

# Analytical Determination of Nicotine Content in Tobacco Brands in Libyan Markets

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**Abstract**— the present research is focused on determination of nicotine content in international tobacco brands and growth tobacco in Libya locally sold in Libyan markets. Extraction method was used to extract the nicotine from the thirteen tobacco brands, three local produced tobacco and ten international brands of locally sold tobacco. The concentrations of nicotine (mg nicotine/g cigarette) in all tobacco brands studied were measured using ultraviolet-visible (UV-Vis) spectrophotometer at wavelength of  $\lambda=602\text{nm}$ . The pattern of nicotine concentrations in Libyan chews were followed green>dry>Ziliten, while were varied in the international tobacco brands. The nicotine content in Libyan tobacco cigarette brand (Al-Riadei) was found to contain on average 4.20 mg nicotine/g cigarette, while the international tobacco brands were found to contain from 3.85E-02 to 1.40E01 mg nicotine/g cigarette. The highest nicotine concentration was found in the international brand named Capital, while the lowest was found in the international brand named Business. The findings in this work showed that the nicotine contents in all brands studied were under a lethal dosage for adult-human, but higher than the significant impact on nicotine intake. Also, the price of tobacco was not related to the nicotine content and quality of tobacco, but related to brand's name.

**Keywords**—about four key words or phrases in alphabetical order, separated by commas

## I. INTRODUCTION

Over three thousand years, tobacco has been smoked and it was found it by Christopher Columbus in the America in 1492 [1]. Different types of tobacco products are available in supermarkets, such as bidis, chew, cigarettes, cigars, dip, dissolvable tobacco, electronic cigarette, hooka, kerteks, pipe, smokeless tobacco, snuff, snus and spit tobacco. The order to use the tobacco products is about 70% of all tobacco, chewing tobacco for 20% and 10% for cigar. However, the composition of tobacco leaf have been identified and characterized. About 3000 components were found in tobacco leaves, while 4000 components are in smoke. These chemicals are classified to be toxic or carcinogenic chemical compounds such as nicotine, nitrogen oxides, carbon dioxide, hydrogen cyanide etc. One of the most dangerous component on human health is nicotine (3-substituted pyridine,  $\text{C}_{10}\text{H}_{14}\text{N}_2$ ) due to more than one dies person every 8 s [2-4]. Therefore, it

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is so important to evaluate the nicotine content in tobacco leaves in order to protect smokers from high dose of nicotine. Nicotine is present in several plants especially in tobacco leaves of *Nicotiana tabacum* and *Nicotinia rustica* plants. Even though, tobacco leaves are extremely hazardous for humans leading to increase heart rate and blood pressure, it is still highly consumed over the world over 10 million pre-year by 2030 being the leading cause of negative impact on economic development. The deep inhalation of cigarette smoke is causing the addiction to nicotine presence in a tobacco leaves due to the nicotine reaches central nervous system within 20 s. For instance, over 85% of American's alcoholics were addicted to nicotine, while 29% of non-alcoholic Americans are addicted. Also, 39.9% of Romanians are smoke tobacco as reported by Center for Health Policies and Services [1]. In U.S., the cost of diseases by smoking tobacco leaves excess of \$150 billion each year. It has been reported by WHO, one of two long term smokers in low income countries will die from tobacco daily intake [5].

The physiological effects on human health by inhaling the nicotine depend upon the dose, rate of administration and elimination, and tolerance level of smoker. The nicotine content determination is an important indicator for quality and usability of industrial tobacco brands [4]. The tobacco products may contain amount of nicotine that is more or less than normal, which cause serious health problem [1]. Therefore, in order to prevent the harmful and toxic behavior on human that is caused by nicotine intake, the evaluation of nicotine concentration is needed. Therefore, it is a very important to evaluate the concentration of nicotine in commercial tobacco products.

Based on the data available in the literature, great concern is claimed recently regarding the nicotine content in tobacco. A global concern on nicotine content in the international tobacco markets has been paid due to the quality of tobacco and quantity of nicotine present in each cigarette. However, 40 to 60 mg of nicotine is considered to be highly toxic for a lethal dosage for adult-human [3]. Also, the significant impact on nicotine intake ranges from 0.3 to 3.2 mg nicotine/g cigarette. The concentration of nicotine in cigarettes is an important factor for determination of absorbed nicotine by human over period of time. Unfortunately, a very little of published studies regarding to the concentration of nicotine in local sold tobacco brands has been done. Many local and international tobacco brands are sold in Libya market. International tobacco brands are mainly imported from Niger, Malta and Turkey, while local brands are grown in different parts of Libya such as

Ziliten, Murzk and Zaweia. The present study is aimed to determine the nicotine contents in local and some international tobacco brands and to compare the nicotine content between the brands for 13 popular cigarettes sold in Libyan market.

