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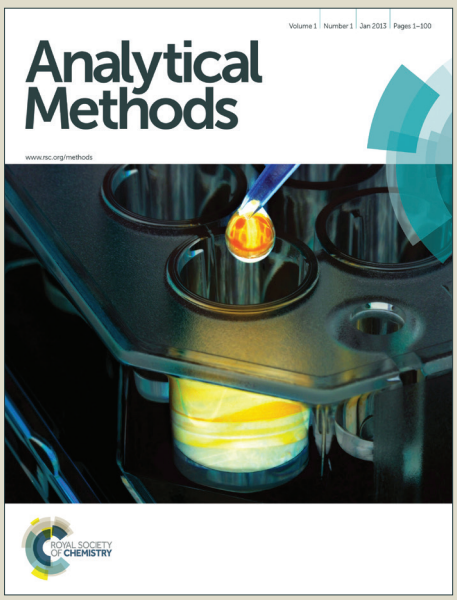

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Establishment of an analytical method for the measurement of tobacco-specific nitrosamines in E-cigarette aerosol

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Abstract

We developed a sampling method for tobacco-specific nitrosamines (TSNAs) in aerosol from electronic cigarettes (E-cigarettes) termed a gas-tight syringe (GTS) method based on extraction with solvent at the same duration of aerosol introduction in a gas-tight syringe. The analytes were dissolved in the solvent injected already in a GTS, and the extract was directly injected into liquid chromatography-tandem mass spectrometry (LC-MS/MS). The optimum extraction conditions of TSNAs were the following: solvent, methylene chloride; volume, 10 mL; shaking time, 3 min. The GTS method and the conventional impinger method were compared. The GTS method showed good recoveries of 98% - 103% with a small relative standard deviation (RSD) of 2%. The per puff (50 mL puff) amounts of TSNAs were 3.5-45.5 pg for *N'*-nitrosonornicotine, 3.5-759.5 pg for 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, 3.5-164.5 pg for *N'*-nitrosoanabasine, and 3.5-612.5 pg for *N'*-nitrosoanatabine in aerosol of 50 brands from 11 electronic cigarette companies purchased in the Korean market. When the developed method was applied to aerosol from E-cigarettes using the prepared replacement liquids containing TSNA precursors, no TSNAs were observed. Therefore, it is suggested that TSNAs were not formed through the operation of E-cigarettes. We also used the same method to detect the levels of TSNAs in the mainstream from 10 cigarettes produced by 5 companies purchased in Korea. Observed total TSNA levels in E-cigarette

were about 100 folds lower than those of the conventional cigarettes.

1. Introduction

Electronic cigarettes (E-cigarettes) are battery-powered devices that transfer liquid containing nicotine, aromas and flavors into aerosol that can be inhaled.[1, 2] The appearance of E-cigarettes was designed to mimic a conventional cigarette.

Tobacco-specific nitrosamines (TSNAs) are very important hazardous compounds identified in the cartridge liquid of E-cigarettes.[3-6] *N'*-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) are, specifically, classified as a human carcinogen (Group 1) by the International Agency for Research on Cancer (IARC).[7] *N'*-Nitrosoanabasine (NAB) is a weak esophageal carcinogen in rats, while *N'*-nitrosoanatabine (NAT) is not a carcinogen.[8] Our preceding research reported concentration ranges for NNN, NNK, NAB, and NAT in 105 replacement liquid brands from 11 electronic cigarette companies.[6] A previous study examined the presence of TSNAs in the aerosol generated from 12 models of E-cigarettes.[9] It is still unknown whether TSNAs present in the cartridge liquid are aerosolized during the operation of E-cigarettes or if they are produced from precursors in a heating system of an E-cigarette.

Until now, analytical methods about the substances that are present in the aerosol of E-

cigarettes are limited. Sampling is one of the most important steps for the quantitative analysis of TSNAs in the aerosol of E-cigarettes. A sampling of TSNAs aerosolized from E-cigarette has been performed by impinger method,[3] in which the aerosol from E-cigarette was collected in buffer solution, which was used to extract analytes with an organic solvent, which was injected after condensation. One of the problems with this approach was that the analytes may not be effectively absorbed into the solution and their recoveries had very low values. Moreover, the method suffered from the disadvantages of being time-consuming and using large amounts of toxic organic solvents.

These shortcomings necessitated the development of a new cost-effective method with special emphasis on speed, usage of negligible volume of organic solvent, and ability to detect analytes at low concentrations. We developed a sampling method, termed a gas-tight syringe (GTS) method, in this study. Sampling with the GTS method was based on aerosol introduction in a gas-tight syringe; thus, the analytes were directly dissolved into the solvent injected in a gas-tight syringe.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been used to determine TSNAs in E-cigarette and mainstream cigarette smoke. It has also been adapted to users with high sensitivity and has been manufactured through a reproducible method in recent years.[3-6, 9-15]

This study aims to develop a sampling method of TSNAs in aerosol from E-cigarette, and to quantify the concentration of four TSNAs in aerosol generated from 50 brands from 11 E-cigarette companies purchased in Korean shops. Several sampling parameters were studied in order to select parameters with a high sensitivity and low interference in the sampling.

2. Experimental

2.1. Materials

NNN, NNK, NAT, NAB, NNN-d4, NNK-d4, NAT-d4, NAB-d4, nicotine, nornicotine, anatabine and anabasine were purchased from Sigma-Aldrich (St. Louis, MO, USA), along with analytical grades of methanol, acetone, methylene chloride, pentane, methyl-*t*-butyl ether, ethyl acetate, propylene glycol, potassium hydroxide, ascorbic acid, dipotassium phosphate, monopotassium phosphate, and HCl. The water used in this study was purified by a Milli-Q-Reagent-Grade water system (ZD20) and had a resistivity of over 17 MΩ. A gas-tight syringe (100 mL) and a Teflon stopcock were purchased from Hamilton (Reno, Nevada, USA) and Labcampus (Seoul, Republic of Korea), respectively. The gas-tight syringe device consisted of a removable plunger inserted in a syringe (0.53 mm in ID) containing extracting solvent and stopcock. This is shown in Fig. 1.

