precision of our method.

3.3.5. *Sensitivity*

The method detection limits (MDLs) were estimated, as described by the U.S. EPA, from the standard deviation (σ) of multiple measurements (n=7) of TSNA solutions within a factor of 5 of the MDL. The MDLs were set equal to 3σ, considering a 99% interval confidence[25]. The estimate MDLs for NNN, NNA, NNK and NNAL were 70, 30, 120 and 340 pg mL^{-1}, respectively. On the other hand, the method quantification limit (MQLs) were estimated by analyzing TSNA standards in the range of 10-100 ng mL^{-1} and defining MQL as 10σ, as indicated by the American Chemical Society for samples with RSD lower than 10% [25]. In all cases, MDLs and MQLs were significantly below the levels determined in the SHS samples (see below). Furthermore, compared to other methods which involve a preconcentration step and clean-up procedure, MDLs for this method are much lower than for the GC/TEA method, are similar to those found using LC/MS/MS [15-17] and are better than those reported by Zhou et al. [19] using GC-IT-

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MS/MS. Lower MDLs may be attained by using an accelerated solvent extraction (ASE) with lower solvent volumes, or employing a preconcentration step using rotary evaporator or solid phase cartridges (SPE). However, this adds another step to the procedure and may increase the time required and uncertainty, which would likely limit the method throughput.

3.3.6. Stability

The stock solutions of TSNAs prepared in methanol and stored at −20 ˚C were found to be stable under short-term (bench-top, 48 h, 20 ˚C) and long-term storage conditions (freezer, 1 month, −20 ˚C). Under these conditions, analysis of these samples consistently provided values with a maximum %RSD of 7 %. On the other hand, diluted standards and sample extracts kept in the freezer at 0 ˚C for more than a week were found to be relatively stable with a %RSD lower than 10 %. This indicates that no significant degradation occurred and confirms the overall stability of analytes during storage and preparation procedures.

Regarding stability during GC-MS analyses, a noticeable loss of analytes was observed for injection temperatures exceeding 210 ˚C, which was attributed to thermal degradation of TSNAs in the injector.

3.3.7. Artifact formation

Artifact formation of TSNAs during sample collection has been investigated by several groups [12, 26]. For example, Caldwell and Conner [26] suggested that artifacts form via nicotine nitrosation during smoke collection. In contrast, other groups [15, 27] did not observe artifact formation during collection of TSNAs on untreated Cambridge Filters. It

has been reported that the formation of artifactual nitrosamines can be reduced with the addition of ascorbic acid to the sample collection system [26]. With our assay, we did not detect a significant difference in the concentrations of four selected TSNAs with or without pretreatment with ascorbic acid (20mg L^{-1} dissolved in methanol). Therefore, we did not use pretreated filters.

3.4.   Application to the analysis of SHS in indoor environment

To date, only a few studies have reported measurements of TSNAs in SHS polluted indoor environments [13, 28-31]. This may be attributed to the low levels of these compounds combined with the lack of methods suitable for routine analysis of TSNAs. To demonstrate the potential of our GC-IT-MS/MS method, we collected samples in a SHS-contaminated environmental chamber and analyzed these samples as described. The chamber experiment was conducted at realistic conditions (see Table 1) indicative of a poorly ventilated room polluted with SHS. Figure 3 depicts relevant segments of GC-MS/MS chromatograms, corresponding to (A) a standard solution of TSNAs, (B) an extract of SHS passively collected onto a cellulose substrate and (C) an extract of SHS particulate matter collected using a TCGF filter.

