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RESEARCH ARTICLE

DETERMINATION OF NICOTINE IN DOMESTIC AND IMPORTED CIGARETTES IN ADEN (YEMEN) MARKETS

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Abstract

The current study aimed at determining the nicotine content of some domestic and imported cigarettes and comparing the nicotine content among 12 types of familiar cigarettes sold in the markets of Aden Governorate (Yemen) using high-performance liquid chromatography linked with a UV detector, (HPLC-UV). The results of this study showed that the amount of nicotine in each cigarette was from 7.29 to 18.74 mg/g cigarettes with an average of 12.75mg/g cigarettes, in imported cigarettes, and between 11.34 to 17.52 mg/g cigarettes with an average of 14.40 mg/g in domestic cigarettes. Although the amount of nicotine in both domestic and imported cigarettes showed a large variation, but when comparing the averages of local and imported cigarettes, the statistical analysis showed that there were no statistically significant differences between the averages of local and imported cigarettes. The Nicotine content of all tested cigarettes (imported and local brands) was higher than the international standard (1mg/g tobacco).

Keywords: Nicotine, Cigarettes, Tobacco, HPLC-UV.

1. Introduction

Tobacco use is a major cause of death worldwide [1]. Tobacco kills more than 8 million people every year of whom more than 7 million are direct users of it and about 1.2 million are non-smokers exposed to second-hand smoke [2]. Most tobacco users live in low- and middle-income countries [3]. Some recent studies concluded that smokers were more likely to be infected with Corona disease (COVID-19) with a significant increase in deaths from the disease between smokers [4-5].

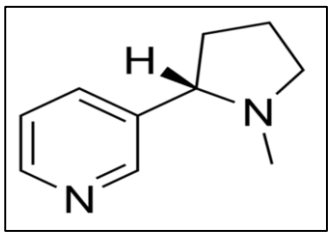

Fig. 1: Chemical structure of nicotine

The chemical structure of nicotine: (C10H14N2, CAS:54-11-5, MolWeight:162) (Figure 1), [6]. It is 3- (1-methylpyrrolidin-2-yl) pyridine, which is a purine alkaloid with stimulating and highly addictive effects [7]. It is water soluble, hygroscopic oily liquid, colorless, less to pale yellow [8]. Nicotine is a secondary metabolite in a variety of plants of the *Solanaceae* family [9]. It is found naturally in the form of the S-(-) isomer in plants from Nicotiana genus such as N. tabacum (4.98–13.50 mg/g dry weight of leaves), *N. rustica* (e.g. 3.17–7.59 mg/g), *N. silvestris* (0.77–23.70 mg/g) or *N. glauca* (11.0–18.2 mg/g) [10]. It is also found naturally in smaller amounts in plants from the family *Solanaceae* (such as potatoes, tomatoes, and eggplant) [11]. Nicotine is the main component of the alkaloids, containing about 95% of the total content of alkaloids in tobacco [12].

The human body obtains nicotine through smoking, chewing and smelling, all of which have dangerous and destructive effects on human health [13]. Consumption of smoking and smokeless tobacco are common all over the world [14]. Long-term tobacco use is associated with increased rates of cancer, chronic obstructive pulmonary disease, atherosclerosis, high blood pressure and low birth weight for babies born to mothers who smoke [15]. Absorption of nicotine across biological membranes through oral mucosa (chewing tobacco and snuff) and through the lungs, presumably because of the huge surface area of the alveoli and small airways, (cigarettes smoke) [16]. When nicotine enters the body, it is quickly distributed through the bloodstream and crosses the blood-brain barrier. It reaches the brain within 10-20 seconds after inhalation [17].

The current study aims to determine the nicotine content of some domestic and imported cigarettes and compare the nicotine content between 12 types of famous cigarettes sold in the markets of Aden Governorate (Yemen), using high-performance liquid chromatography linked with a UV detector, (HPLC-UV).

2. Materials and Method

2.1 Chemicals and apparatus

The chemicals used in the study were: S (-) Nicotine Standard, CAS: 54-11-5 ,≥ (99.0%) from Merck-Schucharde, Germany. Methanol 99.8% of HPLC grade from (Lobal Chemie. India), hydrochloric acid (HCl) 37.0% from BDH England, Ortho phosphoric acid 85% from Merck, Germany, and tri ethyl amine 99.5% from BDH England.