2.2. E-cigarettes and conventional cigarettes

Replacement liquids were purchased in July and August, 2012 from 11 E-cigarette shops in various regions in Republic Korea. These shops imported directly the liquids from the manufacturer in China, which were mixture of solution type containing nicotine and various flavoring agents. All samples were analyzed within two months of purchase after storage in a refrigerator at 4 °C. Two E-cigarette devices (Wetop Power) were purchased from RUYAN (China), and have stainless steel casing and tank-type delivery system.

Ten conventional cigarette brand types were purchased from a cigarette shop in Republic Korea made from Korean tomorrow and global (KT&G), Japan tobacco international, Philip Morris International, and British American Tobacco. These were a blended type containing burley tobacco.

2.3. GTS extraction procedure

The E-cigarette device with the selected cartridge filled with the standard spiked samples or commercial nicotine liquids was connected to the GTS via a short tygon tubing. 50 mL of aerosol was drawn through the E-cigarette into the GTS for 5 seconds. The E-cigarette was observed to assure that the LED lit, indicating that the flow rate was sufficient to activate the heater in the E-cigarette. After 50 mL puffs were trapped in 10 mL of the aerosol-dissolving

solution spiked with internal standards (each 10 ng of NNN-d4, NNK-d4, NAT-d4, and NAB-d4), the syringes were turned upside down five times while being shaken intensely by hand for 30 s before being shaken mechanically. The sample was allowed to mix for an additional 3 minutes mechanically, and the glass in the syringe was rinsed with the trapping solution in the test tubes. The solution was evaporated gently with nitrogen stream, dissolved with 100 μL of 0.01% formic acid in water, and directly injected into LC-MS/MS after filtration.

2.4. Impinger extraction procedure

Impinger extraction procedure was done using a variation of the method published by the US FDA.[3] A trapping device consisted of a 150 mL aerosol washing bottle with sparger. A magnetic stirring bar was added to the aerosol washing bottle along with 50 mL of extraction solution spiked with internal standards (each 10 ng). Extraction solution was prepared by mixing 100 mL of acetonitrile, 11.5 g of phosphoric acid, and 800 mL of water. A draeger 100 mL hand pump was connected to the outlet of the aerosol washing bottle. The E-cigarette device with the selected cartridge filled with the standard spiked samples or commercial nicotine liquids was connected to the aerosol washing bottle via tygon tubing. The magnetic stirrer was turned on. 100 mL of air was drawn through the E-cigarette into the aerosol

washing bottle. The E-cigarette was observed to assure that the LED lit, indicating that the flow rate was sufficient to activate the heater in the e-cigarette. After 100 mL puffs were trapped in the aerosol washing solution, the impinger system was rinsed with the trapping solution back into the aerosol washing bottle. The collected solution was used for analysis. The solution was extracted two times with 40.0 mL of methylene chloride by mechanical shaking for 10 min. The total organic phase was evaporated in a rotary evaporator under vacuum and finally dried in a nitrogen stream. The residue was dissolved with 100 μL of 0.01% formic acid in water, and the solution was transferred into a tapered auto vial, with 10 μL automatically injected in the LC system.

2.5. LC-MS/MS

The analytes were separated using a 50 mm × 2.1 mm Eclipse Plus C18 column with a 1.8 μm pore size (Agilent, USA). A binary gradient with a flow rate of 0.2 mL min^{-1} was used.

The MS-MS detection was performed on an Agilent 6460 series triple quadruple instrument (Agilent, Palo Alto, CA), while the mass spectrometer was operated with electrospray ionization in the positive ion mode (ESI+). The capillary voltage was set to 3.2 kV. The source temperature was 120 ℃ and the desolvation temperature was 350 ℃. Nitrogen was used as desolvation vapor (flow 500 L h^{-1}) and argon was used as collision

vapor at a pressure of 3×10^{-3} mbar. For each analyte, three ion transition pairs were used under multiple reaction mode (MRM). These ion pairs were 178/148, 178/105, and 178/93 for NNN; 208/178, 208/122, and 208/106 for NNK; 192/162, 192/133, and 192/106 for NAB; and 190/160, 190/106, and 190/79 for NAT. For internal standards, the following values were employed: 182/152 for NNN-*d*4, 212/126 for NNK-*d*4, 196/166 for NAB-*d*4, and 194/164 for NAT-*d*4.

2.6. Calibration procedure

The E-cigarette device with the selected cartridge filled with the standard spiked solutions was connected to the GTS via a short tygon tubing. The standard spiked solutions were made by adding 0.05, 0.5, 1.0, 5.0, 10.0, 20.0, and 40.0 ng of each TSNA in 0.5 mL propylene glycol. 50 mL aerosol was drawn through the E-cigarette into the GTS for 5 seconds, and the following procedure was performed as described in the GTS extraction procedure.

3. Results and discussion

3.1. Instrumental conditions

Our previous work[6] showed that TSNAs existing in the liquid content of the E-cigarette (propylene glycol base) could be sensitively detected by LC-MS/MS. All the parameters of

LC-MS/MS used a variation of the method established in our preceding publication. When 0.01 % formic acid in water was used as a mobile phase for TSNA separation, stable MRM could be obtained. The protonated molecular ion $[M+H]^+$ and the fragment ions of 148, 178, 162, and 160 formed by the loss of a NO from the precursor ions ($[M+H]^+$) of NNN, NNK, NAB, and NAT were used as quantitative ions.

3.2. Optimization of the GTS extraction method

The GTS extraction is based on the fact that an organic solvent is mixed with aerosol in the syringe after an aerosol sample from E-cigarettes is drawn in the syringe by withdrawing the plunger. A schematic diagram describing the process of extraction is shown in Fig. 1. If shaking is made to mix extracting solvent with the aerosol sample within the barrel of the GTS, the analyte will be well-dissolved from the aerosol into the extracting solvent, and can be enriched in the solvent. The extraction capacity of the solvent is affected by solvent type, solvent volume, temperature, shaking time, and syringe barrel geometry. For the purpose, we performed triple experiments detailed as follows.