NNN, NNK and NNA were detected and identified in the extract of the TCGF filter whereas only NNK and traces of NNA were found in the extract of the cellulose substrates. The levels of NNN, NNK and NNA in SHS particles reported on a whole-air concentration basis were 4.4 ng m^{-3}, 2.0 ng m^{-3} and 0.58 ng m^{-3}, respectively (Table 5). These concentrations are consistent with those reported in the literature. For example, Piade et al. [30] measured an NNN concentration of 2.3 ng m^{-3} in the SHS generated in

an 18-m^3 chamber using the same cigarette brand. Klus et al. [29] reported mean levels in the range of 0-6.0 ng m^{-3} for NNN and 0-13.5 ng m^{-3} for NNK in offices with poor ventilation conditions. Similar levels of 0-23 ng/m^{-3} NNN and 3-24 ng m^{-3} NNK, were reported in heavily smoke-polluted bars and restaurants [13].

4.   Conclusions

We have developed and validated a sensitive, selective and fast GC-IT-MS/MS method that provides a powerful analytical tool for unequivocal identification and reliable measurement of TSNAs in SHS. MS/MS parameters, ion structures and fragmentation pathways were investigated to achieve sufficient sensitivity and specificity allowing us to avoid the use of cleanup procedures in sample preparation.

Analysis of TSNAs in SHS samples collected from a simulated but realistic indoor environment revealed that most of TSNAs are concentrated in airborne particulate matter. NNK and traces of NNA were also found to be sorbed onto cellulose substrates, a surrogate for multiple indoor surfaces (e.g. fabrics, wallpaper). To the best of our knowledge, this is the first time that NNA has been detected and measured in SHS. Furthermore, this is the first study to report the detection and measurement of TSNAs in SHS directly sorbed onto model indoor surfaces. The method, when applied to particulate air samples and surface samples, may help to develop more accurate models for non-smoker exposures to TSNAs in SHS.

Acknowledgments

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FIGURES AND TABLES

Figure 1. EI mass spectra of NNN, NNA, NNK and NNAL with suggested fragmentation pathways and chemical structures of characteristic fragments.

Figure 2. Dependence of precursor and product ion intensities on the resonant excitation voltage (REV). The optimum REV values for each TSNA are listed in Table 2.

Figure 3. Extracted MS/MS chromatograms of TSNAs obtained from the analysis of standard solution (A), SHS samples collected on cellulose substrates (B) and SHS sample collected on TFGC filters (C).

Table 1. Experimental parameters for the chamber test with secondhand smoke.

Table 2. GC-IT/MS/MS parameters for TSNAs analysis in secondhand smoke.

Table 3. Extraction recovery of TSNAs from TCFG filters and cellulose substrates.

