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Efficiency Improvement of a Gas Chromatographic-Mass Spectrometric Method for Quantification of Nicotine in Hookah (Water Pipe) Tobacco Products

Hazim M. Ali
Department of Chemistry, College of Science, Jouf University,
P.O. Box 2014, Sakaka, Aljouf, Saudi Arabia

Abstract: In the present study, a highly sensitive Gas Chromatographic-Mass Spectrometric (GC-MS) method and single-step extraction procedure have been developed for the determination of nicotine level in Hookah tobacco products samples. The principle focus of the developed method is the lower limit of detection (0.025 ng mL^{-1}) and a wide linear range (0.03-17000 ng mL^{-1}) achieved that can have important implications in the determination of nicotine in any investigated samples. In addition, the accuracy and precision values of the method were in the range of 98.07-103.81 and 0.2660-1.7712%, respectively. While the extraction recovery of nicotine, when spiked to hookah tobacco products was ranged from 94.85-98.00%. The present chromatographic method showed higher sensitivity, linearity precision and accuracy, when compared with the reported chromatographic methods. Finally, the hookah tobacco products samples were used to evaluate the method applicability for the determination of nicotine.

Key words: Hookah tobacco products, nicotine, methyl nicotinate, single-step extraction procedure, sensitivity, recovery

INTRODUCTION

Nicotine is chemically designated as 3-[(2S)-1-methylpyrrolidin-2-yl]pyridine (Fig. 1) and it is the main alkaloid found in tobacco leaves (*Nicotianatabacum L., Solanaceae*) (Levent *et al*., 2009). Nicotine is widely consumed in two patterns, tobacco products in the form of cigarettes, cigars or Hookah (waterpipe) and anti-smoking pharmaceuticals such as dermal patches, tablets, chewing gum and nasal sprays (Magni *et al*., 2016; Marclay and Saugy, 2010; Svorc *et al*., 2014). Tobacco nicotine increases stimulation and pleasure and decreases stress and anxiety because of its effects on the neurotransmitter release (dopamine and others) in brain tissue (Benowitz, 2008; Zuo *et al*., 2004). On the other hand, nicotine has predominant effects on smokers and non-smokers health such as enhancement blood sugar release, blood pleasure and increase heart pulse rate because it is absorbed through the skin, mucous membranes in the mouth and nose or by the lungs during smoke inhalation (Miller *et al*., 2010; Shrivas and Patel, 2010). Tobacco smoking is still the commonly preferred path for intake of nicotine in spite of the fact that it often causes cancer of various organs involving lung, stomach, bladder, colon, kidney, nose and oral cavity (Banerjee *et al*., 2013; Yu and Chang, 2013) and also the difficulties that meet the smokers to quitting the smoking habit (Svorc *et al*., 2014).

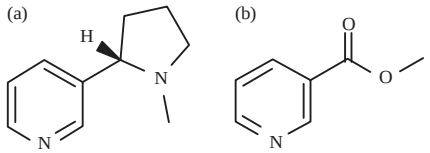

Fig. 1: (a) Chemical structures of nicotine and (b) Methyl nicotinate, IS

In recent years, hookah, also known as a waterpipe, sheesha, chicha, gozah and narghile, smoking is gaining popularity nationwide, especially among urban youth, college students and young professionals (Hadidi and Mohammed, 2004; Ward *et al*., 2007). Hookah tobacco pastes are divided into two main types according to its constituents, known as moassel and jurak. It generally contains tobacco leaves, molasses (black honey or juice of sugarcane), with the addition of glycerin and fruits flavor in case of moassel while in case of jurak, spices, dried fruits and tar are presents (El-Hakim and Uthman, 1999; Farid, 2013). Hookah tobacco smoking is a form of tobacco consumption that utilizes a single or multi-stemmed instrument to smoke flavored or non-flavored tobacco, where tobacco smoke passes through water or another liquid container before reaching the smoker (Auf *et al*., 2012).