Test standard solutions with which E-cigarettes were filled were spiked at the concentration of each TSNA 50 and 100.0 μg L^{-1} in propylene glycol and filled the E-cartridge. After the cartridge was weighed exactly, the E-cigarette device was connected to

the 100 mL GTS via a short tygon tubing, while 50 mL of the aerosol was drawn into the GTS containing the extracting solvent and internal standard through the E-cigarette. The cartridge was again separated from the inhaler unit/atomization chamber, and then it was reweighed. The amount of TSNA transferred into the aerosol was calculated from the weight difference of the cartridge before and after taking a 50 mL-puff.

For the optimum extracting solvent, methylene chloride, acetone, methanol, ethyl acetate, and hexane were tested. The extraction efficiency (in terms of recovery according to the calculated method described above) for the tested extracting solvents is shown in Fig. 2. The best extraction efficiency was determined when methylene chloride was used as an extracting solvent. When the contact area between the extracting solvent and the aerosol was enlarged, the extraction efficiency increases. To optimize the area of contact between the aerosol and extracting liquid phase within the syringe, experiments were preceded using 2.5, 5.0, 10.0, 12.5 mL, and 15 mL of methylene chloride as an extracting solvent. The best extraction yield was reached with 10 mL and there was no appreciable increase when a larger volume was applied. The results of extraction yield (in terms of recovery) for the tested extracting solvent volumes are shown in Fig. 3. To note the impact of extraction time on extraction efficiency, experiments were made over a time range of 1–10 min and the results are summarized in Fig. 3. It was observed that the extraction efficiency reached a maximum in 3 min and there was

no appreciable increase or some decrease when longer extraction time was applied. In ensure adequate mixing we tested syringe flow rate rates between 300-1200 mL min^{-1}. A small increase in extraction efficiency, with an increase in syringe speed was observed. The small increase was probably due to the fact that the extraction process was fast mixing. On the basis of the observations, a syringe speed of 600 mL min^{-1} was selected for subsequent studies.

The following optimum conditions for the GTS method were established: extracting solvent and its volume: 10 mL of methylene chloride; pumping speed: about 600 mL min^{-1}; shaking time: 3 min.

3.3. Method validation

The combination of a high extraction yield and the stable and high sensitive ion formation by ESI-MS/MS permitted the sensitive determination of TSNAs in the aerosol of E-cigarette. Test standard solutions were spiked at the optimum concentration in propylene glycol and filled the E-cartridge. The E-cigarette device with the cartridge filled with the standard solutions was connected to the GTS via a short tygon tubing, while 50 mL of aerosol was drawn into the GTS. The concentration of the analytes in the aerosol from the E-cigarette was calculated from the exhausted amount of the analytes after atomization and after the volume of aerosol was pulled.

The limit of detection (LOD) and limit of quantification (LOQ) were defined as the concentrations resulting in a minimum signal-to-noise ratio of 3 and 10, respectively, whereas the coefficients of variation for replicate determinations (n = 7) of 15% or less, from the aerosol phase of standard solution spiked in pure propylene glycol. The LODs of NNN, NNK, NAB, and NAT were 0.02, 0.02, 0.02, and 0.01 μg m^{-3} in aerosol generated from standard solutions spiked at a concentration of 0.1 μg L^{-1} for each TSNA in pure propylene glycol. Otherwise, LOQs of NNN, NNK, NAB, and NAT were calculated as 0.06, 0.07, 0.06, and 0.04 μg m^{-3} in aerosol generated from standard solutions.

Using the least-squares fit technique, an examination of the typical standard curve was performed by computing a regression line of peak area ratio for TSNAs on the concentration. This analysis demonstrated a linear relationship with correlation coefficients being greater than 0.9997. Linear equations were y = 16.001x + 0.013 (r=0.9999) for NNN; y = 17.611x + 0.006 (r=0.9999) for NNK; y = 16.768x – 0.002 (r=0.9999) for NAB; and y = 15.883x + 0.003 (r=0.9997) for NAT in a concentration range of 0.1–80.0 μg m^{-3} in aerosol generated from standard solutions.

Accuracy and precision were evaluated using spiked samples due to the impossibility of buying a certified standard material. The concentrations in spiked standard solutions were calculated from the calibration curve constructed after the aerosol generation and extraction

of samples spiked with standard solutions in propylene glycol. The concentration of the analytes in the aerosol from the E-cigarette was calculated from the exhausted amount of the analytes after atomization and the volume of aerosol pulled as described before.

Accuracy was assessed as the relative percentage of the concentration of the analytes found in aerosol to the concentration of the analytes in aerosol calculated from the spiked concentration. Precision was calculated as their relative standard deviation. Intra-day accuracy and precision were evaluated by five spiked samples at concentrations of 50 and 100 μg L^{-1} for TSNAs in propylene glycol, while inter-day accuracy and precision were determined by their recovery in spiked standard solutions on 5 different days. The accuracy was in a range of 94–106% and precisions of the assay were less than 14%, as shown in Table 1. The results indicated that this method was sufficiently reproducible to permit reliable analysis of TSNA quantity in the aerosol from E-cigarette.

3.4. Comparison of GTS and conventional impinger methods

GTS and impinger methods were compared to evaluate the efficiency for the trap of TSNAs from the aerosol of E-cigarettes.

Propylene glycol and/or glycerin make up most of the liquid in the nicotine cartridge[20,21] and test standard solutions were made of TSNAs of 50 and 100.0 μg L^{-1} in propylene glycol

and/or glycerin, and they filled the E-cartridge.

For the GTS test, the E-cigarette device with the cartridge filled with the standard spiked solution was connected to the GTS sampling system containing 10 mL of methylene chloride, while 50 mL of aerosol was drawn into the GTS through the E-cigarette. Recovery was assessed by the relative percentage calculated from the amount of TSNAs found in the extracting solvent and the exhausted amount of the analytes after the inhaler atomization.

The optimum conditions established in the extraction method were used for further GTS test. Analytical results of TSNAs showed enough accuracy and precision to have a recovery of above 95% with relative standard deviation within 14% (most of them were within 6%) (Table 2). The GTS sampling method was simple, rapid, and reproducible, and it used small amounts of organic solvent. Total preparation time including extraction was within 10 min.