Table 4. Method performance for the analysis of TSNAs in secondhand smoke

Table 5. Levels of TSNAs in SHS samples collected on TCFG filters and cellulose substrates
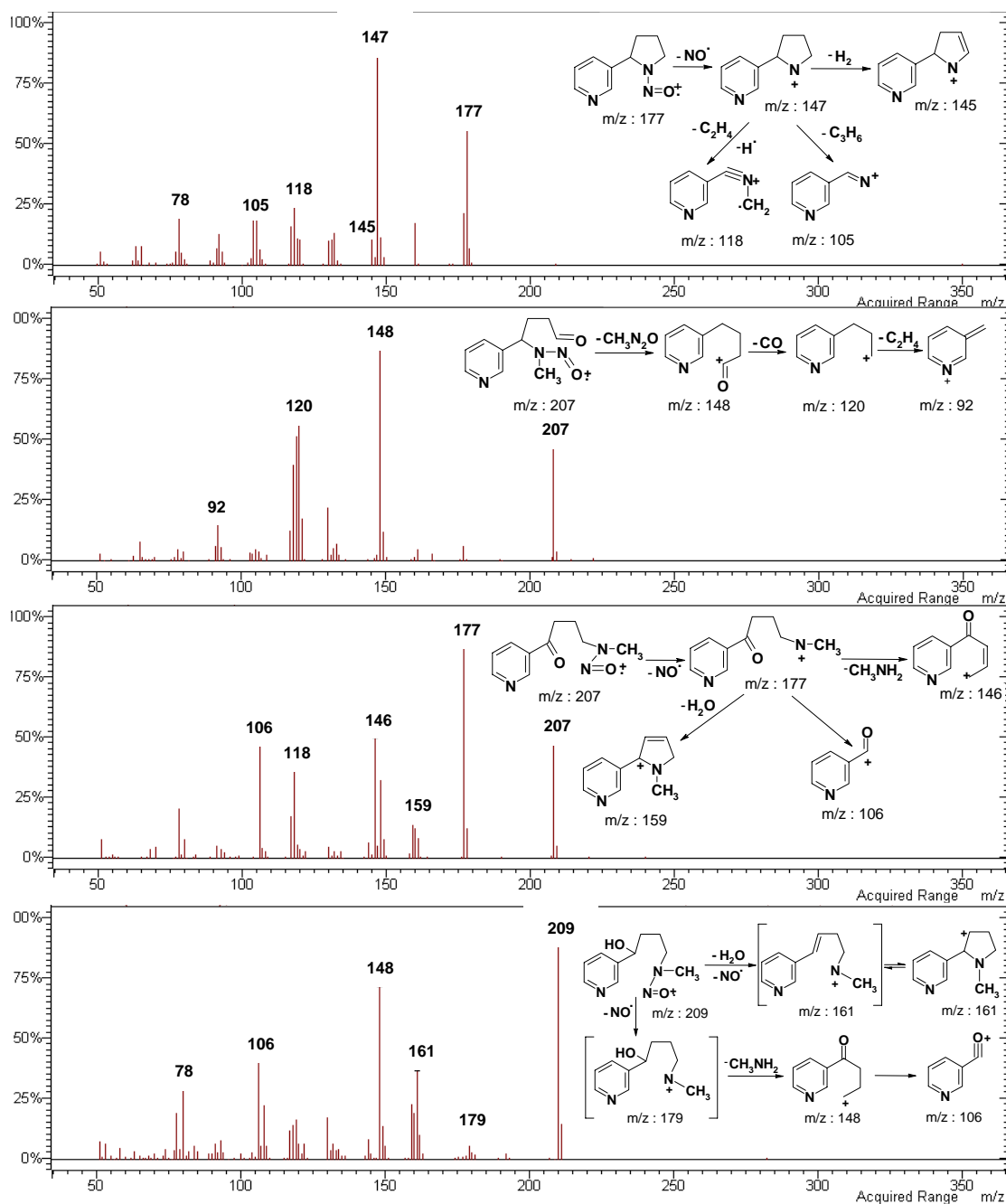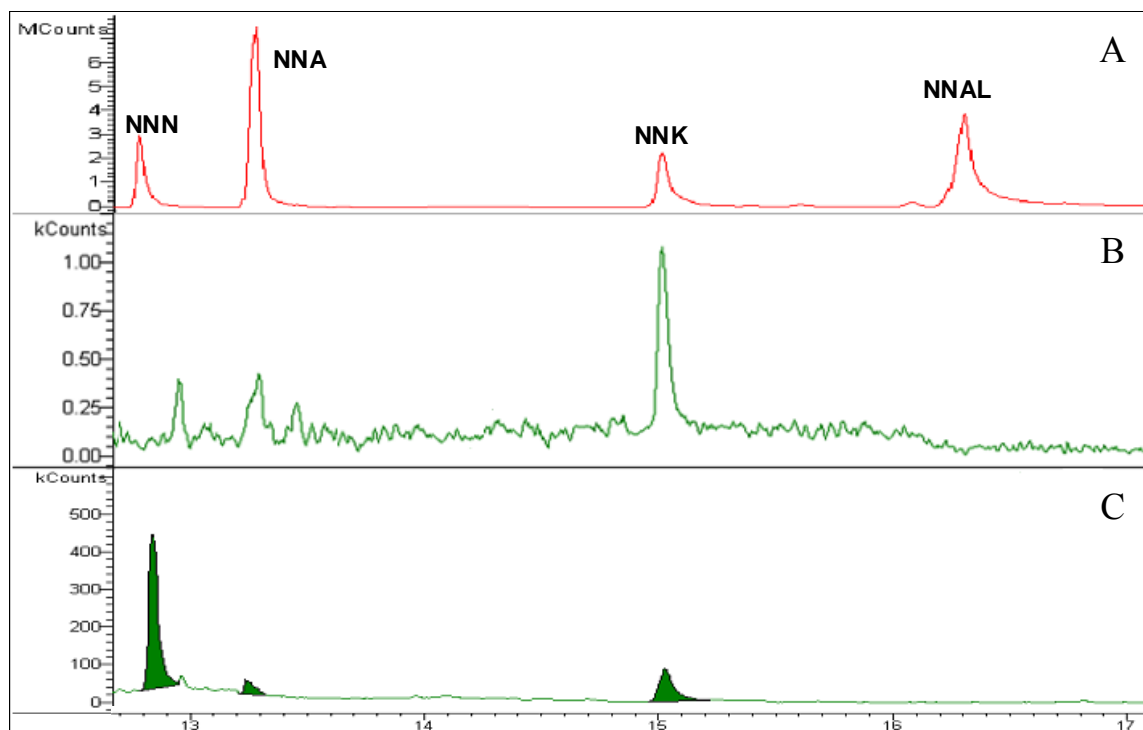