## II. MATERIALS AND METHODS

### A. Materials

NaOH,  $(\text{CH}_3\text{COO})_2\text{Zn}$ ,  $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$ , charcoal and methanol were purchases from and used without further purifications. Deionized water was used in this work. Standard nicotine was purchased from BDH Chemicals Ltd., England with purity 98% and used as received without any further purification. Tobacco brands were purchased from local supermarket with one donation from Fazzan Tobacco Shop.

### B. Instruments

Ultraviolet-visible single beam spectrophotometer of JANWAY 6305 was used at fixed wavelength at 602 nm and 1 cm plastic cell was used for all spectrophotometric analysis. In order to compare the method used in this work, the proposed UV-Vis spectrophotometer method was according to the presented method by Suryani et al 2012 [4].

### C. Samples

Tobacco brands used in this work are summarized in TABLE 1. Tow chew and one tobacco brands of local production and ten international brands of locally sold tobacco were analyzed. Libyan and international Tobacco packs with 20 sticks per pack were bought from local market with one exception of south tobacco collected as green leaves.

### D. Extraction procedure of nicotine

The extraction method of nicotine used in this work was carried out according to the method presented by Suryani et al 2012 with slight modifications [4]. The nicotine present in thirteen commercial products of locally sold tobacco brands was extracted. From each brand, three replicates were made. From 5 to 10 sticks of each different Libyan and international tobacco cigarette brands were weighted (about 3.73 to 3.75 g), dried for 1 h at 450C and grounded using a mortar and pestle. Then, the grounded sample was immersed in 10 ml of methanol. The mixture was stirred for 30 min with 200 rpm and 25 ml of deionized water was added. One ml of 2 N NaOH was added. The mixture was stirred for another 30 min before keeping it in a boiling water bath for 10 min to evaporate methanol. The mixture was cooled and filtered by Whatman filter paper P41. Then, 1 ml of freshly prepared zinc acetate and 1 ml potassium hexacyanoferrate (II) were added, transfer into 50 ml volumetric flask and completed to the mark with deionized water. The mixture was shaken and centrifuged at 4000 rpm for 5 min. The supernatant liquid was carefully collected into a beaker and the residue was discarded. Then, 1.0 mg of charcoal animal was added, mixed well and allowed to sit for 2 min at room temperature. 0.01 N NaOH was added to rise up the solution pH and filtered. Finally, the solution was made up to 50 ml with deionized water. The total nicotine content in the mixture was determined using the calibration curve.

TABLE I  
SAMPLE NAMES AND ABBREVIATIONS

Brand name	Producer	Abbreviation
Business		BUS
Ziliten	North of Libya	ZIL
American Legend		AML
Dry south Libyan Tobacco	South of Libya	DLC
L&M	EU/Philip Morris	L&M
Al-Riadei	Libya	ALR
Karelia	Greece	KAR
Marlboro	Philip Morris	MAR
Rothmans		ROT
Manchester	J.S.S. Tobacco Ltd. London U.K.	MAN
Fisher	Trademark Owner in Germany	FIS
Green south Libyan tobacco	South of Libya	GLC
Capital	Trademark Owner in London U.K.	CAP

### E. Determination of nicotine content

The nicotine concentrations in extracted solution using the above procedure for different brand of cigarette samples were determined using UV-Vis spectrophotometer, JANWAY 6305 at a wavelength at 602nm. 1 cm plastic cuvette was used.

### F. Determination of standard curve for nicotine content

In order to determine the standard curve of nicotine concentration, nicotine standard solution was used. The nicotine standard stock solutions were prepared by appropriate dilution of standard stock solution with methanol and deionized water. Eight standard solutions of nicotine were prepared by dilution of concentrated nicotine solution (1000 mg/L) using concentration in the range of 0.2-1.6 mg/L. In order to eliminate the interference effect by other chemicals on standard curve, all the chemicals used for nicotine extraction was added to nicotine standard as well. The concentration of nicotine was evaluated at selected wavelength of 602nm using JENWAY 6305 UV-Vis spectrophotometer against a reagent blank as reference.

### G. Statistical analysis

The measurements were triplicates and the means of the obtained results were expressed. The data were subjected to two way variance analysis (ANOVA) with  $P < 0.05$  to be considered a significant difference in obtained data.