For the impinger test, the parameters used by the US FDA[3] were selected for evaluating the extraction efficiency of TSNAs in the aerosol from E-cigarette. The extracting solution was constituted with a mixture of 100 mL of acetonitrile, 11.5 g of phosphoric acid, and 800 mL of water. Test samples at a concentration of 50 and 100.0 μg L^{-1} each in propylene glycol were prepared and filled the E-cartridge. After the loading of the prepared E-cartridge, total 100 mL of the aerosol was drawn into two series connected impingers containing 200 mL of absorption solution each through the E-cigarette. Total absorption solution was extracted with

100 mL of methylene chloride and concentrated to a volume of 200 μL. The recovery was calculated by a relative percentage of TSNA, each recovered in the adsorption solution in two impingers connected in parallel and the exhausted amount of the analytes after the inhaler atomization. As a result, the recoveries of the TSNAs were in the range of 14% to 31% with a relative standard deviation within 60% (Table 2). Unsatisfactory recoveries (<31%) and high standard deviations for TSNAs were observed in impinger extraction methods.

The LODs of TSNAs by the GTS were in the range of 0.01 to 0.02 μg m^{-3} in aerosol generated from standard solutions as described in 3.4 method validation, otherwise, those by the impinger were in the range of 0.6 to 0.9 μg m^{-3} in aerosol. The LOD of impinger method were 50 times more than those of GTS method. Moreover, the volume of total solvent was 400 mL, with further extraction step being necessary by the employment of 100 mL of methylene chloride, and a total preparation time of 90 min.

One of the problems with this approach is that the analytes may not be effectively absorbed into the solution, and their recoveries have extremely low values. Moreover, the method suffered from the disadvantages of being time-consuming and using large amounts of toxic organic solvents.

3.5. TSNA analysis from E-cigarettes

Generally, replacement liquids of E-cigarettes were made of the mixture of various flavors in propylene glycol; therefore, they had complicated matrix properties. When this developed method was applied to the aerosol from E-cigarettes, no interfering peak was observed in the chromatograms near the retention times of the analytes due to the discriminatory nature of extraction. NNK, NAB and NAT showed each single peak, otherwise NNN was divided into two peaks, probably due to the basicity of TSNAs and the formation of two structural isomers after their protonation. Fig. 4 shows the LC-MS chromatogram of aerosol from the spiked standard in propylene glycol and commercial nicotine liquids.

We analyzed the levels of TSNAs in aerosol from 50 replacement liquids of E-cigarettes produced by 11 companies using this developed method (Table 3, Fig. 5). The detected concentration range and mean concentration of TSNAs were 0.1-1.3 μg m^{-3} and 0.3 μg m^{-3} for NNN; 0.1-21.7 μg m^{-3} and 2.5 μg m^{-3} for NNK; 0.1-4.7 μg m^{-3} and 0.8 μg m^{-3} for NAB; and 0.1-17.5 μg m^{-3} and 2.7 μg m^{-3} for NAT. The detection concentration range and mean concentration of total TSNAs were 0.4-45.2 μg m^{-3} and 6.3 μg m^{-3}. Observed carcinogenic NNK levels were high compared to the other TSNAs as approximately 40% of the sum of TSNAs.

In order to evaluate whether TSNA compounds could be formed from the heating of E-cigarettes, replacement liquids of E-cigarettes were made of the mixture of TSNA precursors

(nicotine, nornicotine, anatabine and anabasine) in pure propylene glycol. When this developed method was applied to the aerosol from E-cigarettes using the prepared replacement liquids containing TSNA precursors, no TSNAs were observed. When glycerin was used instead of propylene glycol, similar results were obtained. We could conclude that TSNAs were not formed through the operation of E-cigarettes; TSNAs may be formed from their precursors through air oxidation during storage time of E-liquids.

3.6. Comparison to conventional cigarettes

We used the same method to detect the levels of TSNAs in the mainstream from 10 cigarettes produced by 5 companies purchased in Korea. Single 50 mL puffs of 5s duration were taken – TSNAs were captured in 10 mL of the aerosol solvation solution. For the comparison, all parameters developed in this study were selected for detecting TSNAs in mainstream cigarettes. Although this method is a non-standard method for smoking, the matching is thought to be important for the comparison. In analytical results, the concentration range and mean concentration of TSNAs were 60-347 μg m^{-3} and 173 μg m^{-3} for NNN; 31-174 μg m^{-3} and 94 μg m^{-3} for NNK; 10-98 μg m^{-3} and 37 μg m^{-3} for NAB; and 124-668 μg m^{-3} and 355 μg m^{-3} for NAT in mainstream cigarettes (Table 3). The detected range and mean concentration of total TSNAs were 225-1287 μg m^{-3} and 659 μg m^{-3}. Observed total TSNA

levels in E-cigarette were about 100 folds lower than those of the conventional cigarettes.

4. Conclusion

In this paper we have developed and validated a LC-MS/MS method after the GTS extraction for simultaneous determination of four TSNAs in the aerosol of E-cigarettes. Excellent reproducibility and accuracy were achieved with the use of isotope-labeled analogues as internal standard. The LOQs of each TSNAs were 0.04-0.07 μg m^{-3} and the accuracy was in the range of 95-103%, while the precision of the assay was less than 14%. Thus, the method could be suitable for the analysis of TSNAs in the aerosol of E-cigarettes.

The major advantage of this technique can choose the extracting solvent having the compatibility of an analytical instrument and good solubility of the analytes. The most important criterion in the selection of an extracting solvent is that the solvent should dissolve the analytes when injected with aerosol into a syringe. Otherwise, the major disadvantage of this technique is that the amount of the liquid phase exhausted during atomization cannot be maintained constantly. However, the reason may originate from the characteristic of the E-cigarette apparatus.

The TSNAs were identified and quantified in aerosol following generation from 50 replacement liquid brands of 11 companies. The maximum concentrations of total TSNAs in

the aerosol of E-cigarettes were measured at 6.3 μg m^{-3}. TSNAs were not formed from the heating of E-cigarettes; therefore, the replacement liquids should be stipulated by regulators to the lowest concentrations in E-cigarette liquids (i.e. to not detected levels). This point will be the solution for the possibility of the harm-reduction of cigarettes.