The study used Performance liquid chromatography with UV detector,(HPLC-UV): HPLC device produced by the Japanese company JASCO Model LC-NET: LC-NetII⁄ADC S No B323861095 POWER AC100V-240V 50⁄60HZ 28 VA which consists of a fixed-capacity HPLC pump and a detector that works in the ultraviolet field (UV #1: UV-2075) at 260 nm. It is linked to a computer that has the programs for this analysis. The column used is C18, which is 25 cm long and contains the octa decyl silane (ODS) filler containing porous silica. The mobile phase consisted of methanol and Ortho-phosphoric acid (0.2 M) 40: 60 % V/V and Buffered adjust PH 7.25 with tri ethyl amine at a flow-rate of 1 ml/min [18-19].

Table 1: HPLC chromatographic conditions of the method.

Chromatography condition	
Flow rate	1 mL/min
Wavelength	260 nm
Detector	(UV #1: UV-2075)
Column	C18 (250 × 4.6 mm)
Column oven temperature	30 ^{0}C
Injection volume	20 µl

2.2 Analysis of the Samples

2.2.1 Sample Collection

The sample of the study consisted of twelve types of cigarettes (six types are domestic and six are imported), that are more sold in the markets of Aden (Yemen), Three packs of each type of cigarette were selected randomly, one cigarette from each packet taken for testing. Papers and filters have been removed from cigarettes before any extraction. The tobacco of each cigarette was smashed in the blender for half a minute. Extraction of nicotine from cigarette tobacco was carried out by the method described by [18-20-21] with some modifications.

0.5 gram of each sample of tobacco of cigarettes weighted by digital analytical balance was introduced in a 15 ml centrifuge tube. 9 ml of 40% (v/v) methanol and 1ml of 1N HCl were added, shaken for 3mintes, and centrifuged the mixture at 2000 rpm for 10 min. The extraction solution of nicotine was filtered through what man No.2 filter paper in a Buchner funnel. Each extract was taken in 50-ml flask then completed with methanol to mark, filtered through a 0.45 mm Newman syringe filter, and kept protected from light at 4-8 C to be injected in HPLC. (n = 3)

2.3 Procedure:

2.3.1 Preparation of standard solutions

2.3.1.1 Preparation of Nicotine stock solution

Approximately 50 mg nicotine was weighed into a 100-mL flask and dissolved in 100 mL of methanol. Standard solutions of different concentrations (2, 20, 30, 40, 50 mg/L) of nicotine were prepared by serial dilution with methanol for the construction of calibration curve.

2.4 Method validation

The developed HPLC method were validated for linearity, range, sensitivity (limits of detection (LOD) and quantitation (LOQ)), accuracy, precision and robustness as per ICH guidelines [22].

2.4.1 Linearity

After injecting different standard solutions of nicotine and obtaining the area for each concentration, a calibration curve was drawn in Fig. (2) where a linear response was obtained with a correlation coefficient R^2 = 0.9998 and the following linear equation was obtained Y=84817+x6917.3.

Table 2: Linear functions of the standard curve for nicotine

Parameter	Value
Nicotine standards (tr) average (min)	2.32640
Linear equation	y = 84817x +6917.3
Slope a	84817
Intercept b	6917.3
R^2	0.9998
SD	27773.54
P	< 0.05
LOD	1.08 mg/L
LOQ	3.27 mg/L

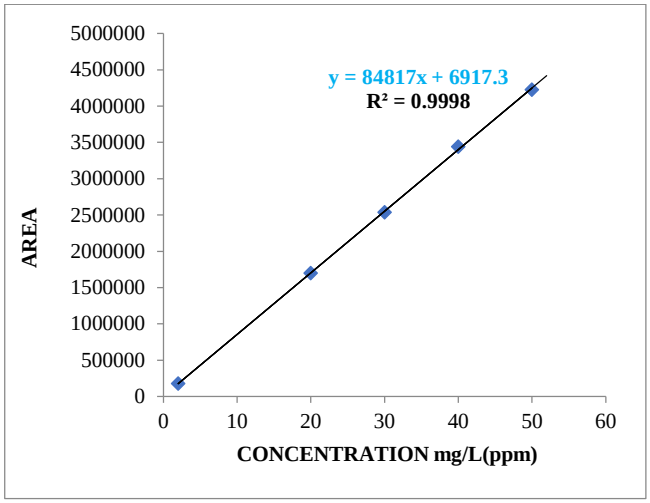

Fig. 2: Calibration curve for nicotine standard.

