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Determination of Nicotine Contents in Local and Imported Cigarettes Smoke in Yemeni Markets Using Gas Chromatography Flame Ionization Detector (GC-FID)

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Abstract

Currently, the number of consumers of tobacco products in the world is growing rapidly. In this regard, the number of tobacco companies and varieties is increasing. Many tobacco companies write a small amount of the ingredients found in a cigarette on the product package. This study aimed to determine the nicotine contents in different brands of cigarette smoke. The paper presents the results of the quantitative determination of nicotine in cigarette smoke samples through the practical application of a validated GC-FID method. The isolation of nicotine was performed by extraction with propanol. The GC-FID and method validation was performed through the parameters: linearity, precision, limits of detection (LOD), and quantification (LOQ). The correlation coefficient was 0.9998 over the nicotine standard range (5 to 500 ppm). The relative standard deviation (%RSD) ranged from 0.71 to 6.67 % and the obtained LOD and LOQ were 0.0926 and 0. 2777 ppm, respectively. For method validation, the correlation coefficient was 0.9987 over the spiked nicotine cigarette range (0.1 to 1.0 mg/cig.). The relative standard deviation (%RSD) ranged from 0.71 to 6.67% and the obtained LOD and LOQ were 0.1056 and 0.3166 ppm, respectively, which is much lower than the accepted value of 0.6 mg/cigarette according to the reference standards based on the results obtained from this work. The content of nicotine from 87 tobacco cigarettes samples was determined. For the local 19 of 21 and imported 45 of 66 tobacco samples exceeded the permitted limit, the variation limits of the nicotine content were more restricted (0.512-1.564 and 0.640-2.415 mg/cig.), respectively.

Article Info

Keywords:
Nicotine, Method Validation, Local Cigarettes, Imported Cigarettes, GC-MS, GC-FID, Yemeni Market.

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1. INTRODUCTION

Tobacco is one of the most widely grown non-food crops worldwide and has enormous economic benefits. Tobacco leaves, which serve as the primary raw material for cigarettes, possess significant value in the global market. Fragments or powders derived from tobacco leaves are commonly used for smoking and chewing [1]. The primary alkaloid in tobacco is nicotine, which accounts for approximately 95% of all tobacco alkaloids and is present in commercial cigarettes at a weight percentage of 1.5% [2]. Nicotine, an extremely poisonous alkaloid with a concentration of 30–60 mg/kg, is chemically known as 3-(1-methyl-2-pyrrolidi-methyl) pyridine [3]. Smoking has detrimental effects on the respiratory and cardiovascular systems, eyes, central nervous system, digestive system, and skin, all of which are frequently irreversible. According to certain estimates, people who smoke live eight years less than those who do not [4]. Compared to alcohol and other drugs, nicotine induces psychologi-

cal addiction more quickly. Because nicotine increases dopamine levels in the reward circuit, particularly in the nucleus accumbens, a substrate for pleasurable experiences experienced by smokers, it can be explained why nicotine has addictive qualities. The urge to repeat ingestion is caused by sustained high levels of dopamine [5]. Research has demonstrated that nicotine, particularly in adolescence, can also affect memory and attention [6]. Nicotine has frequently been used as a replacement therapy for smoking cessation [7] and to ameliorate symptoms associated with Alzheimer's disease [8]. Cigarette smoking is a leading cause of various cancers and diseases associated with the inhalation of toxic chemical substances produced by pyrosynthesis or released during combustion. Tobacco smoke is a source of toxic substances that constitute one of the many classes of carcinogens, toxins, and addictive substances [9]. Smoking is still the most prevalent cause of death, accounting for over four million deaths worldwide per a year [10]. Generally, the nicotine content in tobacco leaves ranges from 2% to 6%. Tayoub et al. determined the nicotine content in the dry weight of five different types of tobacco leaves using HPLC and LS-MS techniques, yielding values ranging from approximately 3.3% to 6.7% [11]. Gas chromatography coupled with flame ionization detection (FID), nitrogen–phosphorus detection (NPD), mass spectrometry (MS), liquid chromatography (LC) coupled with ultraviolet/visible (UV), and multiple scanning electron microscopy have all been used in quantitative evaluations of analytical technologies for nicotine and related alkaloids in tobacco-containing products [12–22]. The use of a gas chromatography-flame ionization detector (GC-FID) simplifies the quantification of the amount of nicotine present [23–25]. Calculating the amount of nicotine present in tobacco products is crucial because the amount of nicotine in cigarettes and other tobacco products varies among brands [26]. Many brands of cigarettes (domestic and imported) are available in the Yemeni market. Therefore, it is important to measure the amount of nicotine in different types of cigarette smoke, which are generally used by people in this country. In this study, a validated GC/FID method was used to quantitatively analyze nicotine in local and imported cigarette smoke samples gathered from the Yemeni market.

2. MATERIALS AND METHODS

2.1. Materials

Nicotine Standard Material 99% purchased from Zedelgem, BELGIUM), and propanol HPLC grade (Sigma).

2.2. Sampling

A variety of local and international brands with a wide range of advertised nicotine content were represented by stores in Sana'a City, Yemen, where packets of cigarettes representing each brand were randomly acquired from the local market. Cigarette samples from 21 domestic and 66 foreign sources were examined in this study.

2.3. Sample Preparation

To prepare cigarette samples, a manual smoking machine setup was used, which included a vacuum pump, liquid trap, and Cambridge filter unit (Glass Filter Paper (GF)). The purpose of GF filter paper is to trap nicotine from cigarette smoke, as illustrated in Fig.1. Nicotine was then extracted using 10 mL of propanol and subjected to GC-FID analysis [27].

2.4. Preparation of Nicotine Standard Solutions

The working standards (5, 10, 20, 50, 100, 250, and 500 ppm) were obtained by diluting the specified amount of nicotine in propanol using a stock standard of nicotine (10000 ppm).