## III. RESULTS AND DISCUSSION

**Figure 1** shows the standard curve for nicotine used in this study to the maximum nicotine concentration of 1.6 mg/L. Several studies and this study have reported the big challenge to obtain a nicotine standard calibration curve [4]. Even though, the same difficulties have been noted in this work as well, a good correlation coefficient is observed. Calibration curve showed a linear response with  $R^2$  of 0.9863 over the range of 0.2 to 1.6 mg nicotine/L water and methanol. This

calibration curve was used to obtain the concentration of nicotine in different tobacco brands. Therefore, it can be reported here as one noteworthy method for a range of nicotine concentrations. However, it should be considered that the cloudy color was started to appear above to 1.8 mg/L of nicotine and the precipitation was occurred above 2.0 mg/L.

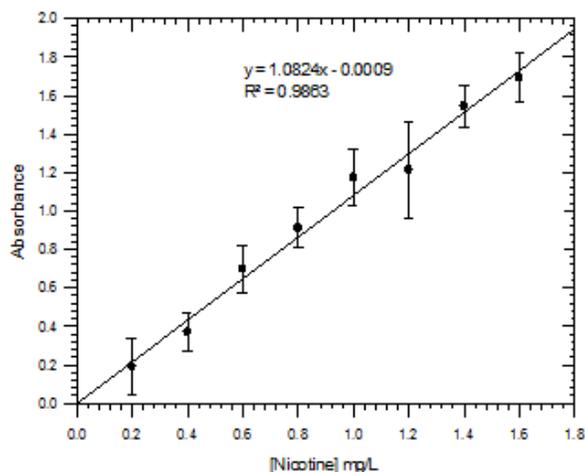


Fig. 1 Standard calibration curve of nicotine content

TABLE II shows the concentration of nicotine in locally sold of commercial cigarette brands and expresses as mg nicotine/g tobacco. The nicotine concentrations in tobacco products were ranged from 3.85E-02 to 14.0 mg nicotine/g tobacco with its mean of 5.31 mg nicotine/g tobacco as listed in TABLE I. It was observed that there is relationship between nicotine content and tobacco brands as shown in Fig II and TABLE II. The difference in nicotine content is not related to the weight of cigarette, but could be directly related to the kind of cigarette used, quality, manufacturing process or packaging tobacco [4]. The nicotine content on cigarette is also based on pH of the manufacturing process of cigarette. Also, some materials like sodium carbonate and ammonium carbonate are added to increase pH of tobacco, which affects the nicotine content [6]. However, Libyan chew showed less nicotine content than other brands with one exception of Libyan green tobacco and Business brand. The increase of nicotine content is in order to: Business, Zlitan, dry Libyan tobacco, American Legend, L&M, Al-Riadi, Karelia, Marlboro, Rothmans, Manchester, Fisher, Green Libyan tobacco (chew) and capital. Also, the price of tobacco box was not related to nicotine content, but it is dependent on taxes and duties due to Capital brand with high concentration of nicotine (14.0 mg nicotine/g tobacco) is sold for 0.5 DL, while Marlboro brand with high concentration of nicotine (4.87 mg nicotine/g tobacco) is sold for 3.00 DL.

To compare the results in the developed standard curve method used in this research and concentration of nicotine on other brands, L&M, Manchester, Marlboro, Rothmans tobacco brands were used in present study as a comparison study. The results showed the concentrations of nicotine found in these brands are almost half of comparable concentration with that found in the same brand of this work. These differences in concentration of nicotine may be due to either the quality of brands or the method used to determine the nicotine content. This could be due to the quality of brands as comparing to

Suryani et al 2012 using the same technique [4]. Different technique LCMSMS by Vlase et al 2005 was used to determine the nicotine content and found different results for M&L, Marlboro and Rothmans brands [1].

Fig 2 shows the relationship between nicotine content per stick of cigarette and cigarette brands. However, the concentration of nicotine in commercial products was found to be in the range of 6 to 18 mg nicotine/g tobacco. While, in the present study, it is ranged from 7.70E-01 to 14.0 mg nicotine/g cigarette with its mean of 5.31 mg nicotine/g cigarette as listed in TABLE II. Therefore, the analyzed brands were either in poor quality or different method used for nicotine detection as comparing to other studies.

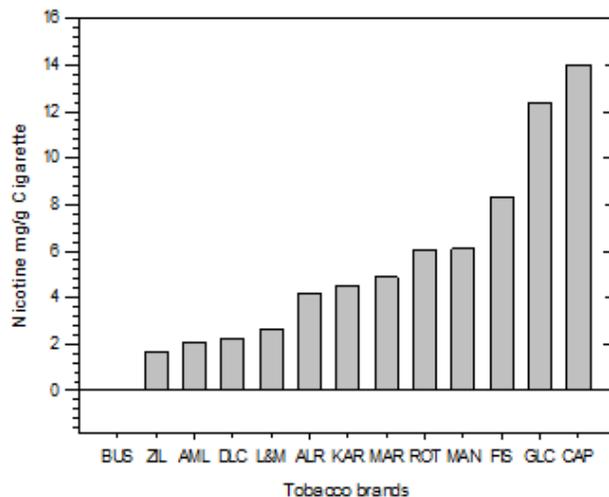


Fig. 2 nicotine concentrations and tobacco brands

TABLE II  
NICOTINE CONCENTRATIONS OF LIBYAN AND INTERNATIONAL TOBACCO BRANDS SOLD IN LIBYAN MARKET.