Legend

Fig. 1 The process of extraction of TSNAs into extracting solvent in the GTS.

Fig. 2 The results of extraction efficiency for the tested extracting solvents.

Fig. 3 The results of extraction recovery of TSNAs as a function of volume and extraction time of methylene chloride**.**

Fig. 4 LC-MS/MS chromatogram from a standard sample quantified in the concentration for each 2.43 μg m^{-3} (A) TSNA, and commercial nicotine liquids quantified in concentrations of 0.99 μg m^{-3} (NNN), 3.04 μg m^{-3} (NAT), and 0.65 μg m^{-3} (NNK) (B).

Fig. 5 The levels of TSNAs in the aerosol of 50 E-cigarette using this developed method.

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Table 1 Intra and inter-day laboratory precision and accuracy results for the analysis of TSNAs in aerosol from E-cigarettes (n=5, puff volume=50 mL, puff duration=5 sec)

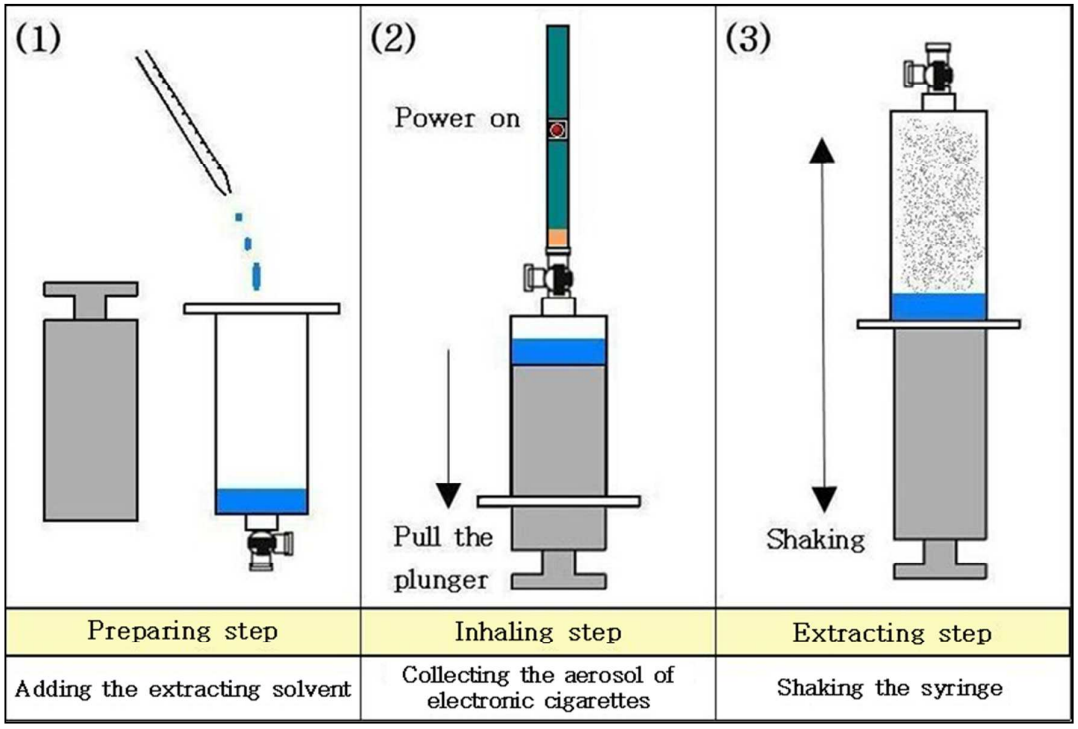
Comp	Conc spiked in EG (μg kg^{-1})	Intra-day measured value				Inter-day measured value			
		Consumption weight of EG phase (mg)	Calculated Conc in aerosol (μg m^{-3})	Measured Conc (μg m^{-3})	Accuracy and Precision (%)	Consumption weight of EG phase (mg)	Consumption amount of of analyte (μg m^{-3})	Measured Conc (μg m^{-3})	Accuracy and Precision (%)
NNN	50	7.2 ± 0.2	7.2	6.9 ± 0.2	95.5 (3.52)	7.3 ± 0.3	7.3	7.2 ± 0.4	99.1 (5.11)
	100	7.3 ± 0.2	14.6	13.9 ± 0.7	95.5 (4.75)	7.4 ± 0.3	14.8	14.5 ± 0.8	98.5 (4.95)
NNK	50	7.2 ± 0.2	7.2	7.1 ± 0.5	99.0 (7.51)	7.3 ± 0.3	7.3	7.4 ± 0.4	101.6 (5.48)
	100	7.3 ± 0.2	14.6	14.0 ± 0.5	95.7 (3.87)	7.4 ± 0.3	14.8	14.6 ± 0.7	98.9 (4.55)
NAT	50	7.2 ± 0.2	7.2	7.3 ± 1.0	101 (14.0)	7.3 ± 0.3	7.3	7.5 ± 0.6	103 (8.85)
	100	7.3 ± 0.2	14.6	15.3 ± 0.5	105 (3.06)	7.4 ± 0.3	14.8	15.0 ± 0.6	102 (4.15)
NAB	50	7.2 ± 0.2	7.2	7.4 ± 0.6	103 (7.95)	7.3 ± 0.3	7.3	7.5 ± 0.4	103 (5.83)
	100	7.3 ± 0.2	14.6	14.7 ± 0.7	101 (4.44)	7.4 ± 0.3	14.8	14.8 ± 0.5	100 (3.24)

Table 2 Recovery comparison of gastight syringe method and impinger method for the analysis of TSNAs in aerosol (n=5)