2.5. Identification of Nicotine Using Gass Chromatography-Mass Spectrometry (GC-MS)

GCMS-QP2010 (Shimadzu, Kyoto, Japan) in electro-ionization (EI) mode was utilized to identify and validate nicotine standards and nicotine in the cigarette extract. Using polysiloxane as a nonpolar stationary phase and a fused silica capillary column DB1 (0.32 mm x 30 m, 0.25µm film thickness, supplied by Agilent, Palo Alto, CA, USA), the analytes were separated. The standard solution was injected in split mode with a sample time of 0.5 min. The injection temperature was set at 250 ℃ and the detector temperature was adjusted at 290 ℃. The temperature program used was as follows: initial 120℃, then increased by 20 ℃ min-1 to 270 ℃, and held for 7.5 min. High-purity helium (> 99.99%) was used as the carrier gas at a flow rate of 1.5 ml min-1. Scanning and selective ion monitoring (SIM) modes were used. In SIM mode, the quantification and confirmation ions used in SIM mode to analyze nicotine were 84, 133, 162, 161, and 119 m/z, respectively.

2.6. Analysis of Sample Using Gass Chromatography-Flam Ionization Detector (GC-FID)

The prepared samples (2 µL) were injected in split mode with a sample time of 0.5 min. The injection temperature was set at 250 ℃ and the detector temperature was set at 290 ℃. The GC-FID. The temperature program used was the same as that used for GC-MS.

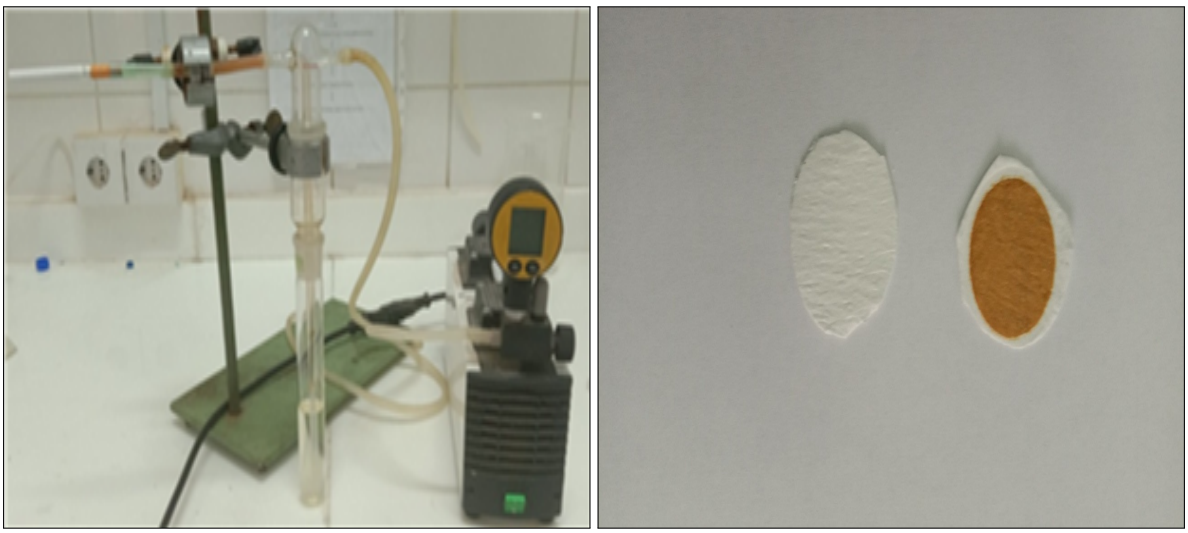

Figure 1. A- Smoking Manual Machine, B- GF Paper Used to Trap Nicotine.

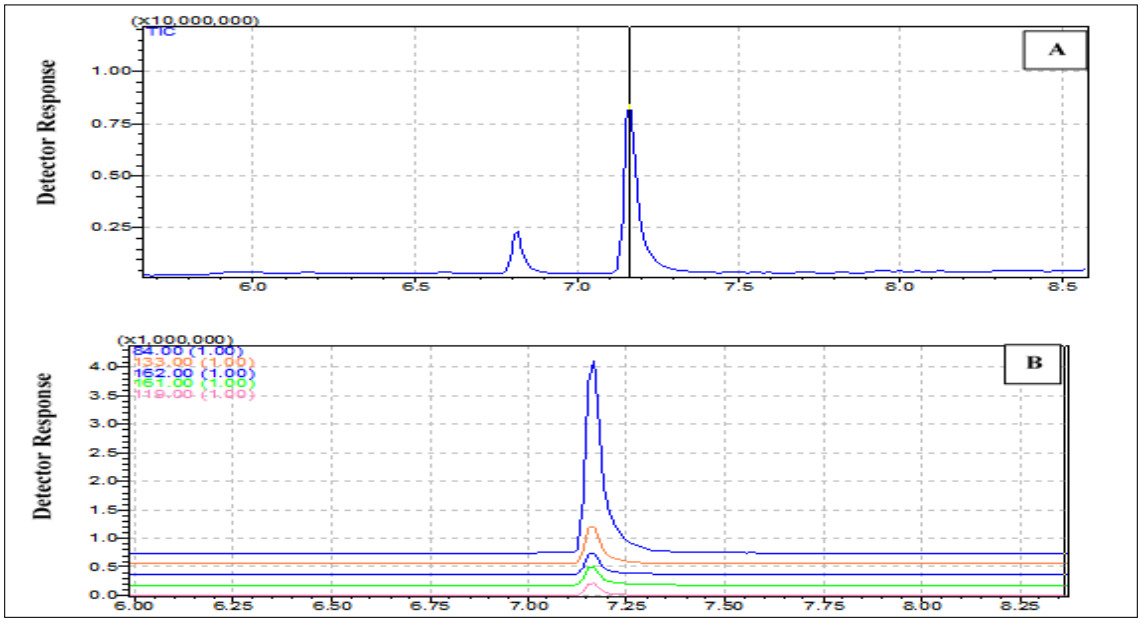

Figure 2. GC-MS Chromatograms of Nicotine in Cigarette Sample: A- Scan mode, B- SIM mode, where the 84, 133, 162, 161, and 119 m/z are quantification and confirmation ions used in SIM mode to analyze nicotine.