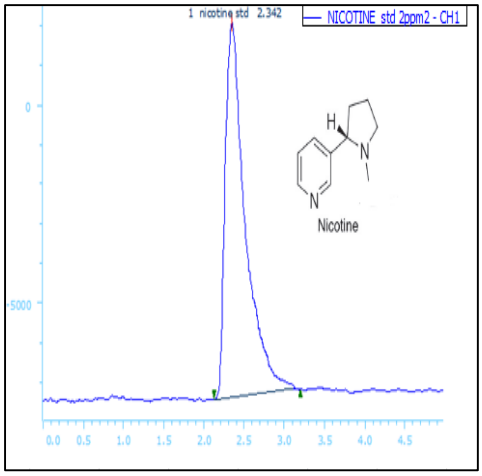

Fig. 3: Chromatogram of Nicotine Standard (2mg/L)

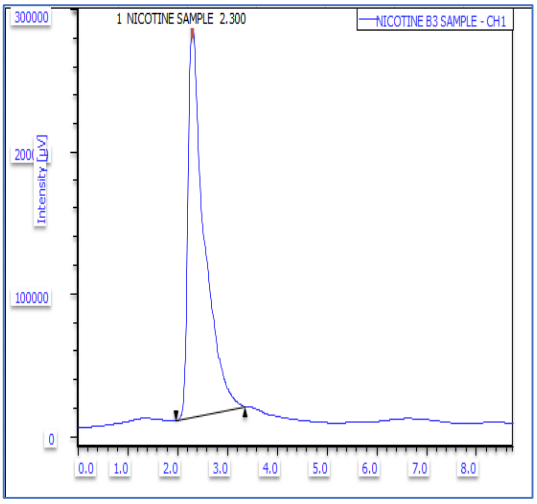

Fig. 4: chromatogram of local cigarette sample (Radfan cigarette)

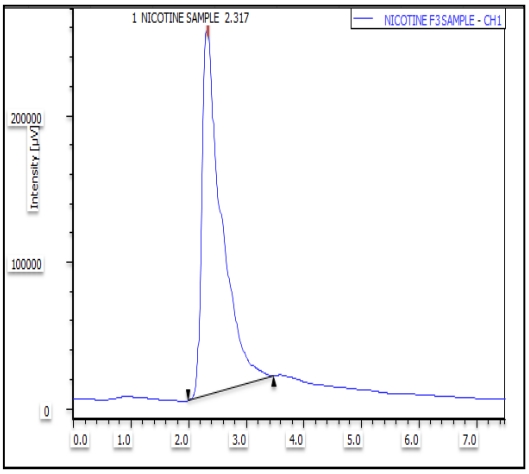

Fig. 5: chromatogram of imported cigarettes sample (Manchester cigarette).

2.4.2 Specificity/selectivity

The selectivity was evaluated as shown in Figures (3), which show the chromatogram of a standard solution and figure (4, 5) which shows the chromatogram of the samples of two of the products under study, respectively, and by comparing the peaks in the product chromatogram with the peaks in the standard solution chromatogram. We notice that there is no overlap between those peaks. This indicates that the separation process is good and appears in the same reservation time, i.e., the nicotine time 2.3 min, which indicates the high selectivity in the determination of nicotine identity depending on the time at which the peak appears in the chromatogram of the standard solution, which is 2.3 min in our study.

2.4.3 Limit of detection (LOD) and Limit of quantification (LOQ).

According to the analytical results revealed in Table (2), shown for the linear functions of the curve, and Figure (2) shown for the standard curve, we calculated the minimum detection limit for low nicotine concentrations using the following equation: [23]

$$\mathbf{LOD = 3.3 \times SD / S}$$

and also calculated the quantitative limit to detect low nicotine concentrations using the following equation:

$$\mathbf{LOQ = 10 \times SD / S}$$

Where SD is the standard deviation of the response and S is the slope.

2.4.4 Precision

To find out the accuracy on the near-term analysis method, (Intra-day), a standard solution of nicotine with a concentration of 20 mg/L and 30mg/L at a rate of three times during one day at a difference of two hours was injected. The nicotine concentration was calculated by the area of the peaks that appeared in the chromatogram, and by using the calibration curve equation or regression, the calculation of Standard deviation (SD), relative standard deviation (RSD%) and relative error (RE). To know the daily accuracy (inter-day), the standard solution of 20 mg/L and 30 mg/L was injected once a day for three consecutive days with a total of three iterations for each concentration, as shown in Table (2).