Various analytical techniques, spectrophotometry (Al-Tamrah, 1999; Asthana *et al*., 2004), spectrofluorimetry (Zhou *et al*., 2009), capillary electrophoresis (Ralapati, 1997; Yang and

Corresponding Author: Hazim M. Ali, Departmentof Chemistry, Forensic Medicine Authority, Cairo, Egypt

Smetena, 1995), voltammetry (Levent *et al*., 2009; Svorc *et al*., 2014), liquid chromatography (Abdallah *et al*., 2016; Dash and Wong, 1996; Miller *et al*., 2010; Tambwekar *et al*., 2003; Yasuda *et al*., 2013) and gas chromatography (Hossain and Salehuddin, 2013; Kim *et al*., 2005; Magni *et al*., 2016; Man *et al*., 2006; Nystrom *et al*., 1997; Siegmund *et al*., 1999) have been used for the detection of nicotine in different matrices (e.g., tobacco product, anti-smoking pharmaceutical products and biological fluids). There are only one published method describing the determination of nicotine content in hookah tobacco products (Hadidi and Mohammed, 2004). This method applied only for flavored and unflavored moassel tobacco samples commercial available in Jordan market and multi-step extraction procedure used for gas chromatography provided with nitrogen phosphorous detector for analysis of nicotine.

The aim of the present study is to develop a simple and fast method based on gas chromatography-mass spectrometry (GC-MS) with highly sensitivity and suitable linearity for the determination of nicotine in flavored, unflavored moassel tobacco and jurak samples. In addition, the method in the present work should characterized by its low cost, simple extraction procedure and minimizing the interference effects.

MATERIALS AND METHODS

Chemicals and materials: Nicotine was obtained from Loba Chemie Co., India. Methyl nicotinate (internal standard), HPLC Methanol and anhydrous sodium sulphate were purchased from Sigma-Aldrich (Spruce street, St. Luis, USA).

Standard and calibration solutions: Standard stock solutions containing 100 μg mL^{-1} of the nicotine and methyl nicotinate (Internal Standard, IS) were prepared daily in methanol. A set of eighteen-calibration solution made up of 0.03, 0.05, 0.1, 0.5, 1, 5, 10, 50, 100, 300, 500, 1000, 3000, 5000, 7000, 10000, 13000 and 17000 ng mL^{-1} nicotine was prepared from stock solution. Subsequently, the methyl nicotinate (IS) was added to each calibration solution to maintain a concentration of 500 ng mL^{-1} and the mixture was diluted with methanol. Three working solutions (25, 400 and 1000 ng mL^{-1}) were used toevaluating the accuracy and precision of the developed method. Extraction recovery was performed by spiking hookah tobacco sample with three different concentration levels (85, 550 and 2000 ng mL^{-1}) of nicotine and with the IS at a fixed amount (500 ng mL^{-1}). All solutions were stored at 4°C prior to analysis.

GC-MS instrumentation and conditions: Analysis of hookah tobacco samples was carried out by gas chromatography (TRACE™ 1310 GC) provided with Single Quadrupole Mass Spectrometer (ISQLT) and AI/AS1310 auto-sampler unit (Thermo Scientific, USA). Separation of nicotine was done by TG-5MS column (60 m, 0.25 mm I.D., 0.25 μm film thickness, Thermo Scientific, USA) with 1 mL min^{-1} helium (carrier gas) flow rate. The applied temperature program was started at 80°C for 3 min, then rising to 250°C (5 min hold) at a rate of 30°C min^{-1}. The temperatures of the injector, transfer lineand ion source were adjusted to 250, 250 and 255°C, respectively. The analysis was performed in Selected Ion Monitoring (SIM) mode using electron ionization with 70 eV energy. The characteristic ions of nicotine and methyl nicotinate (internal standard) were used for identification: m/z 42, 84, 133, 162 and 51; m/z 87, 106 and 137, respectively. The split-less mode of the injector was used with an injection volume of 1 μL. Data processing and analysis data were carried out using Xcalibur Program Version 3.1 (Thermo Scientific, USA).