2.7. Instrument and Method Validation

GC-FID method analysis was performed and validated according to the data from YSMO 820/2004 “Cigarettes,” GSO597/2009 “Cigarettes,” and GSO / ISO 10315: "Determination of nicotine in smoke condensate – Gas Chromatographic methods" [27], and previously published papers [28–33].

2.7.1. Linearity and Calibration Curves

Using the GC-FID system, a series of seven nicotine standard solutions in the range of 5 to 500 ppm and six spiked amounts of nicotine in the range of 0.1 to 1.0 mg/cig concentrations were analyzed to determine the linearities of the instrument and method.

2.7.2. Precision (Repeatability)

The relative standard deviation (%RSD) was used to assess the instrument precision and the method. Three duplicate analyses of the prepared standards and spiked samples were performed and the (%RSD) was calculated for each concentration.

2.7.3. Sensitivity (Detection and Quantification limits)

The instrument and method limits of detection (LOD) and limits of quantification (LOQ) for nicotine were calculated from the chromatograms of the nicotine standard and spiked sample. The LOD and LOQ are the concentrations corresponding to the peak with a height of 3 and the average noise height, respectively. This value was

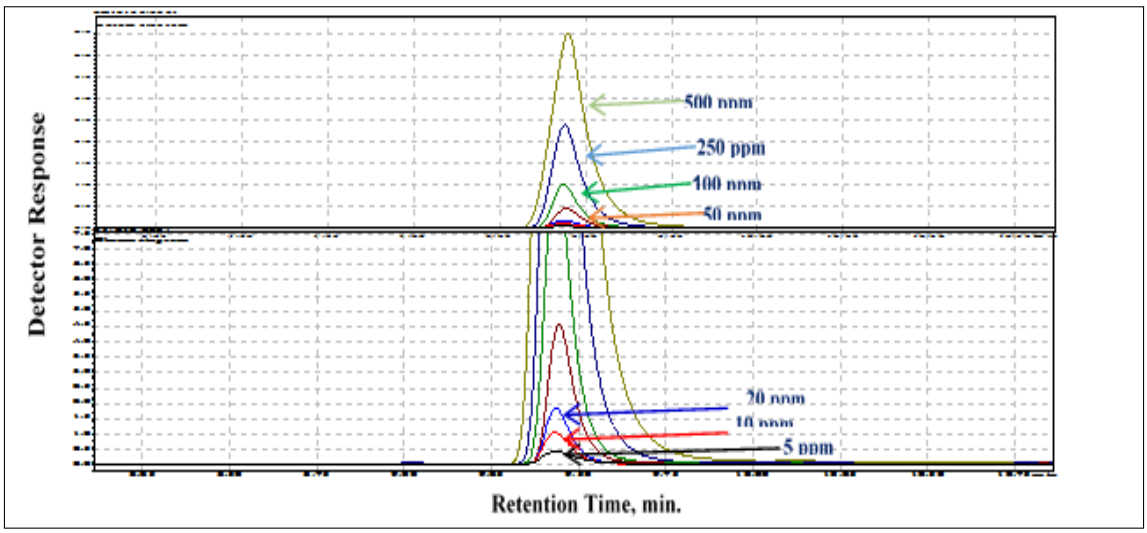

Figure 3. Overlay Chromatograms of Nicotine Standards.

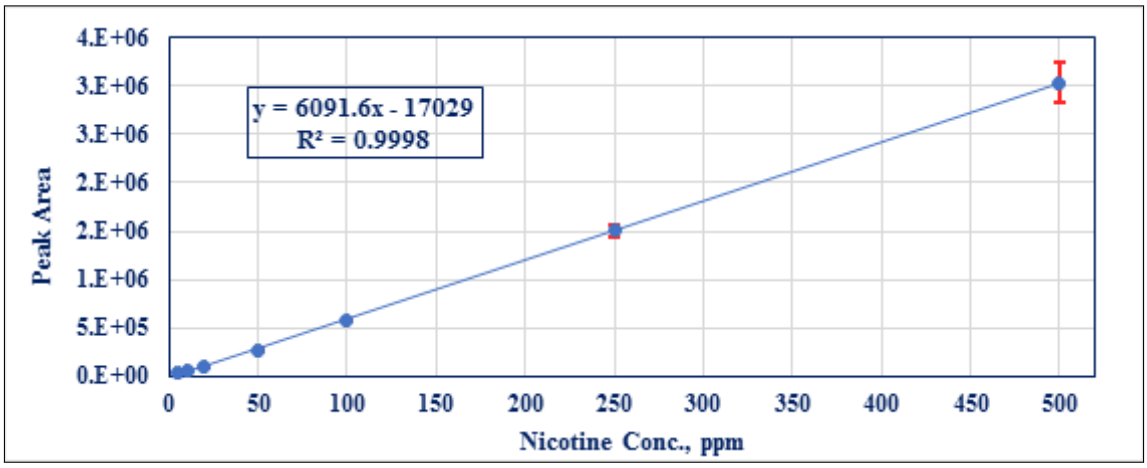

Figure 4. : Calibration Curve for Nicotine Standard Solutions.

calculated using the following equation:

$$LOD = 3 \times Cstd \times Hnoise/Hstd$$

$$LOQ = 10 \times Cstd \times Hnoise/Hstd$$

Where Cstd, Hnoise, and Hstd are the concentration of the standard, the height of the noise peak, and the height of the standard peak, respectively.

2.7.4. Accuracy (Recovery)

Accuracy studies were performed to examine the efficiency of the method. To carry out the recovery, three independent analyses of cigarette samples spiked with nicotine range (0.1-1.0 mg/cig.) were performed. The Recovery (%R) was calculated using the following formula [34]:

$$\%R = (A1 - A2/A3) * 100,$$

Where A1 is the spiked sample's peak area, A2 is the sample's peak area before spiking, and A3 is the standard's peak area.