Table 2: Estimated intra- and inter-day relative standard deviation (RSD%) and relative error (RE) of the assay method.

Concentration Taken	Average of (N=3) in one day (Intera-day)				Average of (N=3) in three day (Inter-day)			
	Nicotine content	SD	RSD%	RE	Nicotine content	SD	RSD%	RE
20 mg/L	19.73	.201	1.018%	1.35	19.53	0.30	1.64 %	2.35
30 mg/L	29.64	0.15	0.506%	1.2	30.43	0.47	1.54 %	1.43

In light of the indicator values shown in Table 3 above, the method of analysis in the near-term (Intraday) and daily range (Inter-day) was considered to be acceptable, sensitive and accurate.

2.4.5 Recovery Test

Recovery of analyte spiked onto the matrix was used as a surrogate measure of accuracy. Recovery was determined by spiking known amounts of standards (before extraction) into an Erlenmeyer flask with tobacco and the nicotine was extracted by the same method as for samples. Unspiked tobacco was also analyzed. The recovery was calculated using the following equation and as shown in Table (3) [24].

= (analytical result-unspiked result / spiked amount) x 100% Recovery

The results of the retrieval test for one of the cigarette samples with pure nicotine added in different concentrations appear in Table (3). It is clear that the recovery rate (%) for the three solutions is (92%, 103%, and 94.66%) respectively with an average of 96.3%, which is an acceptable percentage that confirms that the extraction used in this study either recovered or extracted a large proportion of nicotine from the samples used, which increases the reliability of the results in this study.

Table 3: Amount and recovery of nicotine spiked onto the matrix

Sample	nicotine added (mg)	Amount of total nicotine (mg)	Recovery (%)
Pine	0	7.29	0
Pine1	0.5	7.75	92%
Pine2	1	8.32	103%
Pine3	1.5	8.71	94.66%

2.5 Statistical analysis

Statistical analysis was done using complete random design, and one-way ANOVA statistical analysis was used at a significant level ($P \leq 0.05$). Using Origin 7.5, SPSS, and excel statistical programs.

3. Results and Discussions

Table 4: shows that the amount of nicotine in local cigarette samples ranges from 11.34 to 17.52 mg /g tobacco with an average of 14.75mg/g tobacco, where Radfan cigarettes were found to have the highest amount of nicotine, while Kamaran cigarettes contain the least amount of nicotine. As for imported cigarettes, Table 5 reveals that the amount of nicotine in imported cigarettes ranges from 7.29 to 18.74 mg /g tobacco with an average 12.75 mg/g tobacco, where Best Man cigarettes were proved to have the highest amount of nicotine, while Pine cigarettes contain the least amount of nicotine.

Table 4: Amounts of nicotine mg/g in each type of domestic cigarettes

N	Cigarettes name	Nicotine contents mg/g cigarettes	Std. Deviation	RSD%
1	Radfan	17.52	0.26779	1.528
2	Lucky	15.73	0.1637	1.041
3	Shamlan	14.83	0.060	0.405
4	Rothman	13.62	0.085	0.624
5	Pall Mall	13.32	0.115	0.863
6	Kamran	11.34	0.1345	1.186
Mean	14.391			
Maximum	Radfan	17.52		
Minimum	Kamran	11.34		

Table 5: Amounts of nicotine mg/g in each type of imported cigarette

N	Name	Nicotine contents mg/g cigarettes	Std. Deviation	RSD%
1	Best Man	18.74	0.091	0.49
2	Manchester	13.68	0.115	0.84
3	Bon	13.3	0.141	1.06
4	Modern	12.05	0.13	1.08
5	Marlboro light	11.39	0.168	1.47
6	Pine	7.29	0.141	1.93
Mean	12.75			
Maximum	Best Man	18.740		
Minimum	Pine	7.290		