Comp	Conc spiked in EG (μg kg^{-1})	GTS (puff volume 50 mL)				Impinger (puff volume 50 mL)			
		Consumption weight of EG phase (mg)	Consumption amount of of analyte (ng)	Measured amount (ng)	Recovery	Consumption weight of EG phase (mg)	Consumption amount of of analyte (ng)	Measured amount (ng)	Recovery
NNN	50	7.7 ± 0.3	0.38 ± 0.01	0.38 ± 0.01	98.1 ± 1.1	4.2 ± 0.9	0.21 ± 0.04	0.06 ± 0.02	30.1 ± 11.7
	100	7.4 ± 0.2	0.74 ± 0.02	0.73 ± 0.01	98.7 ± 0.5	3.5 ± 1.5	0.35 ± 0.15	0.08 ± 0.06	19.8 ± 9.1
NNK	50	7.7 ± 0.3	0.38 ± 0.01	0.39 ± 0.01	102.2 ± 0.6	4.2 ± 0.9	0.21 ± 0.04	0.04 ± 0.01	21.2 ± 11.3
	100	7.4 ± 0.2	0.74 ± 0.02	0.72 ± 0.02	98. 4 ± 0.7	3.5 ± 1.5	0.35 ± 0.15	0.06 ± 0.04	16.1 ± 3.7
NAT	50	7.7 ± 0.3	0.38 ± 0.01	0.38 ± 0.02	100 ± 0.3	4.2 ± 0.9	0.21 ± 0.04	0.04 ± 0.01	22.9 ± 12.0
	100	7.4 ± 0.2	0.74 ± 0.02	0.74 ± 0.02	100 ± 1.1	3.5 ± 1.5	0.35 ± 0.15	0.06 ± 0.04	16.6 ± 3.9
NAB	50	7.7 ± 0.3	0.38 ± 0.01	0.39 ± 0.02	101 ± 0.4	4.2 ± 0.9	0.21 ± 0.04	0.04 ± 0.02	20.4 ± 12.3
	100	7.4 ± 0.2	0.74 ± 0.02	0.74 ± 0.01	100 ± 0.3	3.5 ± 1.5	0.35 ± 0.15	0.06 ± 0.03	14.8 ± 3.5

Table 3 Analytical results of TSNAs in aerosol of E-cigarettes and conventional cigarettes (a blended type) (puff volume=50 mL, puff duration=5 sec)

Analyte	E-cigarette					Conventional cigarette				
	Sample No	Frequency detected (%)	Mean ± SD (μg m^{-3})	Minimum (μg m^{-3})	Maximum (μg m^{-3})	Sample Nr	Frequency detected (%)	Mean ± SD (μg m^{-3})	Minimum (μg m^{-3})	Maximum (μg m^{-3})
NNN	50	80	0.3 ± 0.3	0.1	1.3	10	100	173 ± 84	60	347
NNK	50	90	2.5 ± 4.2	0.1	21.7	10	100	94 ± 53	31	174
NAT	50	83	2.7 ± 4.0	0.1	17.5	10	100	355 ± 156	124	668
NAB	50	87	0.8 ± 1.0	0.1	4.7	10	100	37 ± 25	10	98
ΣTSNAs	50	100	6.3 ± 8.4	0.3	44.1	10	100	659 ± 286	240	1287



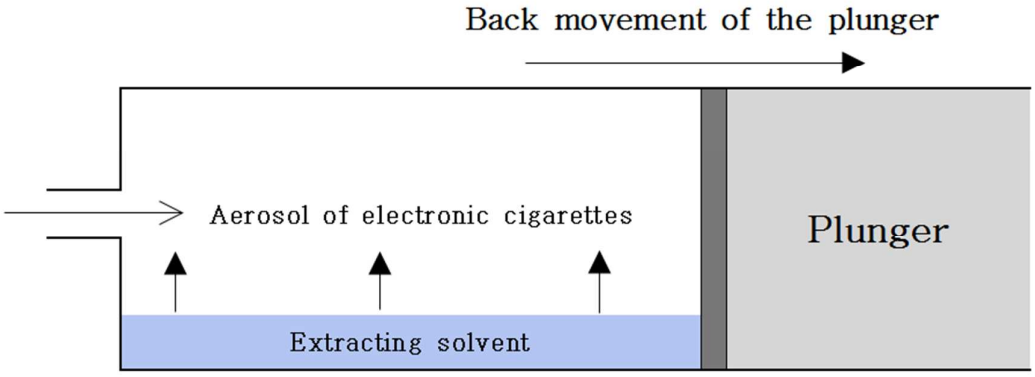


Fig. 1 The process of extraction of TSNAs into extracting solvent in the GTS.

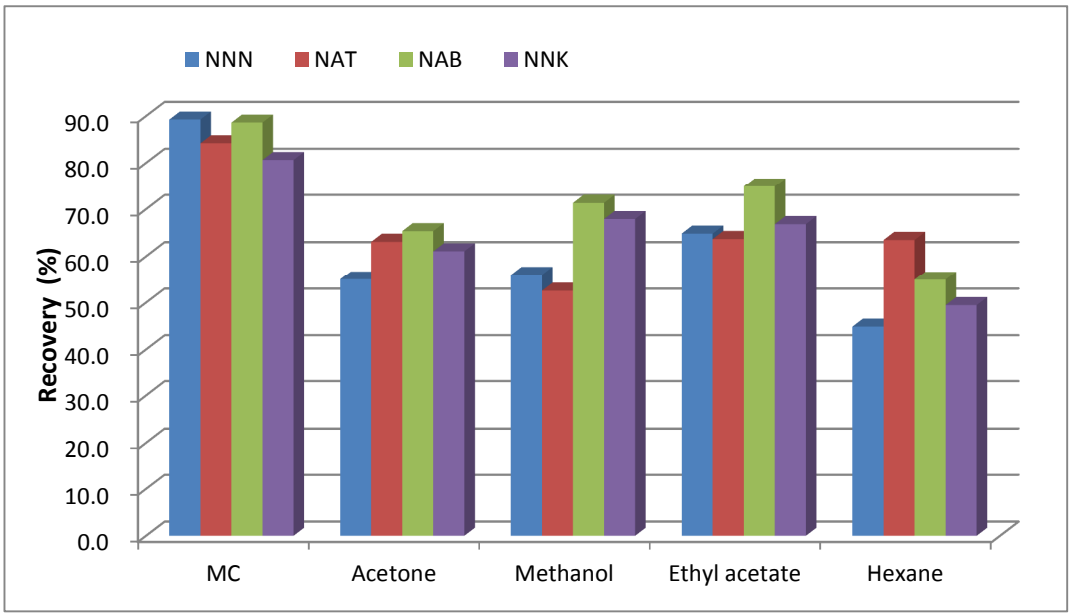


Fig. 2 The results of extraction efficiency for the tested extracting solvents.