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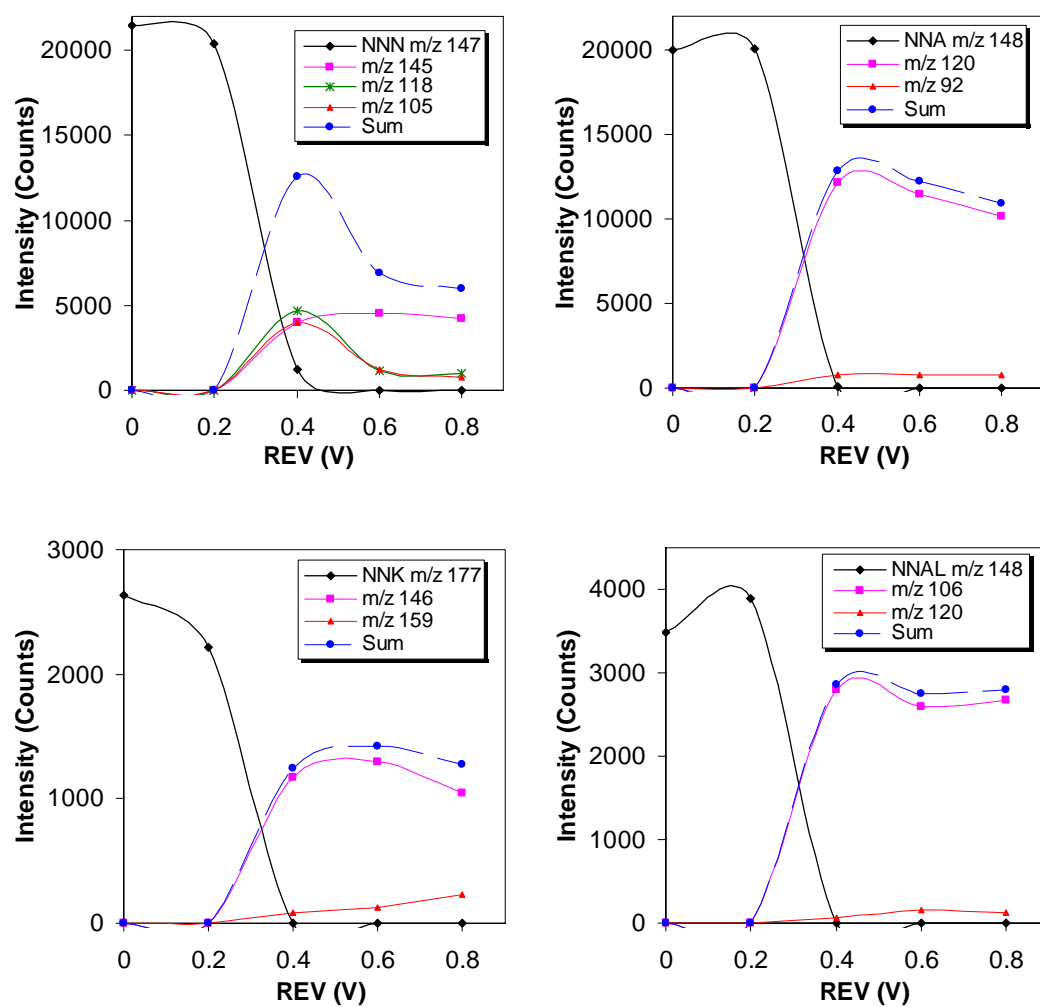
Figure 2.   Dependence of precursor and product ion intensities on the resonant excitation voltage (REV). The optimum REV values for each TSNA are listed in Table 2.