Sample collection and preparation: Twenty-five hookah tobacco products samples were purchased from tobacco product shops. The hookah tobacco products samples were divided into the following groups by fruit-flavored Almoassel tobacco (sixteen samples; F_1-F_{16}), unflavored Almoassel tobacco (Four samples; A_1-A_4) and Jurak (Five samples; J_1-J_5) as shown in Table 1.

The 1 g of hookah tobacco was weighed into a round-bottom flask, equipped with a stirring bar, methyl nicotinate (internal standard, 500 ng mL^{-1} in extracting solvent; 50 mL) was added and completed the volume to 50 mL with methanol. The flask was connected to a reflux condenser and the mixture was stirred and directly heated on stirrer-hotplate adjusted to 350 rpm and 65°C for 30 min. The mixture was left to cool at room temperature and then filtrated. To remove water, anhydrous sodium sulfate was added to the filtrate. Then, the filtrate was further filtered by passing through a 0.2 μm CHROMAFIL®Xtra PTFE syringe filter (MACHEREY-NAGEL GmbH and Co.KG, Düren, Germany) directly to GC vials and 1 μL of the filtrate was used for the analysis.

Method validation: The linearity, Limit of Detection (LOD), Limit of Quantitation (LOQ), accuracy and precision, specificity and extraction recovery of developed method was evaluated according to the US Food and Drug Administration (USFDA) guidelines for the bioanalytical method.

Table 1: Amounts of nicotine in hookah tobacco products

Sample types	Sample codes	Brands	Flavored types	Manufactured country	Concentration (mg/g)±SD[a]
Fruit flavored	F_1	AL Fakher	Orange	United Arab Emirates	1.577±0.040
almoassel tobacco	F_2		Grape with Berry		1.044±0.027
	F_3		Lemon with Mint		1.200±0.021
	F_4		Vanilla		1.338±0.013
	F_5		Mint		1.300±0.044
	F_6	Mazaya	Natural Two Apples	Jordan	0.973±0.013
	F_7		Watermelon with Mint		0.594±0.014
	F_8		Mastic Gum		0.633±0.002
	F_9		Blueberry Exotica		0.560±0.014
	F_{10}		Heavenly Fruit		0.553±0.011
	F_{11}		Gum with cinnamon		0.489±0.003
	F_{12}	J and J (Al-Bazz)	Mint	Egypt	0.516±0.009
	F_{13}		Strawberry		0.378±0.001
	F_{14}		Lemon with Mint		0.518±0.007
	F_{15}		Two Apples		0.620±0.018
	F_{16}		Grape with Mint		0.515±0.015
Unflavored	A_1	Al-Bazz	Without	Egypt	1.625±0.051
almoassel tobacco	A_2	Alborg		Egypt	2.979±0.073
	A_3	Zaghloul		Egypt	2.723±0.033
	A_4	Zawati Elnaama		Egypt	2.815±0.006
Jurak	J_1	Al-Safi	Without	India	0.786±0.007
	J_2	Abou Dallah		India	0.850±0.006
	J_3	Sheesha		India	0.779±0.005
	J_4	Dallah (Alasly)		India	0.648±0.002
	J_5	Alasly Baeshen		India	1.069±0.005

RESULTS AND DISCUSSION

Optimization of sample extraction conditions: Single-step extraction procedure was used for extraction of nicotine from hookah tobacco products samples in presence of methanol as a suitable solvent for the extraction process. Several parameters, including temperature and time, may have an effect on the efficiency of the nicotine extraction. In order to optimize the hookah tobacco samples extraction, different temperatures (30, 40, 50, 60, 65, 70 and 70°C) and different extraction times (5, 10, 20, 30, 40, 50 and 60 min) were tested in this study. Finally, 65°C and 30 min were chosen as an optimal exposure temperature and time for the extraction of nicotine from different hookah tobacco samples.