3. RESULTS AND DISCUSSION

3.1. Identification of Nicotine Using GC-MS

The Scan and selective ion monitoring (SIM) chromatograms for nicotine standard were represented in Fig.2.

3.2. Instrument Validation

The linearity of the GC-FID was investigated by analyzing seven nicotine standard solutions three times on the same day at concentrations of 5–500 ppm. The calibration results of GC-FID are presented in Table 1, overlay the GC-FID chromatograms of the nicotine standards, and the calibration curve is shown in Fig.3. The linearity was excellent, with a correlation coefficient (R2 value of 0.9998, and the linear regression equation (y =6091.6x–17029) was calculated from the calibration curve, as shown in Fig.4. After analyzing each prepared standard in triplicate, the relative standard deviation (%

Table 1: The Calibration and Precision Results for Different Nicotine Standard Concentrations.

No.	Concentration (ppm)	Average of Peak Area, n=3	SD	%RSD
1	5.00	30549	1543	5.05
2	10.00	61887	3304	5.34
3	20.00	100043	3865	3.86
4	50.00	271226	1920	0.71
5	100.00	570152	14102	2.47
6	250.00	1510365	62248	4.12
7	500.00	3032193	202152	6.67

Table 2: The Results of Linearity, Precision, and Recovery of Spiked Cigarette Sample.

Spiked Amount of Nicotine (ppm)	Measured Nicotine (spiked+ unspiked) (mg/cig), n=3	Measured spiked Nicotine (mg/cig), n=3	SD	%RSD	Recovery, %
0.00	0.512	0.000	0.0197	3.84	////////
0.10	0.608	0.096	0.0040	4.17	95.79
0.20	0.701	0.189	0.0042	2.22	94.51
0.40	0.894	0.382	0.0040	1.04	95.53
0.60	1.092	0.580	0.0228	3.93	96.68
0.80	1.304	0.792	0.0376	4.75	98.98
1.00	1.459	0.947	0.0260	2.74	94.75

RSD) for each concentration was determined. The results are presented in (Table 1). The %RSD varied, ranging from 0.71 to 6.67%; low %RSD results show that the analysis procedure was highly precise. The detection and quantification limits for GC-FID sensitivity were determined to be 0.0926 ppm for the LOD and 0.2777 ppm for the LOQ.

3.3. Method Validation

Three independent samples were spiked for each concentration, with nicotine at concentration levels ranging from 0.1 to 1.0 mg of nicotine per cigarette. Each sample was then prepared using a smoking machine to trap nicotine from the cigarette smoke, the GF paper, extracted by 10 ml of propanol and analyzed by the GC-FID system to verify the linearity, accuracy, and precision, and to calculate the LOD and LOQ for the analysis method.

Based on Fig.5 and Table 2, with a correlation coefficient R2 value of 0.9987, the linearity was excellent. The method limit of quantification (LOQ) for spiked samples was 0.3166 ppm (equivalent to 0.003166 mg/cigarette), below the MRLs value (0.6 mg/cig.) shows the sensitivity and suitability of the analytical method. The accuracies calculated as average recoveries (% R) values were between 94.51 and 98.98%, while the RSD did not exceed 5.01 %. These results indicate the high accuracy and precision of the proposed method.

3.4. Real samples analysis results

The analytical approach was then used to analyze the real samples after validation. The amount of nicotine in 87 cigarette samples, 21 domestic and 66 imported, was determined through analysis using a spiked calibration curve equation. Tables 3 and 4 summarize the results obtained. Table 3 and Fig.6 display the results of the nicotine concentration in the local cigarette smoke, which varied from 0.512 to 1.564 mg/cig. The results revealed that two samples (9.5%) had nicotine levels below the acceptable value, whereas 19 cigarette samples (90.5%) had nicotine levels that were primarily higher than 0.6 mg/cig. In Table 3, it is clear that only one out of 21 cigarette backets was labelled as nicotine. The nicotine concentrations in the imported cigarettes, displayed in Fig.7 and Table 4, varied from 0.353 to 2.32 mg/cig. The results showed that 19 samples (28.8%) had less nicotine than the acceptable value and 47 samples (71.2%) had nicotine primarily higher than 0.6 mg/cig. According to the Yemeni Organization for Standards, Metrology, and Quality Control (YSMO) YSMO 820/2004 "Cigarettes" [27], and 46 out of 66 of cigarette backets were labelled the nicotine amount which ranged from 0.1 to 1.2 mg/cig as shown in Table 4. For all samples (87), 24.80% of the local and 75.20% of imported samples had nicotine contents less than the accepted value, while 31.82% of the local, and 68.18% of the imported samples had nicotine contents greater than the accepted value, as shown in Fig.8.

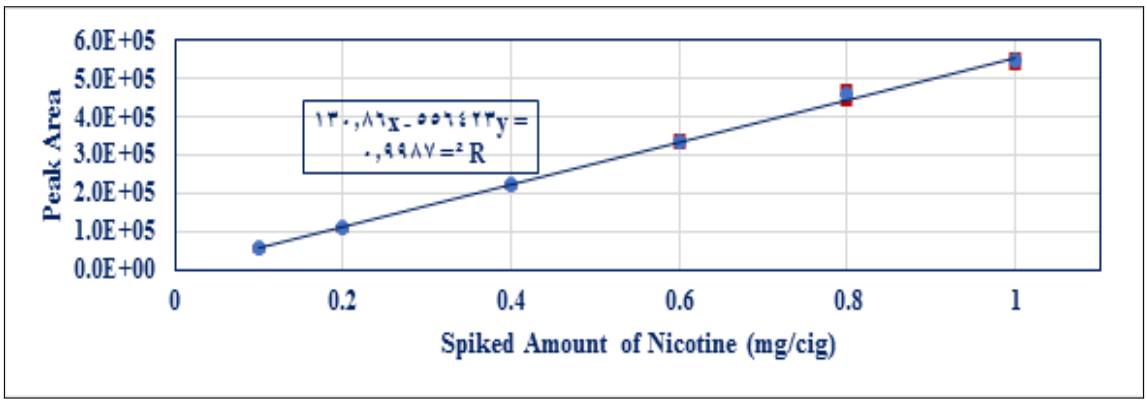

Figure 5. Calibration Curve of Spiked Cigarette with Nicotine.