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Table 1. Experimental parameters for the chamber test with secondhand smoke

Parameter	
Chamber volume (m^3)	18
Cigarettes smoked	9
Temperature (˚C)	24
Air-exchange rate (h^{-1})	0.33
Flow rate through filter sample (L min^{-1})	100
Filter sampling duration (min)	176
Volume of air collected with filter (m^3)	17.6
Net mass of particles on TFGC filter (mg)	17.8
SHS concentrations of TFGC filter ($mg.m^{-3}$)	1.01
Nicotine gas phase concentration ($\mu g.m^{-3}$)	60

Table 2. GC-IT/MS/MS parameters for TSNAs analysis in secondhand smoke

Compound	RT (min)	Precursor ion (m/z)	Product ions (m/z)	REV (V)	"q" value
NNN	12.8	147	145 [a], 120[a], 105 [b]	0.4	0.46
NNA	13.3	148	120 [a], 92 [b]	0.38	0.42
NNK	15.1	177	159 [b], 146 [a]	0.6	0.44
NNAL	16.2	148	106 [a], 78 [b]	0.48	0.39

[a] *quantitation ion.* [b] *confirmation ion*

Table 3. Extraction recovery of TSNAs from TCFG filters and cellulose substrates

Analyte	Recovery ± SD[a] (%)	
	TCFG [b]	**Cellulose** [b]
NNN	88 ± 5	95 ± 6
NNA	114 ± 7	104 ± 4
NNK	107 ± 5	91 ± 6
NNAL	113 ± 11	83 ± 9
Mean ± SD	105 ± 7	93 ± 6

[a] *Mean ± standard deviation of six replicate analysis of separate samples (n=6).*

[b] *100-200 ng of each compound was used for spiking.*

Table 4. Method performance for the analysis of TSNAs in secondhand smoke

Analyte	Spiked (ng mL^{-1})	calculated (ng mL^{-1}) [a]	Accuracy (%) [b]	Precision (%) [c]		MDL [d] (pg mL^{-1})
				Intra-assay	Inter-assay	
NNN	98	93	95	2.8	7.2	70
NNA	102	105	103	2.6	6.4	30
NNK	94	87	93	3.0	8.8	120
NNAL	106	82	77	5.9	11.3	340

[a] calculated from the linear regression standard curves. [b] calculated as: (calculated concentration /spiked concentration) x 100 % . [c] expressed as % RSD. [d] limit of detection determined from injections of TSNA standards in methanol.

Table 5. Levels of nicotine and TSNAs in SHS samples collected on TCFG filters and cellulose substrates

Analyte	Level (ng.m^{-3})	Level (ng.m^{-2})
	TCFG [a]	Cellulose [b]
NNN	4.36	< 20 [c]
NNA	0.58	60
NNK	1.95	990
NNAL	< 0.1 [c]	< 70 [c]

[a] level of nicotine in indoor air was 60 µg m^{-3}. [b] Nicotine concentration on cellulose surface was 16 mg. m^{-2}. [c] below the MLD