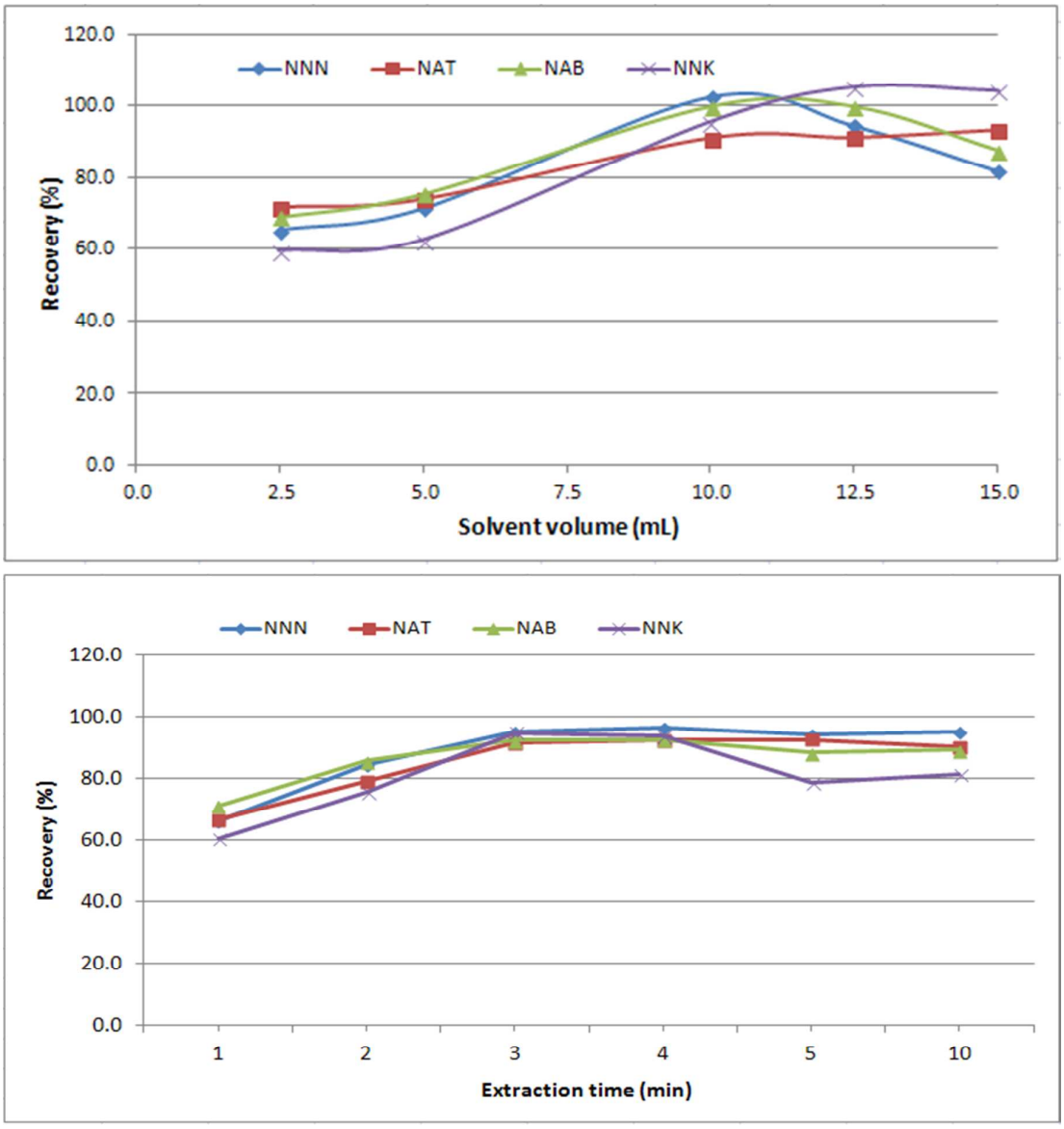


Fig. 3 The results of extraction recovery of TSNAs for volumes and extraction time of methylene chloride.