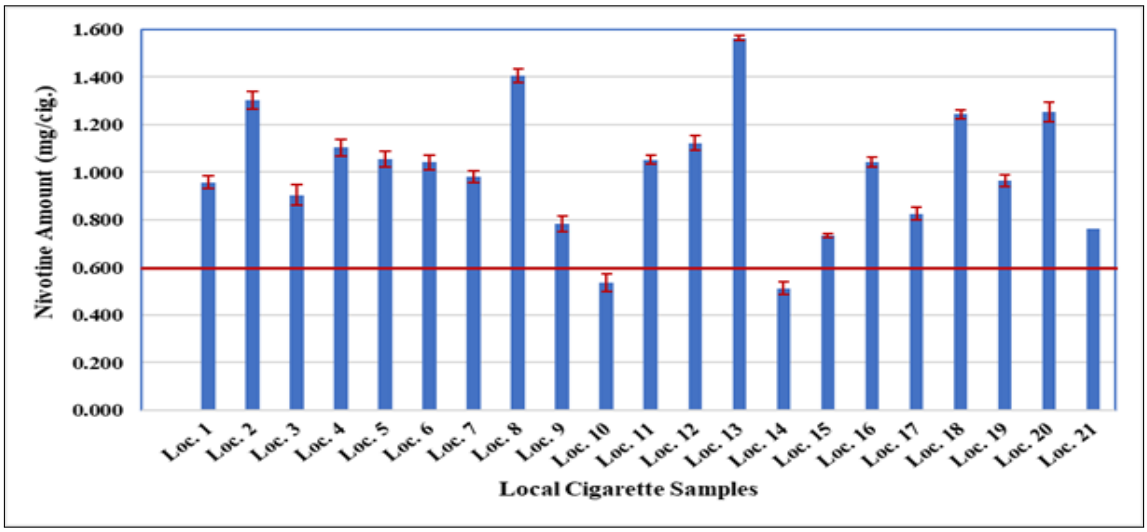

Figure 6. Amount of Nicotine in Local Cigarettes Smoke Samples.

Table 3: Nicotine Content in Local Cigarette Smoke Samples.

Cod.	Measured Nicotine (mg/cig), n=3	SD	%RSD	Labelled Nicotine(mg/cig)
Loc. 1	0.958	23703.72	4.45	*..
Loc. 2	1.303	15365.79	2.12	*..
Loc. 3	0.904	20133.36	4.00	*..
Loc. 4	1.103	23938.11	3.90	*..
Loc. 5	1.056	18831.31	3.21	*..
Loc. 6	1.041	17716.04	3.06	*..
Loc. 7	0.982	16289.30	2.98	*..
Loc. 8	1.405	13800.25	1.77	*..
Loc. 9	0.782	16660.28	3.83	0.8
Loc. 10	0.537	17942.01	6.01	*..
Loc. 11	1.053	21164.25	3.61	*..
Loc. 12	1.123	10373.35	1.66	*..
Loc. 13	1.564	16886.36	1.94	*..
Loc. 14	0.512	6153.52	2.16	*..
Loc. 15	0.732	15249.48	3.74	*..
Loc. 16	1.043	4679.10	0.81	*..
Loc. 17	0.825	11355.14	2.48	*..
Loc. 18	1.243	14950.37	2.16	*..
Loc. 19	0.965	9972.09	1.86	*..
Loc. 20	1.253	13679.95	1.96	*..
Loc. 21	0.765	22203.63	5.22	*..

*..= There is no Nicotine concentration labeled on the bottle, The Permitted Nicotine concentration = is 0.6 mg/cig

Table 4: Nicotine Content in Imported Cigarette Smoke Samples.

Cod.	Measured Nicotine (mg/cig), n=3	SD	% RSD	Labelled Nicotine (mg/cig)
Imp. 1	0.506	0.021	4.22	0.1
Imp. 2	0.640	0.020	3.04	0.5
Imp. 3	0.428	0.020	4.66	0.4
Imp. 4	0.353	0.021	5.83	0.3
Imp. 5	0.599	0.013	2.12	0.4
Imp. 6	0.832	0.021	2.47	0.6
Imp. 7	0.382	0.018	4.75	0.4
Imp. 8	0.456	0.030	6.58	0.5
Imp. 9	0.566	0.027	4.78	0.6
Imp. 10	0.557	0.014	2.56	0.1
Imp. 11	0.568	0.026	4.55	0.1
Imp. 12	1.093	0.024	2.16	0.1
Imp. 13	0.764	0.025	3.29	0.3
Imp. 14	1.104	0.036	3.30	0.8
Imp. 15	0.599	0.022	3.72	0.1
Imp. 16	0.593	0.027	4.59	*..
Imp. 17	0.459	0.023	5.09	*..
Imp. 18	1.013	0.019	1.88	0.9
Imp. 19	0.985	0.009	0.92	0.8
Imp. 20	0.884	0.010	1.17	0.3
Imp. 21	1.506	0.021	1.37	1.2
Imp. 22	1.565	0.025	1.61	*..
Imp. 23	0.764	0.044	5.81	*..
Imp. 24	0.748	0.011	1.45	0.5
Imp. 25	1.083	0.046	4.28	0.4
Imp. 26	0.489	0.026	5.39	0.7
Imp. 27	0.921	0.014	1.48	0.6
Imp. 28	0.830	0.009	1.13	0.5
Imp. 29	0.543	0.021	3.80	*..
Imp. 30	0.587	0.018	3.08	*..
Imp. 31	1.166	0.021	1.83	*..
Imp. 32	0.531	0.021	3.93	*..
Imp. 33	0.730	0.016	2.25	0.5
Imp. 34	0.633	0.019	3.04	0.6
Imp. 35	0.716	0.019	2.72	0.5
Imp. 36	0.540	0.030	5.48	0.6
Imp. 37	0.512	0.020	3.84	0.5
Imp. 38	0.950	0.021	2.22	0.5
Imp. 39	0.980	0.009	0.91	*..
Imp. 40	0.563	0.012	2.09	0.6
Imp. 41	0.535	0.026	4.77	*..
Imp. 42	0.723	0.010	1.37	*..
Imp. 43	2.119	0.042	1.99	0.5
Imp. 44	2.416	0.056	2.32	0.6
Imp. 45	0.959	0.033	3.45	*..
Imp. 46	0.692	0.029	4.26	*..
Imp. 47	1.419	0.010	0.71	*..
Imp. 48	0.892	0.017	1.95	*..
Imp. 49	0.482	0.024	5.08	0.4
Imp. 50	1.000	0.025	2.49	0.3
Imp. 51	0.884	0.021	2.37	0.3