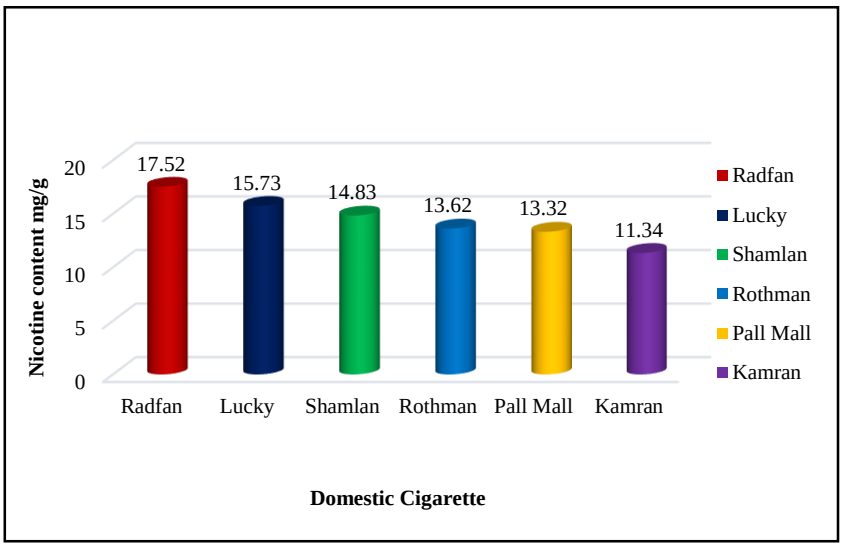

Fig. 6: Bar Chart showing Nicotine Content in domestic cigarette (mg/g)

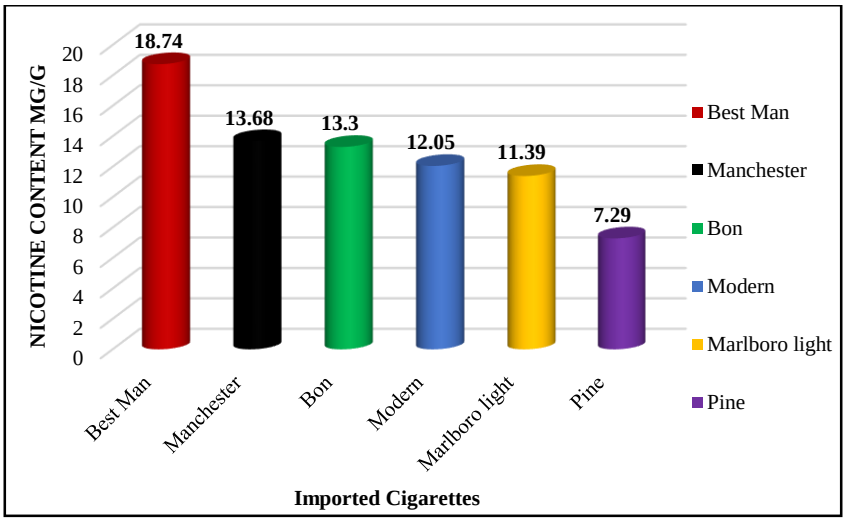

Fig. 7: Bar Chart showing Nicotine Content in Imported Cigarettes (mg/g)

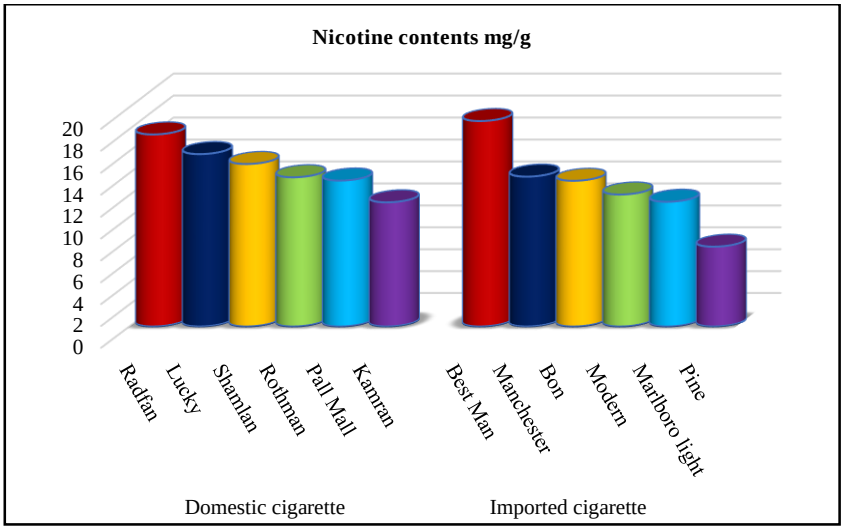

Fig. 8: Bar Chart showing Nicotine Content in all Cigarettes (mg/g)

There are wide differences in the content of nicotine in tobacco between cigarettes sold worldwide [25]. Difference in the content of nicotine in cigarette can be connected directly to the type of cigarettes used or manufacturing process, packaging, or quality of tobacco [26].