Method development: Column type and oven temperature were tested to optimize the chromatographic separation of nicotine and methyl nicotinate (IS). Two different type of capillary column, TG-5MS column (60 m, 0.25 mm I.D., 0.25 µm film thickness) and Rxi®-624Sil MS column (60 m, 0.53 mm I.D., 3.0 µm thickness) were used to check the best separation of nicotine and methyl nicotinate. The TG-5MS column was considered the most suitable column for the separation and quantification of nicotine and methyl nicotinate due to its high selectivity for nicotine determination with no interferences from other hookah tobacco sample constituents as shown in Fig. 2 and 3.

Oven temperature and holding time are important factors in enhancing both the separation speed and efficiency of the analyte. The nicotine peak responses were determined when starting oven temperature were set at 40, 50, 60, 70, 80, 90 and 100°C and hold for 1, 2, 3, 4 and 5 min, the results showed 80°C was the optimal equilibration temperature and 3 min was the suitable holding time. The final oven temperature, temperature-increasing rate and holding time were also tested. The results indicated 250°C, 30°C min^{-1} and 5 min were the suitable conditions for the final oven temperature, temperature-increasing rate and holding time respectively. Finally, the suitable oven temperature was therefore, set at 80°C for 3 min, then rising to 250°C (5 min hold) at a rate of 30°C min^{-1}.

Under the chromatographic conditions described above, the separation of nicotine in pure form and hookah tobacco products samples was achieved within 13.67 min at TG-5MS column as shown in Fig. 2 and 3, respectively. The GC retention times were consistent at 8.71 min and 10.11 min for nicotine and methyl nicotinate (IS), respectively.

Validation of the method

Specificity: Specificity was confirmed by analyzing the chromatograms of nicotine standard solution and hookah tobacco products samples in presence of methyl nicotinate as an Internal Standard (IS). The results showed that the developed method was specific for the detection of nicotine as there was no other peak to interfere with the peak of nicotine as shown in Fig. 3.