Cod.	Measured Nicotine (mg/cig), n=3	SD	% RSD	Labelled Nicotine (mg/cig)
Imp. 52	1.151	0.018	1.56	0.3
Imp. 53	0.892	0.027	3.02	0.3
Imp. 54	1.284	0.028	2.18	0.3
Imp. 55	1.464	0.010	0.70	0.3
Imp. 56	1.182	0.012	0.99	0.3
Imp. 57	1.222	0.025	2.02	0.3
Imp. 58	0.849	0.025	2.97	*..
Imp. 59	1.547	0.039	2.49	0.4
Imp. 60	2.098	0.044	2.10	1
Imp. 61	0.803	0.027	3.36	*..
Imp. 62	0.712	0.026	3.59	*..
Imp. 63	0.780	0.006	0.73	*..
Imp. 64	0.899	0.013	1.44	0.8
Imp. 65	0.957	0.033	3.48	*..
Imp. 66	1.350	0.031	2.27	0.5

*..= There is no Nicotine concentration labeled on the bottle, The Permitted Nicotine concentration = is 0.6 mg/cig

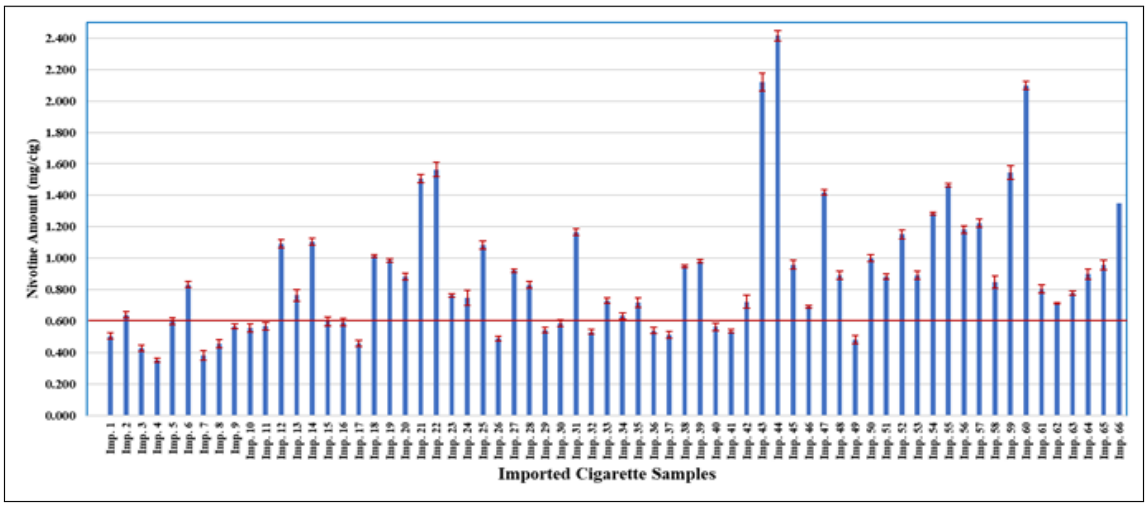

Figure 7. Amount of Nicotine in Imported Cigarettes Smoke Samples.

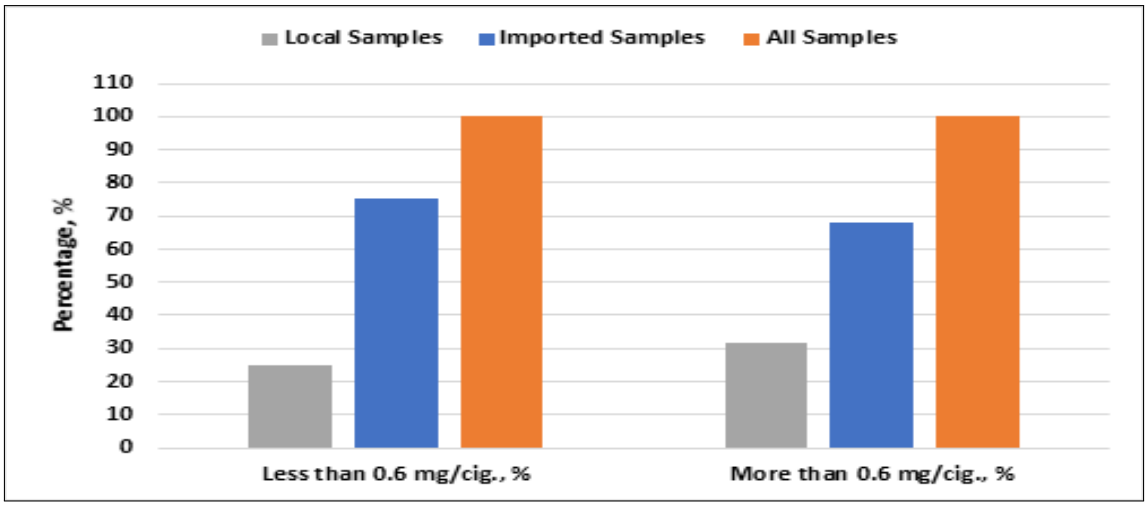

Figure 8. The Summary of Nicotine Content of Cigarette Smoke Samples.