Statistical analysis showed statistically significant differences in the amounts of nicotine between randomly selected domestic cigarettes. The results showed that Radfan cigarettes outperformed the rest of the cigarette samples and Kamran cigarettes got the lowest percentage, and they show statistically significant differences with all the local cigarette samples, while Pall Mall cigarettes and Rothman cigarettes showed close results and there are no significant differences between them. In addition, the statistical analysis also showed statistically significant differences in the amounts of nicotine among the imported cigarettes selected at random. On the other hand, the results showed that Best Man cigarettes outperformed the rest of the samples of local and imported cigarettes, and Pine cigarettes got the lowest percentage, and showed statistically significant differences with all samples of local and imported cigarettes, while Manchester and Bonn cigarettes showed similar results, which are also close to locally made Pall Mall and Rothman cigarettes.

Although the amount of nicotine in both domestic and imported cigarettes showed a large variation as shown in Figure 3.24, it was in the range of (7.29-18.74 mg/g). But when comparing the averages of local and imported cigarettes, the results of the one-way analysis of variance showed that there are no statistically significant differences between the averages of local and imported cigarettes.

To compare the results obtained from this study, which were between 7.29-18.74 mg/ g in 12 types of domestic and imported cigarettes, with previous studies, the results were consistent with the findings of Vlase, et al [27] through the HPLC method, where nicotine values in tobacco between 7.5 and 17.6 mg / g were obtained from 40 brands of cigarettes available in Romania. The results were also close to the findings of Taghavi, et al [28], where the amount of nicotine in 22 domestic and imported brands in Iran was between 6.17 - 28.86 mg / g, as well as with the values indicated by Sharma et al, [29], in 22 brands of cigarettes sold in India, where the results were between 8.8 to 21.2 mg/g. However, Hosu and Cimpiou·s study [15] revealed much lower nicotine rates than the rates this study revealed, i. e., one mg /cigarette as they used thin-layer chromatography (TLC) method performed on silica gel F254 plates. Darmon et al, [26], also obtained (3.85E-02 to 14.0 mg/g) with an average of 5.31mg/g of nicotine in 13 types of cigarettes available in the Libyan market, using the UV and visible spectrum method, where it is less than what we obtained in this study. The decrease in nicotine content in the last two studies may be due to the difference in the methods used to determine nicotine.

4. Conclusion

The present study quantified nicotine in a group of domestic and imported cigarettes consumed in Aden (Yemen) using the method of chromatographic analysis (HPLC). The method of analysis showed high reliability and accuracy for determining nicotine concentrations in the studied samples. The results of this study showed that the amount of nicotine in each cigarette was from 7.29 to 18.74 mg/g cigarettes with an average of 12.75mg/g cigarettes, in imported cigarettes, and between 11.34 to 17.52 mg/g cigarettes with an average of 14.40 mg/g in domestic cigarettes.

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مقالة بحثية

تحديد النيكوتين في السجائر المحلية والمستوردة في أسواق عدن (اليمن)

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المُلخّص

هدفت الدراسة الحالية إلى: تحديد محتوى النيكوتين في بعض السجائر المحلية والمستوردة ومقارنة محتوى النيكوتين بين 12 نوعًا من السجائر المألوفة التي تباع في أسواق محافظة عدن (اليمن)، باستخدام كروماتوجرافيا سائلة عالية الأداء مرتبطة بكاشف الأشعة فوق البنفسجية، (HPLC-UV). أظهرت نتائج هذه الدراسة أن كمية النيكوتين في كل سيجارة كانت من 7.29 إلى 18.74 مجم / جرام سجائر بمتوسط 12.75 مجم / جرام سجائر في السجائر المستوردة، وما بين 11.34 إلى 17.52 مجم / جرام سجائر بمتوسط 14.40 مجم / جرام في السجائر المحلية. وعند مقارنة متوسطات السجائر المحلية والمستوردة، أظهر التحليل الإحصائي أنه لم تكن هناك فروق ذات دلالة إحصائية بين متوسطات السجائر المحلية والمستوردة. كان محتوى النيكوتين لجميع السجائر المختبرة (العلامات التجارية المستوردة والمحلية) أعلى من المعيار الدولي 1مج/جم.

الكلمات المفتاحية: النيكوتين، السجائر، التبغ، الكروموتوغرافيا السائلة عالية الأداء – طيف الأشعة فوق البنفسجية.

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