Brand Name	Number Sticks	Weight per Stick (g)	[nicotine] mg nicotine/g box of Tobacco	Nicotine mg nicotine/stick	STD	Price for box of tobacco (DL)	Suryani (mg/g)	Vlase (mg/g)
BUS	5	0.75	7.70E-01	1.54E-04	2.32	0.50		
ZIL	0	0.375	3.38E01	3.38E-02	5.77E-04	1.00		
AML	5	0.75	4.09E01	8.18E-03	1.15E-03	0.75		
DLC	0	0.375	4.44E01	4.44E-02	5.77E-04	2.00		
L&M	7	0.535	5.23E01	7.48E-03	0.00	2.50		9.16
ALR	10	0.373	8.40E01	8.40E-03	1.15E-03	1.00		
KAR	6	0.625	9.01E01	1.50E-02	5.77E-04	1.00		
MAR	6	0.626	9.74E01	1.62E-02	1.73E-03	3.00		9.86
ROT	5	0.75	1.21E02	2.43E-02	1.70E-17	2.50		1.25E01
MAN	6	0.626	1.22E02	2.03E-02	1.15E-03	0.35	3.50E-01	
FIS	5	0.75	1.66E02	3.32E-02	5.77E-04	0.50		
GLC	0	0.375	2.48E02	4.13E-02	1.70E-17	1.00		
CAP	7	0.535	2.80E02	4.01E-02	3.68E-03	0.50		

a study by Vlase et al 2005 using LCMSMS to determine nicotine concentrations[2]; b study by Suryani et al 2012 [1]; LD: Libyan dinar =0.756 dollar

The statistical analysis listed in table 3 shows some variation within brands. Table 3 shows the values of sum squares (SS) and mean of squares (MS). The results show that SS and MS for columns are 9.03 and 4.52, respectively, which higher than those along to the rows, 3.15 and 2.62E-01, respectively. This suggests that the nicotine content found in those brands can be compared along to columns than rows due to MS along to columns are higher than this of along to rows. Also, the value of Fcal is 9.04E-01, while that of Fcrit. is 2.18 along to rows indicate that the null hypothesis is accepted along to columns and no significant differences along to rows between nicotine content and cigarette brands. The value of Fcal is 13.5 while that of Fcrit is 3.40 along to columns. Fcal is greater than Fcrit and the p-value in columns (p-value < 0.05, one-way ANOVA) indicate that the null hypothesis is rejected and significant differences along to brands, weight and price of cigarette. Statistically, the results indicate significant differences in the nicotine content studied among the cigarette brands, weight and price. These observed variations across all samples testes lead to our differentiation between nicotine content brands was based on the kind of brand.

To compare the results in the developed standard curve method used in this research and concentration of nicotine on other brands, L&M, Manchester, Marlboro, Rothmans tobacco brands were used in present study as a comparison study. The results showed the concentrations of nicotine found in these brands are almost half of comparable concentration with that found in the same brand of this work. These differences in concentration of nicotine may be due to either the quality of brands or the method used to determine the nicotine content. This could be due to the quality of brands as comparing to Suryani et al 2012 using the same technique [4]. Different technique LCMSMS by Vlase et al 2005 was used to determine the nicotine content and found different results for M&L, Marlboro and Rothmans brands [1].

TABLE III  
STATISTICAL ANALYSIS FOR BRAND WEIGHT AND NICOTINE CONTENT

ANOVA						
Source of Variation	SS	Df	MS	F	P-value	F crit
Rows	65.009	12	5.4173	0.8568	0.5971	2.183
Columns	170.19	2	85.097	13.458	0.0001	3.4028
Error	151.75	24	6.3230			
Total	386.96	38				

#### IV. CONCLUSION

The findings in this research indicate that the Libyan produced cigarette brands have less nicotine content than international brands. The amount of nicotine present in locally sold cigarettes was lower than those sold overseas. This method to determine the concentration of nicotine in this work is reliable and may be used for further. The calibration method was successfully applied for determination of nicotine in different brands of commercial cigarettes. The nicotine contents found in different cigarette brands were lower than content found in other study. The finding in this work confirm that the price of cigarette was not dependent on the nicotine content, but on taxes and duties. The estimation of nicotine content might be useful in the development of cigarette quality and control.

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