4. CONCLUSION

The validation results show that the proposed method has good precision and excellent linearity over the studied range. Moreover, the LOD and LOQ of the method are equivalent to 0.000926 and 0.00277 mg/cigarette, respectively, which is far less than the accepted value of 0.6 mg/cigarette as per the reference standards. Based on the results obtained in this study, it can be concluded that the GC-FID method is valid and applicable for the quantification of nicotine in cigarette smoke. The nicotine content of 87 tobacco cigarette samples was determined. For the local and imported samples, 19 out of 21 and 45 out of 66 tobacco samples exceeded the permitted limits.

AUTHOR CONTRIBUTIONS

N. A. and A. A. performed and designed the experiment; F, A, and M.A methodology, A.A. and N.A.; analyzed the data F.A and M.A.; writing-original draft preparation, F.A. and M.A; writing-review and editing, F.A and N.A.; supervision, A. A. All authors have read and agreed to the published version of the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

[1] K. Kim and M. Picciotto, "Nicotine addiction: More than just dopamine," *Curr. Opin. Neurobiol.*, vol. 83, p. 102 797, (2023). DOI: 10.1016/j.conb.2023.102797.

[2] N. Benowitz and J. Henningfield, "Establishing a nicotine threshold for addiction," *N Engl J Med.*, vol. 331, no. 2, pp. 123–125, (1994). DOI: 10 . 1056 / NEJM199407143310212.

[3] S. Mahpishanian and H. Sereshti, "Graphene oxide-based dispersive micro-solid phase extraction for separation and preconcentration of nicotine from biological and environmental water samples followed by gas chromatography-flame ionization detection," *Talanta*, vol. 130, pp. 71–77, (2014). DOI: 10.1016/j.talanta.2014.06.004.

[4] S. S. Hecht, "Tobacco carcinogens, their biomarkers, and tobacco-induced cancer," *Nat. Rev. Cancer.*, vol. 3, no. 10, pp. 733–744, (2003). DOI: 10.1038/nrc1190.

[5] D. Baconi, D. Bălălău, and P. Abraham, *Abuzul & toxicodependen021ba*. Bucureşti: Editura Medicală, pp. 369-372, (2008).

[6] N. Goriounova and H. Mansvelder, "Short-and long-term consequences of nicotine exposure during adolescence for prefrontal cortex neuronal network function," *Cold Spring Harb. Perspect. Med.*, vol. 2, no. 12, pp. 1–14, (2012). DOI: 10.1101/cshperspect.a012120.

[7] G. Thompson and D. Hunter, "Nicotine replacement therapy," *Ann. Pharmacother.*, vol. 32, pp. 1067–1075, (1998). DOI: 10.1345/aph.17382.

[8] P. Newhouse, A. Potter, M. Kelton, and J. Corwin, "Nicotinic treatment of alzheimer's disease," *Biol. Psychiatry*, vol. 49, no. 3, pp. 268–278, (2001). DOI: 10.1016/s0006-3223(00)01069-6.

[9] R. S. Pappas, G. M. Polzin, C. H. Watson, and D. L. Ashley, "Cadmium, lead, and thallium in smoke particulate from counterfeit cigarettes compared to authentic us brands," *Food Chem. Toxicol.*, vol. 45, no. 2, pp. 202–209, (2007). DOI: 10.1016/j.fct.2006.08.001.

[10] M. Carro, G. Guidon, N. Sharma, and D. Shokoohi, *Tobacco control country profiles*, Available at URL: http://www5.who.int/tobacco/page.cfm?sid=57, Atlanta, GA, (2000).

[11] G. Tayoub, H. Sulaiman, and M. Alorfi, "Determination of nicotine levels in the leaves of some nicotiana tabacum varieties cultivated in syria," *Herba Pol.*, vol. 61, pp. 23–30, (2015). DOI: 10.1515/hepo-2015-0028.

[12] V. Ghalop, L. Kosmider, and M. Halquist, "A standardized approach to quantitative analysis of nicotine in e-liquids based on peak purity criteria using high-performance liquid chromatography," *J Anal Methods Chem.*, vol. 9, p. 1 720 375, (2018). DOI: 10.1155/2018/1720375.

[13] F. Marclay and M. Saugy, "Determination of nicotine and nicotine metabolites in urine by hydrophilic interaction chromatography-tandem mass spectrometry: Potential use of smokeless tobacco products by ice hockey players," *J. Chromatogr. A*, vol. 1217, no. 48, pp. 7528–7538, (2010). DOI: 10.1016/j.chroma.2010.10.005.

[14] X. Xu, M. Iba, and C. Weisel, "Simultaneous and sensitive measurement of anabasine, nicotine, and nicotine metabolites in human urine by liquid chromatography-tandem mass spectrometry," *Clin. Chem.*, vol. 50, no. 12, pp. 2323–2330, (2004). DOI: 10.1373/clinchem.2004.038489.

[15] M. Meger, I. Meger-Kossien, A. S. Metz, D. Janket, and G. Scherer, "Simultaneous determination of nicotine and eight nicotine metabolites in the urine of smokers using liquid chromatography–tandem mass spectrometry," *J. Chromatogr. B*, vol. 778, no. 1-2, pp. 251–261, (2002). DOI: 10.1016/s0378-4347(01)00451-0.

[16] A. Abu-awwad, T. Arafat, and O. Schmitz, "Simultaneous determination of nicotine, cotinine, and nicotine n-oxide in human plasma, semen, and sperm by lc-orbitrap ms," *Anal. Bioanal. Chem.*, vol. 408, no. 23, pp. 6473–6481, (2016). DOI: 10.1007/s00216-016-9766-7.