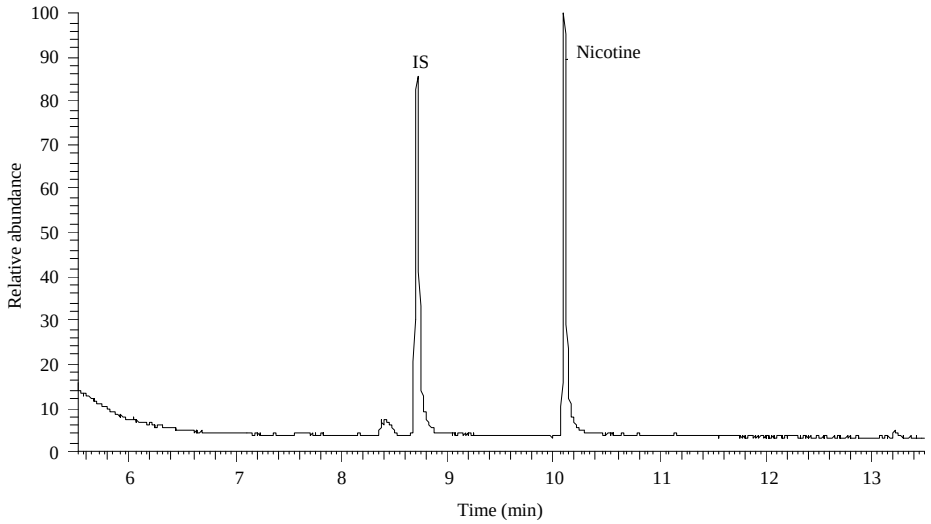

Fig. 2: Chromatogram of nicotine and methyl nicotinate (IS) in pure form

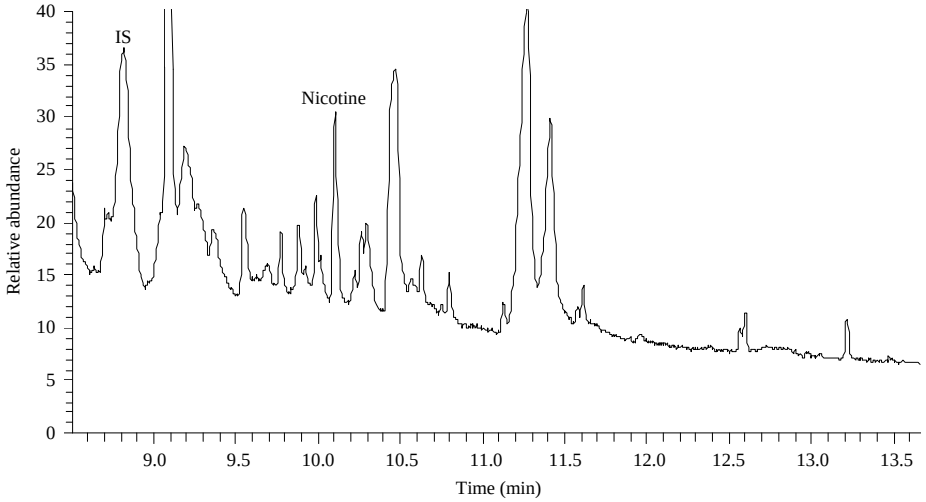

Fig. 3: Chromatogram of nicotine and methyl nicotinate (IS) in sample F_{13} of fruit-flavored almoassel tobacco

Linearity, limit of detection and limit of quantification: The calibration curve of nicotine was constructed by plotting peak area of nicotine versus concentration at eighteen concentration levels. Limit of Detection (LOD) and Limit of Quantitation (LOQ) of nicotine was calculated using the following formulas:

$$3\times SD/S$$

and:

$$10\times SD/S$$

where, SD is the Standard Deviation of intercept and S is the slope of calibration curve), respectively. Calibration was linear in the concentration ranged from 0.03-17000 ng mL^{-1}. The linear regression equation was:

$$Y = 4\times10^{7}\times\text{-}732025 \ (n = 3)$$

with the correlation coefficient was 0.999. The LOD and the LOQ for nicotine under GC conditions were 0.025 and 0.083 ng mL^{-1}, respectively.

Precision and accuracy: The precision and accuracy were determined as the percentage relative standard deviation:

$$RSD = \frac{SD}{\text{Mean measured value}}\times100$$

and the percentage recovery:

$$\text{Recovery (\%)} = \frac{\text{Measured value}}{\text{Added value}}\times100$$

Table 2: Accuracy and precision of the proposed method for the analysis of nicotine

	Intra-day			Inter-day		
Concentration added (ng mL^{-1})	Concentration found (ng mL^{-1}) ±SD[a]	Recovery (%)	RSD (%)	Concentration found (ng mL^{-1}) ±SD	Recovery (%)	RSD (%)
25	24.9±0.2	99.76	0.8855	24.5±0.4	98.07	1.7712
400	405.5±1.1	101.38	0.2660	398.2±3.6	99.54	0.9039
1000	1038.1±3.1	103.81	0.2965	1031.2±8.3	103.12	0.8015

[a]SD: Standard Deviation

Table 3: Recovery analysis of nicotine in Hookah tobacco products using developed method

Tobacco samples	Determined (ng mL^{-1})	Added (ng mL^{-1})	Expected (ng mL^{-1})	Found (Mean±SD, ng mL^{-1})	Recovery (%)
A_4	2252	85	2337	83±4	97.64
		550	2802	535±7	97.27
		2000	4252	1944±64	97.20
J_5	855	85	940	83±5	97.64
		550	1405	539±2	98.00
		2000	2855	1897±29	94.85