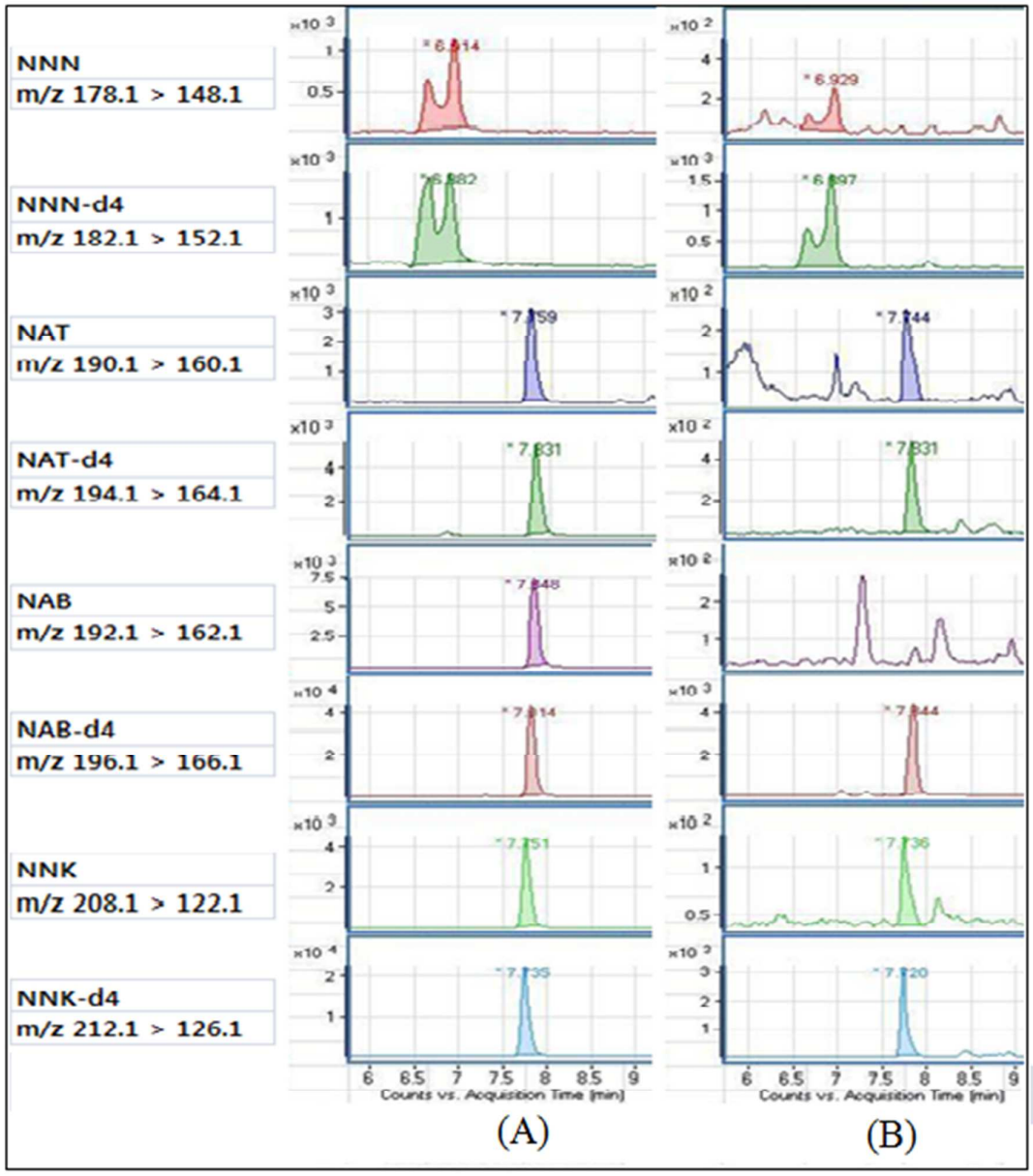


Fig. 4 LC-MS/MS chromatogram from a standard sample quantified in the concentration for each 2.43 µg m^{-3} (A) TSNA, and commercial nicotine liquids quantified in concentrations of 0.99 µg m^{-3} (NNN), 3.04 µg m^{-3} (NAT), and 0.65 µg m^{-3} (NNK) (B).

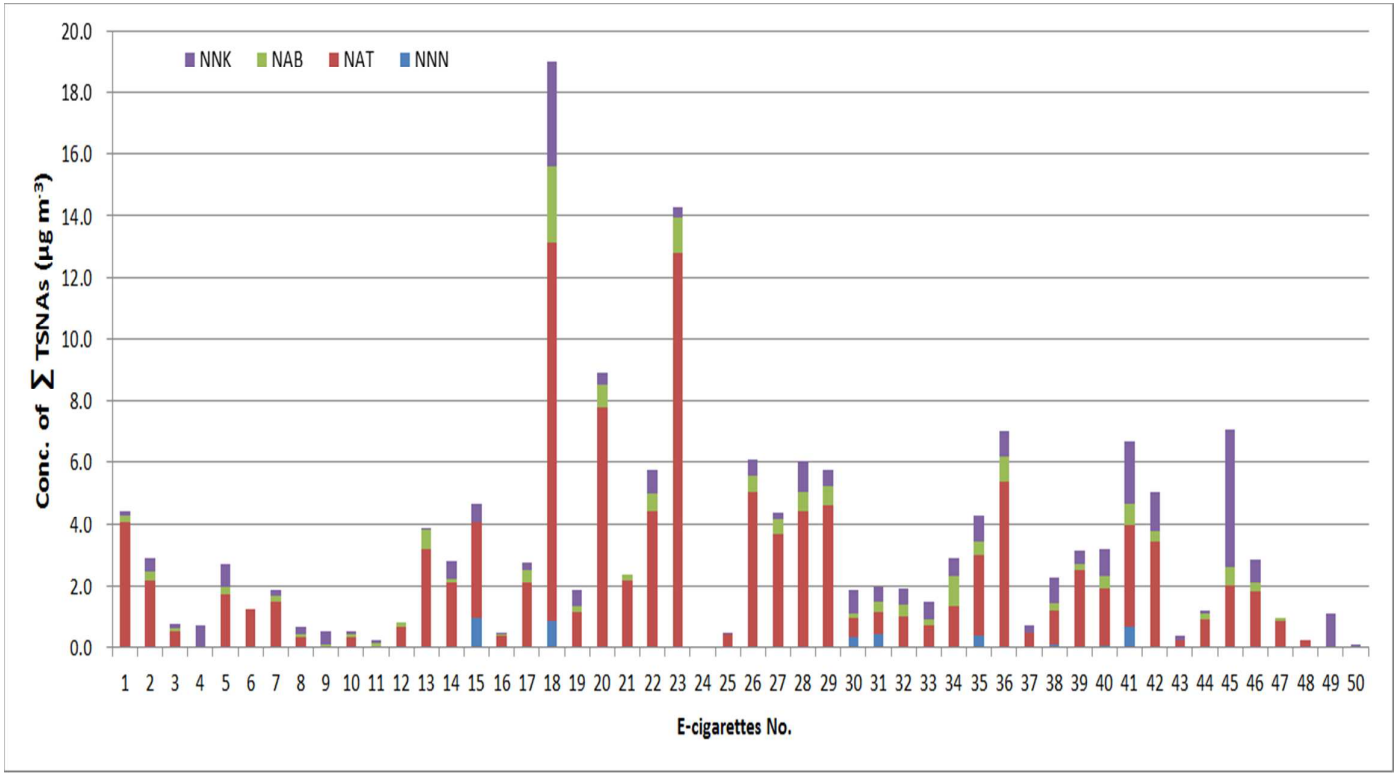


Fig. 5 The levels of TSNAs in the aerosol of 50 E-cigarette liquids using this developed method.