[17] L. Sheng, L. Ding, H. Tong, G. Yong, X. Zhou, and S. Liu, "Determination of nicotine-related alkaloids in tobacco and cigarette smoke by gc-fid," *Chromatographia*, vol. 62, pp. 63–68, (2005). DOI: org/10.1365/s10337-005-0567-y.

[18] J. Cai, B. Liu, P. Lin, and Q. Su, "Fast analysis of nicotine related alkaloids in tobacco and cigarette smoke by megabore capillary gas chromatography," *J. Chromatogr. A*, vol. 1017, no. 1-2, pp. 187–193, (2003). DOI: 10.1016/j.chroma.2003.07.003.

[19] S. Yang, S. Smetena, and C. Huang, "Determination of tobacco alkaloids by gas chromatography with nitrogen–phosphorus detection," *Anal. Bioanal. Chem.*, vol. 373, no. 8, pp. 839–843, (2002). DOI: 10.1007/s00216-002-1380-1.

[20] J. Lisko, S. Stanfill, B. Duncan, and C. Watson, "Application of gc-ms/ms for the analysis of tobacco alkaloids in cigarette filler and various tobacco species," *Anal. Chem.*, vol. 85, no. 6, pp. 3380–3384, (2002). DOI: 10.1021/ac400077e.

[21] W. Wu, D. Ashley, and C. Watson, "Determination of nicotine and other minor alkaloids in international cigarettes by solid-phase microextraction and gas chromatography/mass spectrometry," *Anal Chem.*, vol. 74, no. 19, pp. 4878–84, (2002). DOI: 10.1021/ac020291p.

[22] L. Li *et al.*, "Direct determination of free nicotine content in tobacco," *ACS Omega*, vol. 7, no. 27, pp. 23 061–23 068, (2022). DOI: 10.1021/acsomega.2c00089.

[23] I. Shaffer and Y. Lin, "Quantification of nicotine in e-cigarette liquid sample using gc-fid and hydrogen carrier gas," Shimadzu Scientific Instruments, Shimadzu Corporation, Tech. Rep., (2018). [Online]. Available: https://www.shimadzu.com/an/sites/shimadzu.com.an/files/pim/pim_document_file/technical/technical_reports/13363/sic119019.pdf.

[24] A. Chen, F. A. Morsed, and N. Cheah, "A simple method to simultaneously determine the level of nicotine, glycerol, propylene glycol, and triacetin in heated tobacco products by gas chromatography-flame-ionization detection," *J AOAC Int.*, vol. 105, no. 1, pp. 46–53, (2022). DOI: 10.1093/jaoacint/qsab140.

[25] R. Carter, A. Kovach, and B. Thomas, "A comparison of nicotine content methods to produce a upc2-ms2 method for the analysis of nicotine and minor alkaloids in spectrum nicotine research cigarettes," *Anal Bioanal Chem.*, vol. 414, no. 19, pp. 5817–5828, (2022). DOI: 10.1007/s00216-022-04144-x.

[26] L. Vlase, L. Filip, I. Mîndruţău, and S. E. Leucuţa, "Determination of nicotine from tobacco by lc-ms-ms," *Studia Univ. Babes-Bolyai Phys.*, vol. 4, pp. 19–24, (2005), [Google Scholar].

[27] *Gso597/2009 "cigarettes"*, and GSO / ISO 10315: "Determination of nicotine in smoke condensate – Gas Chromatographic methods", General Organization for Standards, (2009).

[28] M. M. AL-Hammadi, A. A. Alnedhary, A. A. Numan, F. A. Murshed, and R. A. Alalie, "Validation and determination of mercury in yemeni common canned tuna using direct mercury analyzer," *JAST*, vol. 1, no. 4, pp. 339–345, (2023). DOI: 10.59628/jast.v1i4.664.

[29] M. M. AL-Hammadi, A. A. Numan, A. A. Alnedhary, F. A. Murshed, and T. H. Al-Hoded, "Optimization, validation, and application of a quantitative gc/npd method for acrylamide determination in yemeni fried fish samples," *JAST*, vol. 1, no. 4, pp. 377–386, (2023). DOI: 10.59628/jast.v1i4.698.

[30] A. A. Alnedhary, M. M. AL-Hammadi, A. A. Numan, and F. A. Murshed, "Optimization and efficiency comparison of dispersive and cartridge solid phase extraction cleanup techniques in the analysis of pesticide residues in some vegetables using gas chromatography-mass spectrometry," *PSM Biol Res*, vol. 5, no. 1, pp. 40–54, (2020). [Online]. Available: https://psmjournals.org/index.php/biolres/article/view/393.

[31] M. M. AL-Hammadi, A. A. Alnedhary, A. A. Numan, and F. A. Murshed, "Validation and application of combined quechers extraction with cartridge solid phase extraction cleanup for pesticide multiresidue analysis in some vegetables by gc-ecd," *PSM Microbiol.*, vol. 5, no. 1, pp. 14–25, (2020). [Online]. Available: https://psmjournals.org/index.php/microbiol/article/view/414.

[32] A. A. Alnedhary, A. A. Numan, M. M. AL-Hammadi, F. A. Murshed, and H. M. Dubais, "A comparative study to assess the quality of different marketed brands of metformin hcl," *PSM Biol Res*, vol. 6, no. 3, pp. 84–95, (2021). [Online]. Available: https://psmjournals.org/index.php/biolres/article/view/565.

[33] Ş. CEYLAN, R. YARAR, Y. CAMADAN, Ö. SARAL, and Ö. ÖZŞEN, "Determination of antioxidant and antimicrobial activities of some medicinal plants grown in şırnak region of turkey," *Erzincan Univ. J. Sci. Technol.*, vol. 12, no. 2, pp. 628–638, (2019).

[34] W. Bremser, *The fitness for purpose of analytical method: A laboratory guide to method validation and related topics* (Eurachem Guide), 2nd. (2014), Available from http://www.eurachem.ul.pt.