Table 4: Comparison of the proposed method with some reported methods

Methods	Linear range (ng mL^{-1})	Limit of detection (ng mL^{-1})	References
HPLC-UV method	50-5000	6.600	24
HPLC-FLD method	0.3-1000	0.090	25
LC-MS/MS method	0.26-52.5	0.086	21
LC–ESI–MS/MS	10-10000	0.500	2
GC-NPD method	2-125	0.500	30
GC-MS method	0.5-5000	0.200	28
The developed method	0.03-17000	0.025	This work

Three different concentration levels (25, 400 and 1000 ng mL^{-1}) were used for evaluating the intraday and inter-day precision and accuracy of the developed method. The intra-day and inter-day precision were in the range of 0.2660-0.8855 and 0.8015-1.7712%, respectively as shown in Table 2. While the intra-day and inter-day accuracy in the range of 99.76-103.81 and 98.07-103.12%, respectively as denoted in Table 2. The results suggested that the method was reliable, reproducible and accurate.

Recovery: The efficiency of the extraction method was determined based on the recovery:

$$\text{Recovery (\%)} = \frac{\text{Measured value}}{\text{Added value}} \times 100$$

and estimated by addition of nicotine in three different concentration levels (85, 550 and 2000 ng mL^{-1}) to hookah tobacco products samples in three replicates. The extraction recoveries of spiked nicotine ranged from 94.85-98.00% as shown in Table 3. The results indicated that the extraction method is precise and reproducible.

Application to hookah tobacco products samples: The developed method was successfully applied for the determination of nicotine content in hookah tobacco products samples. Figure 3 represents the chromatogram of nicotine and methyl nicotinate (IS) in sample F_{13} of fruit-flavored Almoassel tobacco. The content of nicotine in hookah tobacco products samples was reported in Table 1. The amount of nicotine was found in the range of 0.378-1.577, 1.625-2.979 and 0.648-1.069 mg g^{-1} in Fruit Flavored Almoassel Tobacco, Unflavored almoassel Tobacco and Jurak, respectively. The amount of nicotine in unflavored Almoassel tobacco samples is higher than other hookah tobacco products samples. That may be due to the number of additives and the amount of additives in unflavored almoassel tobacco samples is less than other hookah tobacco products samples.

Comparison with other chromatographic methods: A comparison of the analytical data of the developed method with other reported chromatographic methods (High-Performance Liquid Chromatography-UV detector (HPLC-UV), High-Performance Liquid Chromatography-Fluorescence Detector (HPLC-FLD), Liquid Chromatography-Mass Spectrometry (LC-MS), liquid Chromatography-Electrospray Ionization-tandem Mass Spectrometry (LC-ESI-MS/MS), Gas Chromatography-Nitrogen-Phosphorus Detector (GC-NPD) and Gas Chromatography-Mass Spectrometry (GC-MS) for the determination of nicotine as denoted in Table 4. This method offers a wide linear range and high sensitivity with the lowest limit of detection of 0.025 ng mL^{-1}. Up to date, this developed method was found to be the most sensitive chromatographic methods for the determination of nicotine. The high sensitivity makes this method more qualified for the determination of nicotine in any investigated samples.

CONCLUSION

A simple, rapid and highly sensitive GC-MS method for determination of nicotine presented in hookah tobacco products has been developed and validated. The GC-MS analysis of nicotine was carried out in 13.67 min with no interference from hookah tobacco products constituents and additives. The amount of nicotine was found in the range of 0.378-1.577, 1.625-2.979 and 0.648-1.069 mg g^{-1} in Fruit Flavored Almoassel Tobacco, unflavored almoassel tobacco and Jurak, respectively. The amount of nicotine in unflavored Almoassel tobacco samples is higher than other hookah tobacco products samples. The GC-MS method exhibited a good linearity, precision, accuracy and extraction recovery. This method was successfully applied to the determination of nicotine content in hookah tobacco products with satisfactory results and recommended to be applied in any investigated samples.

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